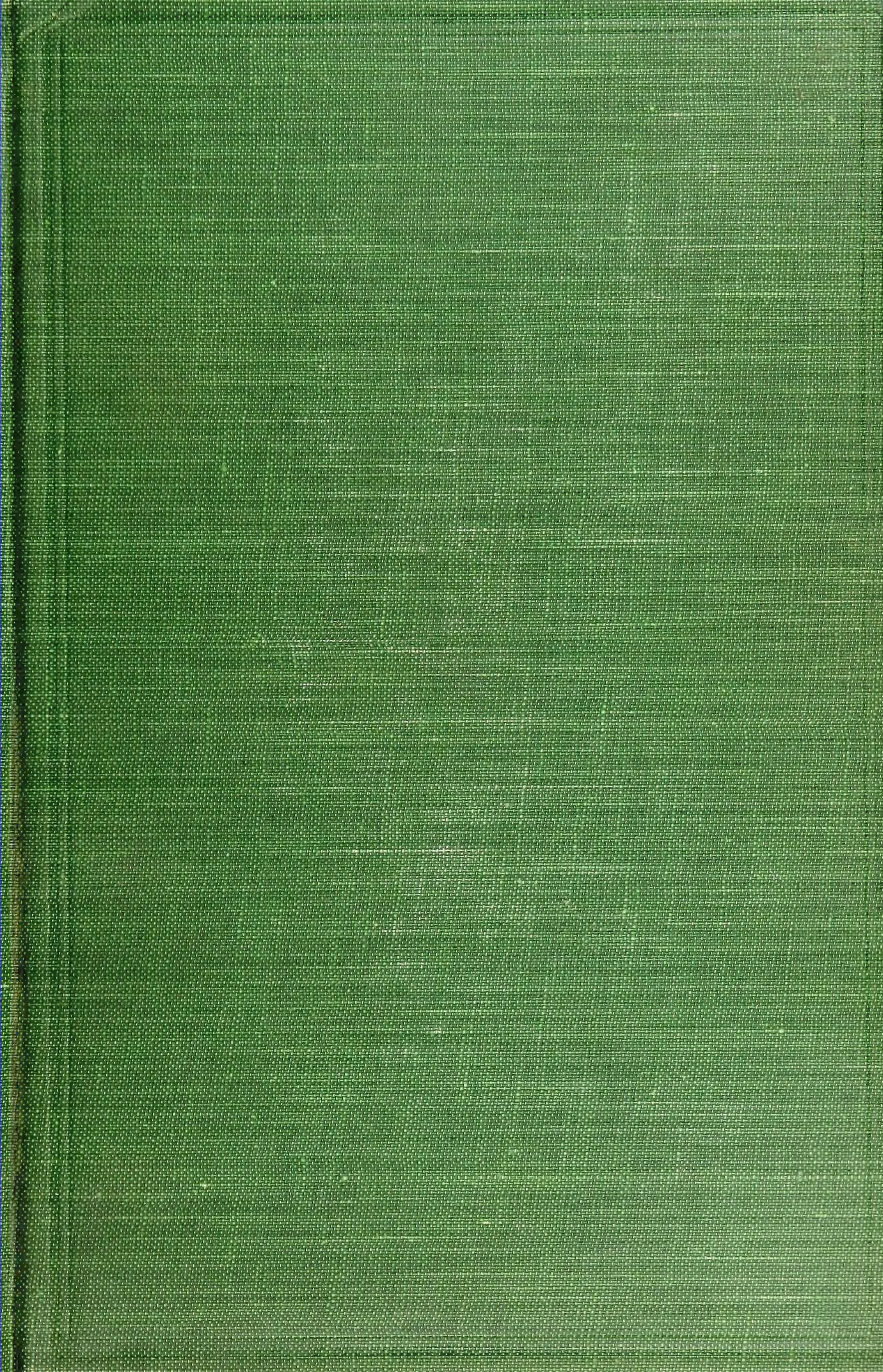

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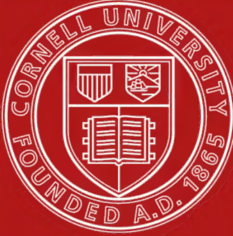

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PHARMACEUTICAL BACTERIOLOGY

SCHNEIDER

PHARMACEUTICAL BACTERIOLOGY

BY

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WITH 97 ILLUSTRATIONS

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PREFACE TO SECOND EDITION

The recent progress in bacteriological science has made it necessary to make certain changes and additions in the present volume. The following chapters have been added. Chapter III, The Origin of Bacteria; Chapter VII, Symbiology; Chapter IX, Zymology; and Chapter XII, Adenology. In addition to this wholly new matter, not given in the first edition, other additions have been made in the text. The subject of soil bacteriology and of milk and water analysis, has been treated more fully. The chapter on sterilization and disinfection has been enlarged. A brief statement of sensitized bacterins, of anaphylaxis, of aggressins and of storing biologies has been introduced. Some of the disputed points have been cleared up or have been entirely omitted. The many imperfections of a first edition of a book covering a new field of scientific endeavor, or to put it more correctly, a new application of a science, namely the science of bacteriology to pharmacy, have been largely corrected.

A text-book deals with the established facts of science, giving just enough of the theoretical to indicate the further advance in the near or perhaps remote future. The present volume has adhered to this requirement. It is possible to present the facts of science intelligently without using difficult words or complicated phraseology. A suitable text must not be encumbered by unnecessary technical terms, nor should it be a mere glossary of scientific terms. If definitions were all that is required of a text-book, then Webster's Unabridged Dictionary would be the ideal universal text-book for all grades and classes of students. It is believed that the present volume is not faulty in these regards. It is believed that the book will be found quite "readable" and intelligible to the student of ordinary ability.

Grateful acknowledgments are hereby made to Dr. Aubrey H. Straus of the Medical College of Virginia, for many suggestions and for calling attention to some of the more glaring imperfections in the first edition. The present volume is believed to be complete for the purpose for which it is intended.

LINCOLN, NEBRASKA,
December, 1919.

PREFACE TO FIRST EDITION

The recent growth and development of the professional side of pharmacy has made new text-books necessary. The present volume is the product of such progress.

The illustrations have been selected with a view to a fuller explanation of the text. The descriptions of the illustrations have been made unusually complete. This is to make it possible for the student to ascertain the use of every article illustrated without the necessity of searching for additional information in the text itself. Some of the illustrations are from original drawings, others are from electros supplied by the Bausch & Lomb Optical Company and the Cutter Biological Laboratory of Berkeley, California. Still others are taken from recent works on bacteriology, notably Williams' "Manual of Bacteriology."

Attempts have been made to adhere strictly to the subject from the standpoint of the pharmacist, with only enough treatment of general bacteriology to make clear the collateral relationships, especially as it pertains to medical, and commercial or industrial bacteriology.

While this volume is primarily intended for students in colleges of pharmacy, it is hoped it will also be found useful by practising pharmacists.

San Francisco.

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PHARMACEUTICAL BACTERIOLOGY

CHAPTER I

GENERAL INTRODUCTION

The science of bacteriology is not new, but its introduction into pharmacy is of comparatively recent date.

About 1896 a few of the colleges of pharmacy in the United States gave optional courses of instruction in bacteriology. At the present time nearly all of the leading colleges of pharmacy give instruction in bacteriology and in many of these institutions the courses are compulsory, forming a part of the prescribed curriculum, represented by lectures and laboratory work. In some universities the students of pharmacy receive their bacteriological instruction in the department of medicine or perhaps dentistry. However, pharmaceutical bacteriology and medical bacteriology are quite distinct. Medical students study this subject from the standpoint of pathology and disease, matters which concern the pharmacist but little. Students of pharmacy do not have the time necessary to devote themselves extensively to special laboratory methods and technic, nor is it advisable that they should receive extensive laboratory instruction in pathology. Pharmaceutical bacteriology must be suitably adapted to the practice of pharmacy.

The pharmacist should have a knowledge of general bacteriology, in order that he may realize what important relationships bacteria bear to human activities in general, to medical practice more especially, and in order that he may comprehend quite fully the significance of these minute organisms in pharmaceutical practice. He should know what pharmaceutical preparations and what medicinal substances are likely to be attacked by bacteria, and what changes they are capable of producing in such substances. He should have some knowledge of the effects that bacterially deteriorated substances may have when introduced into the human organism. He should be qualified to sterilize pharmaceuticals as is now required in the U. S. P. and in the pharmacopœias of several foreign countries as Austria, Italy, and Belgium. He should know some-

thing of the comparative value of the numerous disinfectants and antiseptics used and found upon the market and should know how to standardize these agents according to recent bacteriological methods. The pharmacist should know that bacteria, yeasts, and related organisms develop very promptly and profusely in all aromatic waters; in carelessly manipulated boiled and distilled water; in dilute solutions of all acids and alkalies; in dilute alcohol and alcoholic liquids; tinctures; infusions; extracts, solid and liquid; decoctions; in dilute salt solutions; in plant juices; mucilages; emulsions; elixirs; wines; in syrups of all kinds; in carelessly manipulated vegetable drugs, crude and powdered; in drugs from the animal kindgom, as ox-gall, lard, oils, fats, pepsin, etc. He should have a clear comprehension of antiseptics as germ destroyers, and should know how to prepare and use them. He should have a general knowledge of phagocytosis; of leucocytosis in inflammatory processes, in pus formation, necrosis, etc. He should comprehend immunity, natural and acquired; he should know about opsonins and the opsonic index. He should have a general knowledge of bacterial enzymes; of toxins, ptomaines, leucomaines; of antitoxins; of bacterial vaccines. He should have a special knowledge of the source, manufacture, and use of antitoxins and toxins, modified toxins, vaccine virus, and related products used in medical practice. He should have a general knowledge of the causation of the more common bacterial and protozoic diseases. He should have special instruction in the disinfection of public and private dwellings, and should be able to coöperate with the physician in stamping out threatened epidemics and in carrying out public prophylactic and hygienic measures. To attain these ends a knowledge of bacteriology, specialized to suit the needs of the pharmacist, is absolutely essential.

It is not the so-called practical side of bacteriology, represented by dollars and cents, which should interest he pharmacist in this science, but rather the broader view of his profession which will enable him to perform his duties more intelligently and more efficiently. The man whose actions are altogether prompted and directed by the dollar sign has no place in pharmacy or in medicine. He should turn to some non-professional enterprise.

Text-books on bacteriology for use in universities, medical colleges, and technical schools are not suitable for use in colleges of pharmacy. Some of these books are excellent collateral reading for pharmacists, but most of them are of such a highly specialized nature that they would no doubt do more harm than good should the average pharmacist attempt to use them as a practical guide in the performance of his duties. Bacteriology must not be made discouragingly difficult to the pharmacist, in order that the best results may be attained.

Wherever possible the college instruction in pharmaceutical bacteriology should be supplemented by visits to biological laboratories for the manufacture of sera and bacterial vaccines, to board of health laboratories, quarantine stations, garbage reduction works, etc. Students should also be assigned special reading. Journals and special treatises on bacteriology and on public sanitation should be consulted. The reports on bacteriological and related subjects issued from time to time by the United States Public Health Service are of special interest.

The following references are given for the benefit of those students who may desire further information regarding the earlier conceptions of pharmaceutical bacteriology. It will be found that the opinions advanced by the authors cited differ considerably.

1. Bacteriology for Pharmacists. *Pharm. Journ. Trans.*, 23 (III), 565, 865; 24 (III), 101, 1893.

Largely a description of the apparatus employed in bacteriological work, giving special attention to the value and use of the compound microscope in such work.

2. H. P. Campbell. Bacteria Dangerous to Medicines. *Am. Journ. Pharm.*, 72, 113–118, 1890.

3. R. G. Eccles. Pharmaceutical Bacteriology. *Proc. A. Ph. A.*, 42, 225–230, 1894.

A very interesting paper on the theoretical possibilities of pharmaceutical bacteriology.

4. J. L. Hatch. Bacteriology. *Pharm. Journ. Trans.*, 22 (III), 271, 289, 330, 1891.

A series of lectures delivered before the alumni association of the Philadelphia College of Pharmacy, devoting the major attention to the morphology, physiology, and classification of bacteria.

5. R. T. Hewlett. Bacteriology in its Practical Aspects. *Pharm. Journ. Trans.*, 25 (III), 819–820, 893–894, 1895.

A general retrospect of bacteriology as a possible source of financial gain to the pharmacist.

6. Smith Ely Jeliffe. Moulds and Bacteria. *Druggists Circular*, 94–95, 1897.

A description of some of the more common moulds and bacteria found in medicinal solutions. Good illustrations.

7. E. Klein. Bacteria, Their Nature and Function. *Pharm. Journ. Trans.*, 23 (III), 15, 35, 1893.

8. W. H. Lymans. Bacteriological Culture Apparatus. *Pharm. Journ. Trans.*, 1893. (*National Druggist*, 173, 1893.)

9. Albert Schneider. Pharmaceutical Bacteriology. *Proc. A. Ph. A.*, 48, 186–189, 1894.

10. Specialism in Pharmacy, begotten by Progress in Bacteriology. *Pharm. Journ. Trans.*, 25 (III), 625, 1895.

Points out the necessity of a suitable preparatory training; the importance of a knowledge of the use of antiseptics.

11. R. Warrington. The Chemistry of Bacteria. *Pharm. Journ. Trans.*, 23 (III), 402, 1893. (*Pharm. Era.*, 104, 1894.)

CHAPTER II

HISTORICAL

It must be evident that the science of bacteriology had its inception with the discovery of the compound microscope. For some time the progress in bacteriological investigation continued parallel with the progress in the mechanical perfection of the microscope and with the advance in microscopical technic. Gradually, however, the chemical and physiological investigations pertaining to bacteria gained in importance and significance. Our knowledge of the morphology of bacteria as revealed through the compound microscope has been practically stationary for two decades, but not so our knowledge of bacterial products and bacterial action. The methods of bacteriological technology have been gradually perfected, and the progress along this line has kept pace with the chemical and physiological investigations.

Although, as indicated, the science of bacteriology is of comparatively recent origin, yet we must not lose sight of the fact that many of the ideas underlying this science, as now comprehended, were advanced in remote antiquity. For this reason it is desirable to set forth these earlier concepts in a historical review. Most of the writers on general bacteriology, who make reference to the history of the subject, almost invariably mention the older ideas regarding spontaneous generation as being the forerunners of the modern ideas of bacteriology. It is, however, the ancient theories and beliefs pertaining to the cause of decay, disease, and epidemics which are even more directly associated with the first more important discoveries pertaining to modern bacteriological pathology.

For the purposes of simplification, condensation, and greater clearness the historical review is divided into periods or epochs. It is not possible, in the following brief outline, to cite all investigations of importance. Only a few of the epoch-making specialists are mentioned.

Period I

From Hippocrates (300 B. C.) to Leeuwenhoek (1656). (The earliest ideas regarding epidemics and spontaneous generation.)

From the earliest times the more scholarly writers mentioned certain noxious gaseous, and odoriferous substances or effluvias as being the cause of epidemics. These effluvias were supposed to emanate from the soil,

from the air, from water, stagnant pools, marshes, from decaying and putrescent substances, from crowded habitations, army camps, etc. The common people throughout the world and throughout all ages have held the belief that pestilence and disease was the manifestation of divine or supernatural influence, the judgment of an angry deity, a punishment inflicted on mankind for their sins and iniquities, beliefs which are occasionally asserted even at the present time. Changes of season, climatic conditions, and the influence of heavenly bodies were also considered as causative of diseases of an epidemic nature.

Animals, such as rats, mice, and insects, have long been recognized as possible carriers of disease. An English investigator has recently discovered some very excellent sanitary rules in the Vedas of the Hindus. The following is a translation from Book VI, verse 50, of the Atharva-Veda.

"Destroy the rat, the mole, the boring beetle; cut off their heads, O asvins.

"Bind fast their mouths; let them not eat our barley; so guard ye twain our growing corn from danger.

"Hearken to me, lord of the female borer, lord of the female grub! Ye rough-toothed vermin.

"Whate'er ye be, dwelling in woods, and piercing, we crush and mangle all those piercing insects."

By "piercing insects" no doubt mosquitos are meant. If the injunctions were literally obeyed, plague, malaria, and certain protozoic diseases would be abolished from India.

Hippocrates (460–377 B. C.), the father of medicine, considered seasons and winds as the cause of pestilence, particularly the long continued southerly winds (for Greece), and a warm, humid, clouded atmosphere. Galen (130–220 A. D.) held similar beliefs. He declared that diseases arose from a putridity of the air or from atmospheric and weather conditions. Marcellinus (359 A. D.), a warrior as well as philosopher and historian, declared that the decomposing bodies left on the battlefield were the cause of "pestilential distempers," also caused by extremes in weather, by marsh effluvias, violent heat, and a vitiated atmosphere. Aetius (fifth century), an eminent physician, declared that epidemics or common diseases were caused by bad food, bad water, immoderate grief, hunger, excesses, particularly abundance following extreme want, lack of exercise, excessive humidity, and putrid substances. Alpinus, a Venetian physician of the sixteenth century, explained how the cause of plagues and epidemics may be carried by persons or in cargoes. He pointed out that a given disease from one country is more malignant than the same disease from another country. During the dark and middle ages various ecclesiastical and lay writers ascribed epidemics and pestilence to a variety of causes—the wrath of

God, to demons or evil spirits, comets, meteors, earthquakes, volcanic eruptions, cyclones, eclipses of the sun, terrific storms, wars, famines, great fires, etc. Even as late as 1799 no less an authority than Noah Webster makes the following declaration: "All the great plagues which have afflicted mankind have been accompanied with violent agitations of the elements. The phenomenon most generally and closely connected with pestilence is an earthquake. From all the facts which I can find in history, I question whether an instance of any considerable plague, in any country, can be mentioned which has not been immediately preceded by, or accompanied with, convulsions of the earth. If any exceptions have occurred, they have escaped my researches. It does not happen that *every place* where pestilence prevails is shaken; but during the progress of the disease which I denominate *pestilence*, and which runs, in certain periods, over large portions of the globe, some parts of the earth, and especially those which abound most with subterranean fire, are violently agitated." Were Noah Webster alive, he would certainly cite the recent plague on the Pacific Coast as bearing out his assertions. On April 18, 1906, the coast region about San Francisco was certainly "violently agitated," and this phenomenon was followed by the plague (black pest, bubonic plague). But what were the actual facts? The plague had, in all probability, existed in a sporadic form in "Chinatown," in San Francisco, and in other places on the Pacific coast for many years. In 1903 several authentic cases came to notice and were reported. The reasons why the disease had not previously gained a stronger foothold in San Francisco are several. Chinatown is more or less isolated (socially, at least) from the rest of the city, and the poorer, more filthy class of the Chinese do not as a rule mingle with the white population. The disease is an Oriental filth disease. After the earthquake and fire of April 18–22, 1906, the Chinese of all classes, the plague-infected rats and fleas of the Chinese quarters, became thoroughly intermingled with the rest of the stricken population, and as a result there were established several new foci of plague infection, which accounted for the increase in plague cases in 1907, a condition which was soon under control, thanks to the strenuous efforts of the federal government, the board of health, and various citizens, organizations.

Several writers of remote times, as well as occasional writers of the dark and middle ages, held the opinion that the cause of disease, the disease-producing effluvias, might be carried long distances by air currents, in ships, or by caravans, and that the poison may enter the system *via* the air passages, through the skin, or through the digestive tract. Hodges, an Englishman, who wrote a treatise on the London plague of 1665, declared that some essential alteration in the air is necessary to the propaga-

tion of this disease. That is, the "nitro-aerial" principle, which causes or invigorates plant and animal life, is supposed to become vitiated.

The corrupting principle is a "subtle aura or vapor" which is "extricated from the bowels of the earth." This plague-causing poison was said to affect trees and other plants, fishes and other animals, as well as man. Dr. Mead declared that epidemics were caused by (1) diseased persons, (2) goods imported from infected places, and (3) a vitiated or poisoned state of the air, notions which may be considered as the direct forerunners of the germ theory of disease.

Let us now go back and consider the ancient ideas regarding spontaneous generation. Anaximander, of Miletus, who lived during the forty-third Olympiad (610 B. C.), believed that many animals developed *de novo*, from moisture and water acted upon by sun and warmth. The extremist.

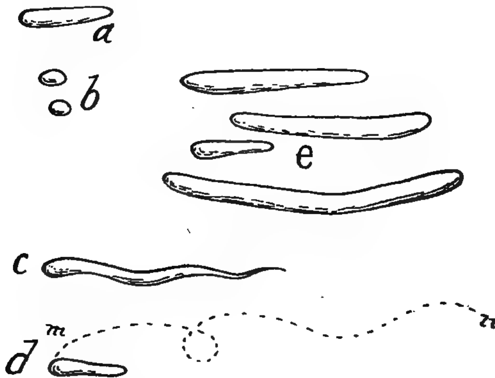

FIG. 1.—From the *Arcana Naturæ* of A. van Leeuwenhoek. The first published illustration of bacteria. These bacilli of the mouth cavity were seen with the aid of simple lenses only. *a*, *b*, bacilli; *c*, a spirillum; *e*, perhaps chain forms of bacilli; *d*, illustrating the characteristic motion of certain bacilli (*n* to *m*).

Empedocles of Agrigentum (450 B. C.), declared that all living things upon the earth were capable of originating spontaneously. Aristotle (384 B. C.) taught that *some* plants and animals originated spontaneously. Ovid, some three centuries later, gives instructions how to create bees spontaneously in the carcasses of horses. To within recent times the belief that certain animals could originate spontaneously, that is, without a pre-existing parent, was quite general, and differed only in grotesqueness. Cardan as late as 1542 declared that water created fishes, and that many fermentative processes created animals. Van Helmont gives instructions how to produce mice artificially. Kircher boldly declared that he had seen certain animals develop spontaneously before his eyes. Paracelsus gives instructions how to make homunculi. The instructions are quite

simple. Certain substances are placed in a bottle, the bottle is well stoppered and buried in a manure heap. Every day certain incantations must be pronounced over the bottle in the manure heap. In time, Paracelsus declared, a small living human being (homunculus) will appear in the bottle. Paracelsus, however, naïvely admits that he has never succeeded in inducing the homunculus to continue alive after being taken from the bottle. Gradually these grotesque and extreme opinions regarding tpontaneous generation were abandoned, and it was declared that only she lower plants and animals, such as seaweeds, algæ, lichens, lice, mites, maggots, etc., could develop spontaneously. In fact, we can find fairly intelligent individuals to-day who firmly believe that certain animals, as lice, mites, etc., can originate without a parent, and that the hair from the tail or mane of a horse will change into a worm or snake if placed in a bottle of water and exposed to light and warmth.

From the earliest records we learn that the value of disinfectants in preventing the spread of infectious diseases (epidemics and plagues) was known. Ovid states that the shepherds of his time used burning sulphur for bleaching wool and to free it from infectious diseases. In time of plagues, big fires were made to stay the ravages of pestilential diseases. The Mosaic law is replete with instructions regarding cleanliness as a means of preventing disease. Wine was highly valued as a dressing for wounds, having the effect of preventing or checking pus formation.

Period II

From Leeuwenhoek (1656) to Schwann (1837). (Discovery of micro-organisms and the early investigations regarding their activities.)

As early as 1646 Kircher suggested that certain diseases might be due to very minute organisms which were supposed to originate spontaneously under certain conditions. Anton van Leeuwenhoek is very justly called the father of microscopy, and to him must undeniably be given the credit of first having discovered and actually figured microbes and other micro-organisms. His *Arcana Naturæ* was published in 1656 in four volumes. It is a most interesting work, and contains many good illustrations showing microbes of the mouth cavity, infusoria of stagnant water and cellular structure of vegetable tissues. He observed the motion of bacteria and infusoria, made measurements, illustrated capillary circulation in the web of the frog's foot, etc. He was closely followed by Robert Hooke, who published his *Micrographia* in 1658. The discoveries of Leeuwenhoek and Hooke were certainly epoch-making. A new world of minute organisms was made known, the question of spontaneous generation received a new turn, and the way to the discovery of the causes of disease and fer-

mentation was paved. In 1660 Leeuwenhoek discovered yeast cells. From 1660 to 1760 the microscope was actively employed by a few investigators, and additions were slowly made to the list of micro-organisms. Audry (1701) designated microbes worms. Müller of Copenhagen (1786), grouped them under two divisions, *monas* and *vibrio*. In 1743 Henry Baker, of England, published his work, "The Microscope Made Easy," from which it would appear that very little progress had been made since the time of Leeuwenhoek (1656).

As early as 1686 Franceso Redi doubted that maggots were generated *de novo* in putrid meats. He noticed that the presence of the maggots was preceded by swarms of flies which, he concluded, had something to do with the development of the maggots. He found that meat from which the flies

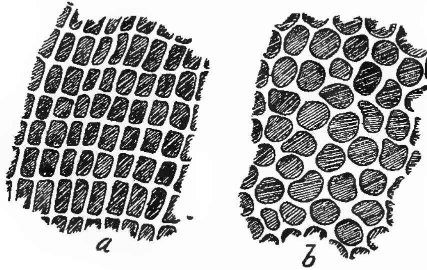

FIG. 2.—From *Arcana Naturæ*. Cell structure of cork. Cell-lumen is shaded and cell-walls are shown light.

were excluded by means of paper or a very fine mesh wire screen, simply decayed without any development of maggots. The paper cover and the fine screen kept the eggs of the flies from being deposited on the meat, and the meat was not infested by maggots, which, as Redi rightly conjectured, developed from the eggs of the fly-like imago. This very simple but reliable experiment did much to create doubt as regards the correctness of the theory of spontaneous generation and other related beliefs.

Spallanzani (1777) was among the first to demonstrate experimentally that boiling and hermetically enclosing fermentable liquids prevented fermentation. Ehrenberg (1828) discovered microscopic organisms in dust and in water, and in 1833 he classified all known bacteria under four orders, bacterium, vibrio, spirillum, and spirocheta. Cagniard-Latour and Schwann (1836) discovered the vegetable nature of yeast, and in 1837 Schwann declared that yeast was the direct cause of fermentative changes resulting in the liberation of alcohol and CO_2, and that the causes of decay were to be found in the atmosphere. Berzelius (1827) declared that the yeast cells were the direct cause of fermentation. F. Schulze (1836) prevented decay in liquids containing certain organic substances by first heat-

ing or boiling them and excluding the air by means of a layer of oil or by closing the container with cotton and supplying it with air which had been sterilized by passing through sulphuric acid. Braconnot (1831) advanced the theory that yeast cells had the power of holding, and condensing within the cell-substance, the oxygen of the air and conducting it to the substances undergoing fermentation, resulting in the splitting up of sugar into alcohol and carbonic acid gas.

The question of spontaneous generation was again discussed with renewed energy. The belief that larger animals could originate *de novo* was quite generally abandoned, but it was very persistently argued that micro-organisms, maggots and a few other very small animals could thus develop. Bastian was perhaps the leader in the arguments in favor of spontaneous generation, opposed by Schwann, Pasteur, and others. Schroeder and von Dusch demonstrated that decay could be prevented by boiling and sup-

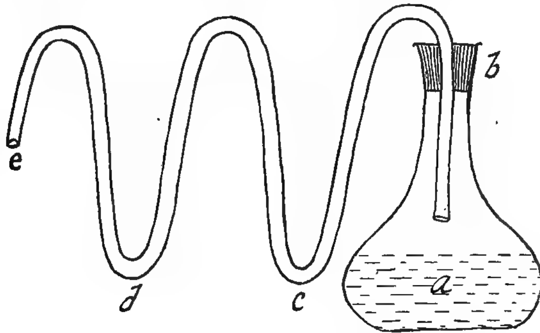

FIG. 3.—Flask, containing an organic substance, *a*, hermetically closed by means of a stopper, *b*. The bent tube is open at *e*, admitting air. Dust and microbes lodge at the bends *d* and *c*.

plying air that had been filtered through cotton. Pasteur (1862) used bent tubes to supply air to the previously sterilized (by heating) substance, as shown in Fig. 3. The microbes in the air passing through the tube are deposited (by gravity) in the lower bends of the tube. Those favoring the theory of spontaneous generation nevertheless continued their arguments. It was pointed out that changes of decay took place in eggs, in internal tissues and organs of the dead as well as in the living, etc., where, it was supposed, microbes could not possibly have access. However, further convincing experiments gradually silenced all opposition. Bastian and a few followers took practically their last stand in 1875, and since that time no scientist of repute has ever argued in favor of spontaneous generation, though the question of the primal origin of living things remains unanswered.

Vaccination as a protection against virulent small-pox was practised early in the eighteenth century in Turkey and other Oriental countries, and was introduced into Europe *via* England through the influence of Lady Mary Wortley Montagu. A. von Humboldt stated that the Mexicans practised vaccination at a very early period. This early vaccination material was obtained from a pustule of a small-pox patient, and not from the cow, as at present. The immunity against subsequent attacks was established, but the disease transmitted through this older method of vaccination was severe and often fatal; besides, the general vaccination was a source of spreading the disease. In 1840 this form of vaccination was prohibited in England by act of Parliament.

In 1768 Jenner's attention was attracted to the value of vaccination, and after a series of patient researches he perfected the method of vaccination by means of the virus obtained from a cow which had been inoculated with small-pox (vaccinia). Jenner established the first public institution, for vaccination in 1799, and in the following year the practice was introduced into France, Germany, and the United States. Vaccination with vaccinia material is now universal in all civilized countries and in countries under civilized control, and as a result small-pox in an epidemic form does not occur in these countries, and the disease has become less and less virulent, so that it is no longer the dreaded scourge that it was two centuries ago. In spite of the beneficient influence of vaccination, there are individuals who oppose this simple, harmless operation with all the energy that ignorance is capable of. Civilized countries are beginning to raise the long-enforced small-pox quarantine as a wholly unnecessary infliction, because vaccination makes the spreading of small-pox impossible. France has raised the quarantine, and so have several other countries, examples which will no doubt soon be followed generally. In conclusion, it is of interest to note that the primary cause of small-pox is unknown even to this day. Ho organism has thus far been isolated from diseased tissues to which small-pox manifestations could be ascribed.

Period III

From Schwann (1837) to Pasteur (1862). (Investigations pertaining to the relationship of micro-organisms to fermentation and disease.)

The discoveries of the cause of fermentation, of decay, and of wound infection, are closely associated. Many centuries ago Varro expressed it as his opinion that certain minute animals, breeding in marshy places, got into the system through mouth and nostrils and caused the disease and decay of the tissues. Theodoric (1260) taught that wound infection came from the air. To prevent such infection he applied wine, which is known

to be somewhat antiseptic. John Colbach (1704) described a "new and secret method of treating wounds by which healing took place without inflammation or suppuration."

From earliest time up to as late as 1860, it was quite generally taught that all normal healing of wounds and cuts must be preceded by pus-formation. A "laudable pus" was recognized, the presence of which was looked upon as a hopeful sign and indicated that repair was proceeding favorably. If the laudable pus which was of a whitish creamy consistency changed to a watery consistency, it was considered an unfavorable sign.

After Schwann and others had demonstrated that fermentation was due to the presence of yeast cells, and it was proven conclusively that decay was caused by bacteria, the relationship of bacteria to disease began to receive consideration. Rayner and Devaine (1850) found bacterial rods in animals suffering from splenic fever. As early as 1840 Henle, who is by some considered the father of modern bacteriology, made some very valuable deductions regarding the relationship of micro-organisms to disease. He recognized a "contagium" (the active cause of the disease associated with micro-organisms), which was supposed to be air-like and yet at the same time fixed. It was supposed to retain its activity for years in the dry state. An unweighable and unmeasurable quantity of this substance may cause an extensive epidemic. Air currents can carry the contagium great distances and cause epidemics in widely separated areas. Bassi (1835) declared that a fungus was the cause of the muscardine disease of silkworms. Pollender (1855) reported that bacteria caused anthrax, verified by Devaine in 1863. Hallier, an enthusiast but not reliable as an investigator, declared that scarlet fever, measles, typhus, and cholera were caused by bacteria. His deductions were, however, not based upon scientific research and proof. Rindfleisch (1866) and Waldeyer (1868) gave considerable attention to wound infection, which, they declared, was due to microbic invasion. In 1869 Pasteur demonstrated the microbic cause of the silkworm disease which interfered very seriously with the silk industry in France. Pasteur and Klebs demonstrated experimentally that bacteria could be grown in artificial culture media, and Robert Koch proved that the pathogenic microbes actually secreted the disease-causing substance. This was demonstrated by transferring an infinitely small quantity of the germ material from a diseased organ to a suitable culture medium and making sub-cultures, until the last culture must contain less than the trillionth part of the original substance. Nevertheless, inoculations from the last culture developed the disease with full energy. This experiment was made to meet the assertions that the cause of the disease did not reside in the bacterium, and that the bacterium, if present

in the disease, was merely incidental to and not causative of the disorder.

A heated controversy continued for some time. Such authorities as Liebig, Nägeli, Bastian, Cohn, Billroth, Hiller, Schroeder, Hoppe-Seyler, Kühne, Tiegel, Sanderson, Nencki, Serval, and Paschutin declared that micro-organisms were not the cause of decay, fermentation, and disease; that these changes were due to chemical substances. However, such men as Pasteur, Koch, Panum, Klebs, and others forged link after link in the chain of evidence connecting the causative relationship of bacteria to disease.

Period IV

From Pasteur (1862) to Behring (1890). (Period of remarkable activity in pathological bacteriology.)

It would be impossible in a brief review to cite all of the important investigations of this period. Pasteur, Koch, and others had already given the subject of bacteriological technic considerable attention. The most suitable culture media, laboratory apparatus, stains, etc., were determined. The compound microscope had now reached a high degree of perfection, and the oil-immersion lenses made the closer study of the morphology of bacteria possible.

As might be expected, the importance of germicides in surgery received first attention. The "laudable pus-" formation ideas were abandoned. It became the surgeon's duty to induce "primary union" or healing by "first intention," that is, healing without any pus formation whatever. This demanded that the surfaces of the incision be brought in close contact, and that all bacterial infection be prevented by the use of antiseptic dressings, antiseptic solutions in the form of irrigations and sprayings, etc. Sir Joseph Lister, of Scotland (1875), brought the use of disinfectants in surgery to a high degree of perfection, and modern antiseptic surgery is often designated "Listerism." The chief antiseptic of Lister and his followers was carbolic acid, which was used for free wound irrigation and general disinfection. He operated in a spray of carbolic acid solution. As late as 1890 there was to be found an occasional lecturer in a college of medicine who held out against the germ theory, and not a small number of the eminent opponents mentioned in the previous period carried their mistaken notions with them to the grave.

The name of Robert Koch will stand throughout the ages as the leader in modern bacteriological science. Early in life he was convinced of the correctness of the germ theory of disease, but his first contributions to bacteriological science awakened a storm of opposition. Billroth, of Vienna, and others persisted in declaring that microbes were not causative of pus-

formation or of the development of disease; but that microbes might be accidentally present, due to the action of a "phlogistic zymoid" which developed in the animal organism.

In 1882 the French government sent a medical commission to India to determine if possible the cause of Asiatic cholera, but the commission returned with a negative report as far as a bacterial cause of the disease was concerned. In 1883 the German government sent a similar commission, headed by Robert Koch, and the report of this commission was that Asiatic cholera was caused by a bacillus, the famous comma bacillus of Koch. The work of Koch in connection with the study of cholera seemed to act as a wonderful stimulus and other eminent investigators made important discoveries within the year or two following. Klebs and Löffler discovered the diphtheria bacillus in 1884. Fraenkel, Weichselbaum and Friedländer discovered the pneumococcus in 1884. Nicolaier and Kitasato discovered the tetanus bacillus in 1884. Löffler and Schütz discovered the glanders bacillus in 1882, and the bacillus of hog erysipelas (Rothlauf) in 1885.

Pasteur in 1881 made his first experiments in reproducing rabies in susceptible animals by inoculation with material obtained from the spinal cord, medulla oblongata, and lobes of the brain of animals dead from rabies. In 1884 he reported his experiments pertaining to the modification of the virulence of rabies by successive inoculations into susceptible animals. His use of this modified rabies virus as a means of preventing a severe and fatal course of the disease in those bitten by animals suffering from hydrophobia, is familiar to all. Thousands of cases have been treated successfully at Pasteur institutes established throughout the larger cities of the civilized world.

The above are only a few of the important investigations of this period. The causative relationship of microbes to certain diseases was undeniably established. The voices of opposition were silenced.

This period is especially notable for the development of antiseptic surgery. As a result, operations were no longer dreaded as in former times. Fatal infections following operations now became rare. Thousands of lives are saved. To remove or destroy the pus germs in open wounds or to prevent the access of germs to wounds, cuts, and abrasions, has become a simple matter, a simple mechanical application of suitable antiseptics.

The progress of purely medical bacteriology was not so marked. Although it was proven that certain diseases were due to bacteria, there were no satisfactory means of destroying them in the system. Internal antiseptics were tried, but without satisfactory results, as a rule. However, preventive medicine based on a bacteriological knowledge gave good results.

Period V

From Behring (1890) to Wright (1907). (Discovery of serum therapy, bacterial vaccines, and development of utilitarian bacteriology.)

The subject of immunity from disease received early attention. Age immunity, race immunity, animal immunity, individual immunity, artificial immunity, natural immunity, acquired immunity, etc., attracted attention and received careful consideration. Metchnikoff (1884) explained immunity on the supposition that certain white corpuscles (leucocytes, phagocytes) of the blood devoured the microbes which entered the system. These white blood corpuscles are the guardians of health. They attack and feed upon any disease germs which may enter the body, either *via* the digestive tract, the respiratory tract, or *via* the circulatory system. If the leucocytes are deficient in number, or if the microbes are excessive in number, disease will develop. This theory had numerous followers, as well as opponents. It is now generally accepted as correct, borne out by observation and by experimental evidence.

The next important discovery was that blood serum had bactericidal properties in a varying degree, and that in addition to this there was something in the blood which had a tendency to neutralize or destroy the action of the poisons or toxins formed by pathogenic microbes. No one particular bacteriologist can be said to have made these discoveries. We can only name a few of the leading investigators who worked along these lines, leading to the discovery of the relationship of immunity and antitoxins—Behring, Brieger, Buchner, Calmette, Chamberland, Ehrlich, Emmerich, Flügge, Fränkel, Hüeppe, Jetter, Kitasato, Klemperer, Löffler, Rankin, Roux, Wassermann, and others. These eminent authorities have demonstrated the possibility of developing or aiding the antitoxic or immunizing power of the blood or of the body cells by introducing sera obtained from the blood of animals in which the antitoxic power is naturally high or is made so as the result of special treatment. Numerous sera (containing antitoxins and toxins) were tried; the one which first proved entirely satisfactory was the diphtheria antitoxin of Behring, which is now in universal use. Others are used more or less successfully, and some are still in the experimental stage.

In 1890 Koch reported on a "lymph" to be used in the treatment of tuberculosis. This lymph was a glycerin extract of the toxin of the bacillus of tuberculosis, and was to be used in the treatment of this dread disease, but the hopes of Koch were not realized, as the remedy proved a failure, and it soon fell into disuse, to be again taken up very recently. In 1907 Wright made known his discovery of opsonins. According to this authority, there exist in the blood certain substances which have the power of acting

on the invading bacteria in such a manner as to render them more liable to be attacked and assimilated by the white blood-corpuscles or leucocytes. There are possibly as many opsonins as there are microbes capable of being digested by the leucocytes. The microbe-devouring power of the leucocytes can be increased by the use of bacterial vaccines, which consist of suspensions of microbes. Very minute quantities are injected into the system, and the resulting reaction increases the power referred to.

Toxins of bacterial origin received the attention of investigators, and antibodies (antitoxins) were extensively discussed as to their possible relationship to health and disease. Enzymes, in their relationship to life processes in plants and in animals, were investigated. It is now supposed that soil toxins of plant origin, as well as those of bacterial origin, influence plant growth. Glandular preparations (ductless glands) have been carefully tested, and several of these are in use.

As the result of Wright's discovery of the use of bacterial vaccines in increasing the opsonic index, the tuberculin (lymph) of Koch was again tried in the treatment of tuberculosis, apparently with some success.

It was found that there were many bacteria other than those which caused disease in animals and plants. Some were found to be decidedly beneficial. Bacterial cultures were employed in butter-making (ripening of cream), in cheese-making, in tanning, in paper-making, siloing, etc. Some bacteria are employed to exterminate certain pest animals. A microbic chintz bug exterminator was tried in 189–597, but it proved a failure. Microbic rat and mice exterminators (azoa, ratite, mouratus, etc.) are now being tested, and they appear to be quite succesful, at least in certain localities and under certain conditions. A microbic rabbit exterminator has been tried in Australia.

In 1879 Dr. Frank, of Berlin, began his investigations of the leguminous root nodule microbes. In 1893 the writer attempted to utilize these microbes in increasing the yield of certain gramineous crops. In 1896 Nobbe and Hiltner, of Germany, introduced a patented microbic fertilizer for leguminous plants. In 1907 a California soil microbe was isolated which appears to be especially active in promoting the growth of sugar beets. This experiment led to the supposition that perhaps every species of plant has its peculiar bacterial flora, symbiotically (mutually beneficent) associated with the root system, mutually essential to active development. The importance of soil bacteria in setting free plant foods has been demonstrated by numerous investigators of Europe and of the United States. Yeast and mould organisms are practically utilized in the manufacture of beer, saké, and other food and drink products.

The above condensed outline of the history of bacteriology may be summed up as follows:

1. Ancient conceptions of disease and of spontaneous generation, dating back to 500 years B.C.

2. Discovery of micro-organisms about 1660 by Leeuwenhoek, followed by the work of Robert Hooke and a few others.

3. Discovery of bacteria in air, dust, and decaying substances, and the causal relationship of microbes to decay, and of the yeast organisms to fermentation.

4. Disproving the theory of spontaneous generation, by Schwann and others, about 1840.

5. Discovery of the bacterial origin of certain diseases—1862 to 1880.

6. Introduction of small-pox vaccination into England by Jenner in 1796.

7. Development of antiseptic surgery or Listerism—1875.

8. Period of great activity in pathological bacteriology—1880 to 1890.

9. Discovery of the causes of immunity to disease, antitoxin of diphtheria and other antitoxins, serum therapy, etc.—1886 to 1894.

10. Introduction of the use of certain bacteria in commerce and agriculture.

11. Discovery of opsonins and the use of bacterial vaccines. Reintroduction of Koch's lymph in the treatment of tuberculosis.

Nothing epochmaking has been discovered in the micro-biological sciences within the last decade. Nothing new has developed during the world war. The cause of cancer remains unknown. The so-called ultramicroscopic organisms remain unidentified. The one bright ray in sanitary science is the typhoid fever control by means of the immunizing bacterins. The primary cause of influenza may soon be discovered.

Useful Works of Reference to Bacteriology and Related Topics

The following references are selected for collateral reading. A few of these works are rare, and can be found only in some of the leading libraries. A reading of these and other related works will serve as a supplement to this text-book. It is not intended to imply that all of the works cited should be procured. Others besides those mentioned may be consulted as opportunity presents itself. Some of them can be obtained from public libraries; others may be ordered through the local book dealer, and a few may be borrowed from professional friends.

HENRY BAKER. The Microscope Made Easy. London. 1743.

Like the work of R. Hoke, this is of great historical interest, and is quite rare. Much of it is a copy of the work of Leeuwenhoek.

B. M. BOLTON (H. U. Williams). A Manual of Bacteriology. P. Blakiston's Son & Co., Philadelphia. 1910.

A most excellent work for medical students, also of value to students of pharmacy.

H. W. CONN. Agricultural Bacteriology.

This is a most excellent little work treating of bacteria in water, in the soil, in

farm products, in the dairying industry, and in plants and domestic animals. It is well written in a simple, clear style.

H. W. CONN. Bacteria, Yeasts and Moulds in the Home. Ginn & Co. 1903.

This is of special value to the pharmacist, as the organisms described may also be found in pharmaceutical preparations.

H. W. CONN. The story of Germ Life. D. Appleton & Co., New York. 1905.

Very useful and interesting general reading on bacteriology.

S. M. COPEMAN. Vaccination, Its Natural History and Pathology. London. 1899.

Of historical interest, besides explaining the subject very fully.

E. M. CROOKSHANK. Text-book of Bacteriology. Philadelphia. 1897.

This is much used as a college text-book on bacteriological technic. Not especially adapted for general reading. Would serve as a laboratory guide.

CHAS. S. DOLLEY. The Technology of Bacteria Investigation. S. E. Casino & Co., Boston. 1885.

Good reference work on bacteriological technic. Somewhat out of date.

DUNHAM, and DAKIN. A Handbook on Antiseptics. The MacMillan Company. 1917.

A small hand book on war time antiseptics, laying special stress upon the dakin solution. The methods for testing antiseptics are entered into very briefly. Water sterilization and disinfection and the disinfection of carriers is explained.

PAUL EHRLICH (Chas. Bolduin). Collected Studies on Immunity. John Wiley and Sons, New York. 1906.

An extensive discussion of the theories pertaining to the action of toxins and antitoxins. Ehrlich's side-chain theory is quite fully treated. The subject is too technical for the average reader, and is of great value only to the specialist in this branch of bacteriology.

DAVID ELLIS. Outlines of Bacteriology. London, New York and Calcutta. 1909.

An excellent English work on general bacteriology especially valuable from the technical and agricultural standpoints.

J. W. EYRE. The Elements of Bacteriological Technic. W. B. Saunders & Co., Philadelphia. 1902.

An excellent laboratory guide for the use of medical, dental, and technical students, and which will serve many purposes of the student of pharmacy.

DANIAL DE FOE. History of the Plague in London. London. 1857.

Of historical interest. Well written.

W. D. FROST. A Laboratory Guide in Elementary Bacteriology. MacMillan Company, New York. 1903.

An excellent laboratory guide. It contains no general information regarding bacteria, and can be used profitably only under the guidance of a laboratory instructor.

W. H. HARROCKS. An Introduction to the Bacteriological Examination of Water. J. A. Churchill, London. 1901.

Of value to anyone interested in the bacterial contamination of water supplies; also useful for general reading.

ROBERT HOOKE. Micrographia. London. 1665.

A very rare and very interesting work treating of the earliest discoveries through the use of the microscope. Some of the illustrations are excellent. Of great historical value and interest. Can be found only in a few of the larger university and public libraries. In English.

L. O. HOWARD. Mosquitoes: How They Live and How They Carry Disease. McClure, Phillips & Co., New York. 1901.

Contains valuable information regarding these pests and how they carry diseases. Of special value in yellow fever and malarial districts.

E. O. JORDAN. A Text-book of General Bacteriology. W. B. Saunders & Co., Philadelphia. 1908.

For medical students. Contains much information of interest to the pharmacist.

F. LAFAR (Salter). Technical Mycology. London. 1903.

Rather technical for general reading. Treats of fermentation and fermentation products, use of yeast organisms and bacteria in the industries, etc. Especially valuable to those interested in beer-making, etc., the dairying industry, etc.

MILLARD LANGFELD. Infectious and Parasitic Diseases, Including Their Cause and Manner of Transmission. P. Blakiston's Son & Co., Philadelphia. 1907.

Contains much valuable information on preventive medicine, sources of infection, disinfectants and disinfection, animal parasites, etc. Excellent collateral reading for the pharmacist.

ANTON VAN LEEUWENHOEK. Arcana Naturæ. Four volumes. London. 1656.

This is by far the most important historical work on the use of the microscope. In Latin. Some very good illustrations. Very rare; found in a few libraries only.

CHARLES E. MARSHALL (and collaborators). Micobiology. P. Blaskiston' Son and Company. 1912.

A very readable and fairly complete guide to the study of micro-organisms, vegetable as well as animal. This book should be in the hands of every student of microbiology.

K. C. MEZ. Mikroskopische Wasser Analyse. Brrlin. 1898.

An excellent German work treating of the bacteriological investigation of drinking water and sewage waters.

GEO. NEWMAN. Bacteria. G. Putnam's Sons, New York. 1899.

Treats of bacteria in industrial processes, bacteria in public health, in nature, in soil, etc. A very valuable work, excellent for general reading.

SAMUAL CATE. PRESCOTT Elements of Water Bacteriology. John Wiley and Sons. 1913.

T. M. PRUDDEN. The Story of Bacteria. G. P. Putnam's Sons, New York. 1889.

Very interesting reading on general bacteriology and on the relationship of bacteria to health and disease.

M. J. ROSENAU. The Bacteriological Impurities of Vaccine Virus. U. S. Public Health and Marine Hospital Service. Hygienic Lab. Bul., No. 12. 1903.

Of special interest to pharmacists. It should be borne in mind, however, that since the publication of this report the methods of vaccine manufacture have been modified somewhat, and the figures and results given may no longer apply.

M. J. ROSENAU. An Investigation of a Pathogenic Microbe of Rats and Mice (*B. typhimurium* Danysz.) Washington, D. C. 1903.

This treatise is also of special interest to pharmacists, as the microbe referred to is the active ingredient of several patented rat and mouse exterminators sold under proprietary names as Azoa (Parke, Davis & Co.), Rattite, Mouratus (Pasteur Co.), etc. These exterminators are still under investigation, testing, etc., and the findings in the above report should not be considered final or conclusive.

M. J. ROSENAU. Preventive Medicine and Hygiene. D. Appleton and Company. 1917.

W. G. SAVAGE. The Bacteriological Examination of Water Supplies. Philadelphia. 1906.

A valuable treatise. Contains a citation of the more important literature on the subject. An excellent laboratory guide for the specialist.

DR. C. STICH. Bacteriologie und Sterilization im Apothekerbetrieb. Berlin. 1904.

In German only. Contains many valuable suggestions but too incomplete and too much lacking in detail for the student.

E. R. STITT. Practical Bacteriology, Blood Work and Animal Parasitology. P. Blakiston's Son & Co., Philadelphia. 1917.

Primarily for medical students, especially those interested in the parasitology of the tropics. Complete on methods. Full details regarding blood work and use of hemacytometer.

FRED W. TANNER. Bacteriology and Mycology of Foods. John Wiley and Sons. 1919.

A fairly complete summary of the bacteriological methods employed in food laboratories, including the examination of disinfectants. The direct methods of microanalysis are explained.

JOHN TYNDALL. Floating Matter in the Air. London. 1881.

A very interesting popular work on the micro-organisms of the air and their relationship to fermentation and putrefaction. For general information.

NOAH WEBSTER. A Brief History of Epidemics and Pestilential Diseases. Two volumes. Hartford. 1799.

Of great historical interest, though entirely antiquated and of no scientific value.

GEORGE CHANDLER WHIPPLE. The Microscopy of Drinking Water. John Wiley and Sons. 1914.

H. W. WILEY. Food and Their Adulteration. P. Blakiston's Son and Company. 1907.

The following are a few select references to naval and army sanitation and hygiene which will serve as excellent collateral reading for the student of pharmacy (course in sanitation).

JOSEPH H. FORD. Elements of Field Hygiene and Sanitation. P. Blakiston's Son and Company. 1917.

VALERY HAVARD. Manual of Military Hygiene. (Third revised edition.) William Wood and Company. 1917.

JAMES CHAMBERS PRYOR. Naval Hygiene. P. Blakiston's Son and Company. 1918.

EDWARD B. VEDDER. Sanitation for Medical Officers. (War Manual No. 1.) Lea and Febiger. 1917.

CHAPTER III

THE ORIGIN OF BACTERIA AND OF OTHER MICRO-ORGANISMS

In consideration of the recent revival of the interest in the question of the origin of living matter, a work on bacteriology would be incomplete without a brief mention of the subject, for the origin of bacteria is one with the origin of life. Despite the interest mentioned we must from the outset admit that the answer to the riddle of life has not yet been solved. Many theories have been advanced, some apparently ridiculous and wholly without scientific warrant; others interesting but not plausible; some apparently scientifically sound but lacking in one or more essentials and hence inconclusive. The following are a few of the more important hyoptheses and theories.

1. Spontaneous Generation.—This has already been referred to in Chapter II. According to this idea, not only microbes, but highly complex organisms, as insects, fish, mice, etc., might, under suitable surroundings arise *dė novo*, without a preëxisting parent. Paracelsus gave specific instructions as to the creation of the "homunculus." These old time absurdities need not be entered into further. Until quite recently, the belief in the spontaneous origin of *simple* forms of life (bacteria, protozoa, lower algæ and fungi) was common among the leading scientists. However, the rapidly accumulating evidence of scientific investigations soon proved the entire erroneousness of this belief also. As soon as the scientific world became convinced, in the face of incontrovertible evidence, that life does not originate spontaneously, and that it could not be developed artificially in the laboratory, a tendency began to manifest itself to dispose of the entire matter as follows. Although life does not originate spontaneously *at the present time*, it must have originated spontaneously at some time in the distant past. Conjectures were advanced as to what the remarkable life giving conditions of this remote past might have been, but none of the suggestions proved satisfying. There was nothing tangible or significant proposed or stated, and at the present time this idea is no longer discussed among scientists. It receives a fleeting mention in the class room perhaps, but it is no longer considered worthy of a place in scientific literature. The entire concept is scientifically dry and meatless and shall be disposed of with this brief mention.

2. The Vitalistic Hypothesis.—This has also been designated the "vital spark" theory or hypothesis. According to this concept it was assumed that some mystical energy or force, or unknown and unknowable power, gave rise to life. It was supposed that the difference between a bit of dead organic substance and a bit of living organic substance was that the living substance had been activated by some mysterious stimulus, the vital spark. It was even suggested that this vital spark might be electrical in nature, and some misguided scientists, in an attempt to harmonize biological science with this essentially eclesiastical hypothesis of life, suggested that life was due to the action of electrical discharges upon organic matter. No scientist today subscribes to the vitalistic concept of life.

3. The Panspermistic Theory.—This is also known as the cosmozoic theory and is one of the very latest attempts to explain the origin of life upon our particular planet, namely the earth. Despite the boldness and daring of the theory, it is well founded in the physical sciences and it is well worth while to give it a more detailed consideration.

According to the panspermia or cosmozoa theory there is an interplanetary distribution of germs. Only within comparatively recent times has the idea become intelligently or rationally formulated. Flammarion suggested that most of the planets were inhabited. De Montlivault (1821) declared that the first terrestrial life came from the moon. Richter (1865) impressed by the writings of Flammarion, conceived the idea that meteors might be the interplanetary carriers of seeds and perhaps of plants and smaller animals. Ferdinand Cohn (1872) strongly supported the idea of Richter which idea is beginning to receive serious attention on the part of scientists generally.

When a larger cosmic body collides with a planet, the impact causes great disturbance and broken masses and particles are driven into space in all directions. The heat generated by the impact would no doubt kill all organisms in the immediate vicinity of the point of impact, even should they survive the mechanical shock. However, there can be no doubt that seeds and many of the lower forms of life could survive and these might be transported to some neighboring planet. It is, however, not conceivable how any of the more highly organized plants and animals could possibly survive such a journey. Suppose a large mass, bearing upon it plants and insects, should become detached from a planet and finally approach the earth entering the layer of atmosphere, the heat generated by the friction would certainly destroy all life present.

The objections above set forth as to the meteoric origin of higher pan-germs, such as higher animals, plants and seeds, does not apply to bacteria, and other similar small organisms. Should a meteor or other planetary

fragment having upon its exterior deposits of organic matter with bacteria endospores, and filterable viruses, enter the atmospheric zone of a planet like that of the earth, the resistance offered by the air would at once remove the now absolutely dry pulverulent germ-bearing portions of the meteor, leaving them behind as a very fine invisible dust which would be very much retarded in its motion toward the earth's surface; somewhat larger particles would follow faster and become more or less incandescent during their flight through the layer of atmosphere, constituting the fiery trail of the meteor. It is highly probable that the very finest particles, as the germs measuring less than 0.05μ. in diameter, would never reach the earth, being prevented from doing so by the earth's electronic (electro magnetic) repulsion. Some spores and micro-organisms would, however, finally reach the earth due to the action of convection currents, electrical action and the direct and reflected repulsion energy (radiation pressure) of light.

Arrhenius in his book entitled "Worlds in the Making" very lucidly sets forth the pros and cons of the idea of panspermia and his general conclusion is that the interplanetary dissemination of bacterial spores is within the possible.

Schwarzschild has determined mathematically that spherical particles measuring 0.1μ in diameter are most markedly affected by the radiation pressure of sunlight. As is well known to bacteriologists, the endospores of bacteria do not measure more than from 0.2μ to 0.3μ in diameter, even less in the dry state. The compound microscope reveals living plasmic particles which are less than 0.025 in diameter. The ultramicroscope enables us to visualize particles of gold and of other minerals in colloidal suspension, which particles are said to approximate molecular dimensions.

The filterable viruses of yellow fever, rabies, smallpox, the mosaic disease of tobacco, foot and mouth disease of cattle, and others, are even smaller than the endospores. For example the cytoryctes (*Cytorhyctes vaccineæ*) which is presumed to be the primary cause of smallpox, measures from 0.5μ to less than 0.2μ in diameter when suspended in liquids. In the dry state the smaller specimens would measure less than 0.065μ in diameter.

Very naturally the question arises how long a period of time is required for these pangerms to be carried from one planet to another. It has been determined mathematically that a germ from the earth would reach Mars in twenty days, Jupiter in eighty days, Neptune in fourteen months and our nearest solar system (Alpha in Centauri) in 9000 years. Can spores and low organisms survive these periods and the conditions known to exist in interstellar space? The answer is in the main in the

affirmative. Spores of higher fungi and of lichens have survived herbarium conditions for many years, according to some authorities, for nearly 100 years. Endospores and some of the filterable viruses are known to survive for several years and longer, in cloth, in dry dirt and in paper. The microscopical examination of the cloth of Egyptian mummies showed the presence of numerous spores of some mold, also endospores of bacteria, and bacteria, apparently in good morphological condition. Some of these apparently developed when introduced into gelatine media, though it was also evident that most of them had lost the power to germinate or to septate. In interstellar space the conditions are almost ideal for the preservation of the life of micro-organisms in the resting stage. There is absence of oxygen of moisture and the temperature is very low (perhaps less than −220° F.). It is true they are exposed to light, more especially the chemically active ultraviolet rays, but even these are checked in their destructive action on life because of the absence of warmth, moisture and of atmospheric oxygen. There is, therefore, no plausible reason why the minute particles detached from the meteoric mass should not be carried through cosmic space by the radiation pressure of sunlight. This radiation pressure would, however, act only in one direction within our solar system, namely, in the direction away from the sun. This force could therefore transport germs to the earth from Mercury, Venus, Moon and such meteoric and other heavenly bodies which move between the earth and the sun. None could reach us from Mars, Neptune or Jupiter, unless perhaps through the radiation pressure of light *reflected* from these planets but since only an infinitely small amount of reflected light reaches us from these sources the likelihood of this carrying life to the earth is correspondingly slight. However, the radiation pressure from a neighboring sun might carry germs to the planets of our system.

Very naturally the question arises how may spores and other similarly small organisms get away from the so-called force of gravity which holds all substances of a planet together and attracts matter in space to its surface. Air currents could readily carry these particles to the upper air zone but could not project them beyond. Arrhenius assumes that the electrical currents of the earth, more especially the negative currents of the north pole, are more than sufficiently strong to carry very small particles against the force of gravity and it is suggested that these electrical currents are constantly scattering innumerable spores and germs into cosmic space. These can never reach the sun because in time, in their flight toward that body, the radiation pressure of light will arrest them and even turn them back upon their own path.

The following is quoted from the book by Arrhenius:

"In the vicinity of solar bodies seeds would be checked in their cosmic

flight by the radiation pressure of sunlight and tend to accumulate in great numbers. The planets rotating about the suns would thus be likely to receive such seeds, more especially those planets which were somewhat more remote from the sun. In these positions the seeds would also have lost much of their original speed and would in all probability not become excessively heated, probably less than 100° C., in their motion through the atmosphere.

"In the vicinity of the suns, the intercosmic seeds now entering upon their return journey to the planets would meet with particles whose weight is somewhat less than the repelling force of the radiation pressure and which for that reason have begun the return journey to the sun. Just like the seeds, these small particles tend to accumulate in the vicinity of the suns.

"These very small seeds and the yet smaller particles clinging to them such as spores and bacteria, would be more likely to reach the planets nearer the sun.

"In this manner life may have been carried from planet to planet, from solar system to solar system, throughout the ages. But, as in the case of the billions of pollen grains which escape from a single oak, and which are distributed by the air currents, perhaps only one will give rise to a new tree; so likewise of the billions and trillions of germs which wander about in cosmic space, only one may ever reach a planet where it may develop and give rise to many new forms of life."

According to the above idea of panspermia all of the organisms in the entire universe are related and consist of cells which are built up through chemical combinations of carbon, hydrogen, oxygen and nitrogen. The supposition that there may be worlds in which, for example, living organisms are formed from chemical combinations in which carbon might be displaced by silicon or titanium, is highly improbable. Life upon other inhabited planets is in all probability similar to that upon the earth.

It is generally known that several planets of our solar system are closely similar to that of the earth, as Venus and Mars. Mercury, like the moon, turns one side to the sun at all times and hence the sides have a constant temperature, one side hot and the other cold, one side in constant darkness and the other constantly illumined.

Even admitting that the idea of panspermia is well founded and that very minute forms of life can be transmitted from one planet to another, the question of the origin of life still remains unanswered. We have merely pushed the problem into a dark corner. By relegating the matter to one or many distant planets we have not thereby escaped the responsibility of the final scientific proof and unimpeachable explanation of the origin of life.

Bacteriologists have from time to time commented upon the fact

that bacteria are morphologically as well as physiologically essentially different from other living things upon the earth, more especially those species which form endospores. As already suggested, bacteria apparently have no phylogenetic relationship to any of higher forms of planet or animal life, nor yet to any of the recognized protozoa or protophyta. May it not be possible, for example, that the xerophytic and anaërobic pathogens, such as the *Bacillus tetani*, *B. botulinus*, *and B. œdematis maligni* have reached us from the moon, where the meteorological conditions are suitable for the existence of this type of organism. May it not even be possible that these pathogens of comparatively high specific gravity and endowed with extreme temperature resistance were the chief actors in the final lunar struggle for the survival of higher life. As the moon was bombarded by planetary bodies most, if not all, of the higher organisms were no doubt killed by the shocks of impact and the heat generated. Such higher forms as survived were unable to continue when the life sustaining atmosphere and moisture become more and more reduced, thus favoring more and more those lowly organized living structures which could thrive in this rarefied atmosphere and dry condition.

As suggested the comparatively large and highly refractive toxigenic spore forming bacilli may have reached us from the moon. The smaller plasmodia and very small non-spore-forming bacilli may have come to us from Mars and Venus, and perhaps also from Mercury. The temperature on Venus is higher than it is on the earth whereas the mean temperature on Mars is lower, even though the polar snows of that planet occasionally disappear entirely during some seasons, which never happens on the earth. These differences in planetary temperatures suggest that the germs of yellow fever, of amebic dysentery, of the African sleeping sickness, of leprosy and perhaps also of malaria, may have come from Venus; whereas the germs of rabies, of la grippe, of whooping cough, of scurvy, of the plague and perhaps also of smallpox, may have come from Mars.

4. The Theory of Universal Evolution.—The belief in the genetic or evolutional relationship of all things in the entire Universe, is of extreme antiquity. According to an ancient Hindoo myth, at the beginning of things there existed a mundane or cosmic egg or germ from which all animate as well as inanimate things successively emerged. The scholars of Athens, of Rome and of Alexandria held similar ideas. Later, the idea became more definitely formulated by such master minds as Huxley, Spencer and others. The same idea is further developed and matured by the teachings of the physicists and now we find ourselves compelled to go back in our study of evolution and bring with us all matter, organic and inorganic, living and dead, atom, molecule and compound.

Biological science has convinced us that death and decay are essential

to the renewal of life and to the advance or evolution of living things and now the physicist presents a similar idea applied to the atom and to the molecule. The atom is no longer considered to be the ultimate unit of matter, unchangeable, indivisible and undestructible. The atom is rather a miniature universe consisting of a definitely known electric charge composed of a positive nucleus about which revolve the negative electrons in definite orbits. The newer teaching of physics is to the effect that the only difference between hydrogen and oxygen, for example, is that the oxygen atom has 16 times more negative electrons grouped about the positive nucleus. The radium atom differs only in the greater number of electrons. The molecule, no matter how simple or how complex, is nothing more than a grouping of electrons. If this be anywhere near the actual facts, and much of the experimental evidence is confirmatory, we at once obtain a new significance of the periodic grouping of the elements (as given by Mendeleeff and Lothar Meyer) and the relationship of molecules. Life is thus resolved into elcetronic groupings and electronic action. Life is thus nothing more nor less than the manifestation of properties peculiar to certain definite electronic groupings as represented by certain molecules. Living things appeared at the precise moment when the electronic activity represented by the appropriate atomic grouping in the molecules was such that it could be designated by the term life. When man shall have acquired the ability to imitate this grouping he will then have developed a living thing. Life is thus nothing more nor less than a problem in physical science.

The physicist tells us that the disintegrating radium atom breaks up into free electrons manifest as beta rays, and perhaps into free or comparatively free positive nuclei and that some of these disrupted atomic constituents are again rearranged or recombined into new atoms of helium represented by the alpha rays. What starts the radium atom on its unalterable explosive disintegrating course? It is assumed that under certain conditions, perhaps, occasioned by the change in the orbital position and rotation of the negative electrons about the positive nucleus, due to the mutual repulsion of the electrons, there is a sudden explosion of the atom and the electrons are shot off into space. These free electrons moving at a speed approximating that of light and obeying the laws of "falling" bodies, may again reform in orbits about a free or comparatively free positive nucleus, perhaps a nucleus from which they have just been shot forth, but the new atomic orbital grouping of the electrons is now entirely different, resulting in the formation of a new atom. Only the dying (disintegrating) atoms make the formation of new atoms possible, for as far as we know at the present time, no new matter is added to the universe nor is there any destroyed (annihilated) or taken therefrom.

It is no pathological stretch of the imagination to suppose that the macrocosm (as represented by a stellar system) is mirrored in the microcosm (as represented by the atom and the molecule). There can be little doubt that old stellar systems disintegrate and new ones form. Nebulæ, comets and stellar dust are not newly created substances as is generally taught, but rather newly reformed or rearranged substances, resulting from explosively disintegrated stellar systems, in similitude to disintegrating atoms (radium, thorium, polonium, lead, etc.).

Bacteriology is the newest of the sciences, dating back to about 1875. And since then this science has made a series of explosive advances. It is generally taught that bacteria are plants and it has been customary to class them with the fungi, and some scientists have even suggested that they are somatically reduced or degenerate algæ. There is, however, no good reason for assuming that they are degenerate algæ nor are we justified in designating them as plants. We are justified in stating that the various groups of bacteria are phylogenetically related and that they, in all probability, had a polyphyletic origin in many different areas of the earth's surface, or mayhap upon other planets as has been explained above. We are at the present time not in a position to state definitely whether or not any of the higher fungi are phylogenetically derived from any of the groups of bacteria. The Leptothrix and Streptothrix groups are perhaps of bacterial origin. We are amply justified in saying that bacteria were among the first, if not the first, living things which made their appearance upon an originally lifeless earth. We know that the large group of nitrifying bacteria will grow in a medium composed of water to which is added 0.1 per cent. each of ammonium sulphate, potassium phosphate and magnesium carbonate, a medium wholly free from sugar and nitrogenous compounds containing only such ingredients as existed on our plant prior to the development of living things. Out of these substances the nitrifiers formed (in the presence of air) ammonia, sugar, fatty acids and proteins, which substances in turn serve as media for the development of other bacteria and of higher organisms. This statement will serve to indicate the potentialities of this group of bacteria in the way of assimilating dead inorganic matter and converting it into or utilizing it as a life-sustaining pabulum. We are, however, still confronted with the problem of the source of the life, the life principle or whatever it may be styled, which made it possible for bits of organic matter to utilize these dead inorganic materials for the purpose of maintaining life and as the building material of a living substance, for life activities imply the existence of a living organic substance.

It may be recalled that some years ago Huxley thought he had discovered the primal living substance in the slime taken from the ocean's bed

and which substance he named *Bathybius Hæckelii.* This supposed deep sea life was found to consist largely of colloidal deposits. The theory of spontaneous generation is fully disproven though considerable effort has been expended in an attempt to form a living substance in the laboratory. Recently Dr. Burke claimed to have succeeded in instilling life into gelatine solutions by means of radium, but this proved to be an error.

Bütchli's "artificial protoplasm" was once of considerable laboratory interest, but it was not intended that this substance (an emulsion of oil) should be considered as being endowed with life. The artificial continuation of tissue growth according to the laboratory methods of Dr. Carel and others, of course, has no bearing on the creation of living cells by laboratory methods.

According to the theory of universal evolution, the origin of bacteria was simply an incident in the series of events (electronic) which resulted in the development of the particular substances which occur in that particular group of organisms, and the theory could be appended to the preceding one or to the one which follows, since there is nothing in the concept of universal evolution which conflicts with the ideas therein presented.

5. The Colloid Theory.—The word colloid is derived from the latin collo (glue) and was applied by Thomas Graham, the father of colloidal chemistry, to non-crystalizable substances of low diffusibility and generally of great viscosity, and other characteristic properties. Graham's experiments on dialysis and the general properties of colloids, were read before the Royal Society of London in 1861, from which it becomes evident that colloidal chemistry is the youngest of the modern sciences. The still more recent investigations in the field of colloids has been fruitful in suggestions and theorizations regarding the nature of living organic substances. In order to comprehend the basic principles of the colloid theory of living matter, it is necessary to set forth the fundamentals of colloids. Gelatine may be taken as a type of colloidal matter. Colloids never crystallize, they will not pass through animal membranes. A colloid dissolved in water is designated a "sol," and as water is gradually abstracted the sol changes into a "gel." A sol is a colloid system consisting of two phases, the continuous phase called the dispersing medium or dissolving medium, and the disperse phase or the colloidal particles in solution. Two classes of colloids are of special importance to biology, namely the emulsoids and the suspensoids. The emulsoids are viscous, gelatinize readily and are not easily coagulated by electrolytes. Among the important emulsoids are gelatine, agar, albumin, histons, and other proteins. The suspensoids do not gelatinize, they are not viscous, but are readily precipitated by electrolytes. Among the suspensoids are the sols of metals generally,

and of some of the dyes. It may also be stated that the colloid state is not restricted to any one group or even many groups of matter, rather it is universal. Any and all substances may be converted into the colloid state, including the chemical salts. In general it is however true that substances with small molecules prefer the crystalloidal state or form, whereas the substances consisting of large molecules prefer the colloidal state. Matter in the colloidal state consists of minute molecular aggregrates (not chemical combinations) varying in size from macroscopic, that is large enough to be visualized by the naked eye (at least when the light rays are properly adjusted) down to aggregates which are so small that they may not even be visualized by means of the ultra-miscoscope. Another property of colloidal matter is the manifestation of the very striking Brownian motion. In a general way colloidal particles are decidedly opposed to chemical combinations. In fact the study of colloids lies almost wholly in the realm of physics, rather than chemistry.

The recent investigations in bio-chemistry and colloidal chemistry have shown that life processes take place in colloid systems. Life, death and decay, and renewed life, form one continuous kalaidoscopic colloidal transformation series, in which the organic molecules, water and enzymes play the leading roles. The secrets of living matter are bound up in colloids. Not the coarser colloids recognizable microscopically or even ultra-microscopically, but in those protein and histon colloids which abound near the border line of the true (molecular) solutions. The living basic substance in which all of the life processes take place, that is the plasm (stroma), is a protein emulsoid in water. It is itself a dialyzing substance which will take up and will allow to enter and pass the smallest colloidal particles only. It is a labilely stable substance. That is, it is chemically stable under certain conditions of light, temperature and environment. The plasmic granula which may be seen under the high power of the compound microscope, or visualized under the ultra-microscope, and all of the known formed cell constituents, inclusive of nuclear matter, are precipitation and coagulation products of plasmic activity. The cell-walls, the starch granules, crystals of calcium oxalate, etc., of plant cells, represent refuse material, rejected by the living plasm. Gradually, the plasm is unable to find sufficient dumping space for the refuse, the cell-wall growing thicker and thicker, the cell lumen smaller and smaller, and finally the plasm dies upon the heap of refuse of its own building. It is true, that the starch and other plasmic rejecta may again be utilized in subsequent growth processes, and the cell-wall serves as a protection against the loss of moisture and also assists in colloidal filtration so essential to the life of the plasm. The nucleus of the cell is nothing more nor less than a series of colloidal coagulation changes, of colloid coagula tem-

porarily thrown off by the plasmic base or stroma, a temporary resting condition of a portion of the plasmic stroma, to take on renewed activity as soon as the conditions shall have become favorable. The nucleus and all that pertains thereto may be likened to the cell plastids and other living inclusions of the cell, in so far as they are plasmic rejecta or storage matter, temporarily set aside to be activated by the plasmic base when the conditions are, for example, suitable for cell division to take place. That plasm is king, and not the nucleus, is proven by the fact that enucleated cells may be induced to grow and septate, whereas nuclei separated from the cell plasm will not divide and develop into new cells. It is highly probable that the substances which a cell deposits in the nucleus, leaves the vitality of the plasm very much weakened and unless the plasm makes use of this stored nuclear substance, it will, as a general rule, not be able to survive for long periods, and under usual or natural conditions finds itself incapacitated for cell division, or even for cell growth. The sphærocytes (nucleated living cell inclusions common in fruits) are apparently extra deposits of the cell plasm which have the power to continue the life of the cell for a time, thus enabling fruits to remain alive for many months after they have ripened and separated from the mother plant. If the living cell could dispose of the coagulation and precipitation products as rapidly as they are formed then the cell would continue to live indefinitely. The Ameba does this to all intents and purposes, because the dispersing medium in which it lives (namely water) disperses the rejected products at once. Death among the multicellular organisms is inevitable, unless some condition develops which will enable the organism to get rid of the coagulation and precipitation products. In this lies the solution to the problem of eternal life and eternal youth. We may indeed reduce the precipitation changes to a minimum, by reducing the plasmic activities to a minimum. Such conditions exist in spores, in seeds, and in the resting stages of various organisms.

From the foregoing it must be apparent that life, as we know it, could not have come into existence until certain highly complex molecules had developed, which complex molecules must be in the proper colloidal comminution and dispersed in water and associated with other colloidal particles, including minerals, as sulphur and phosphorus. Water takes no active part in the life processes, although it is the absolutely essential dispersing medium for the plasmic base or stroma. Unless plasm is actually immersed in water from which it can draw unreservedly, it must constantly have water brought to it, otherwise it cannot continue in the living state. We may make certain statements as to the properties of the living stroma in which all life activities take place and from which all plasmic formations are derived.

1. It is a true colloid phase in which water is the dispersing medium, and in which the dispersoids are complex protein molecules in aggregates so small as to be wholly invisible by any of the optical aids to vision.

2. It is very slightly permeable to water and is even less permeable to the solutes which may be in the water.

3. It is labilely stabile within certain conditions, as water supply, temperature, and food supply. If the conditions become excessive the precipitation and coagulation changes are such as to destroy the disperse phase peculiar to a living substance.

4. In brief, the plasmic stroma manifests properties of a colloidal filter, in which the pores are so small as to permit entrance and passage to the very smallest colloidal particles only.

It may be assumed that life came into existence upon the earth at the precise moment when the particular proteid molecules appeared in water, which in the presence of certain other colloidal suspensions, formed perhaps eons earlier, together with a certain temperature and other environmental conditions, gave rise to the coagulation change in the disperse phase to which we have come to ascribe a living state. The much discussed "spark of life" is thus nothing more nor less than the coming into being of the particular colloid substance (protein) which was essential to bring about the particular physical changes to which we ascribe life. Life is thus not a chemical change although the changes in matter which are essential to give rise to those substances which may be used as food by the living substance, or rather which are essential for the purpose of maintaining those constantly varying physical (colloidal) conditions which we designate as manifestations of life, are chemical. Enzymes in particular, play a very important part in those chemical and physical changes so essential to higher life.

The artificial production of life in the laboratory is not so much a matter of providing a suitable environment to which organic matter must be exposed, as it is a matter of finding a suitable colloidal filter. Should we prepare a colloidal filter having the filtering qualities of the plasmic stroma, we might then be prepared to begin upon a series of laboratory experiments with a view to producing a living colloid substance. Other factors altogether too numerous to mention come into play also. The above is a mere outline of the present concept of the colloidal nature and origin of living substances.

Many other theories have been presented but none of them have anything new or essentially different from the ideas above outlined. Among the investigators who have given the subject considerable thought may be mentioned Osborne who dwells at considerable length on the probable conditions on the earth's surface during the geologic ages. He is of the

opinion that the prototrophic group of bacteria were the first living things upon the earth's surface. Troland is of the opinion that enzymes played the leading part in the creation of life. The source of the first enzyme (protenzyme) is however not known. If all enzymes now known are of living origin then we must assume that the protenzyme came into existence before the protoplasm (first plasm). Thus the Troland suggestion is really of no help in explaining the orgin of life. There is however no reason why we should not assume that protenzyme and protoplasm developed at one and the same time. Allen held that the conditions which maintain life are also conducive to the creation of life, and should by chance all life on the earth's surface at the present time be destroyed, a new cycle of life would begin forthwith. Pflüger suggests that there is a fundamental difference between a living protein and a dead protein and that the cyanogen radical is the distinctive part of the molecular complex of living proteins. Moore was one of the first to give serious consideration to the importance of colloidal changes in the development of living matter. While none of the investigators have yet been able to solve the problem of life, yet the numerous propositions which have been made from time to time, and the yet larger number of theories which will be offered in the near future, are indications that the trend of science is in the same direction and it is but reasonable to expect that some one will in the not very distant future find the solution.

CHAPTER IV

GENERAL MORPHOLOGY AND PHYSIOLOGY

1. INTRODUCTION

Microbiology in the true and comprehensive sense, is the science which treats of microscopic organisms, micro-organisms or microbes, vegetable as well as animal. It, therefore, comprises a study of bacteria, of yeasts, of the lower molds, of the lower algae, of protozoa, of flagellata, of ciliata, and of other low forms of plant and animal life. Applied in a more limited sense, the term has more recently come into use in place of the term *bacteriology*. The latter word is derived from *bacterium* (from the Greek, bactros), meaning a small rod because the earlier students assumed that all microbes were rod shaped, which is not the case, and the Greek word *logos* meaning discourse. Microbiology (from *micros*, small, bios, life; and *logos* discourse) even when applied in the narrower sense, is therefore etymologically more nearly correct than is the word bacteriology. The only excuse for using the words bacteria and bacteriology is established usage.

By *microbes* or *microscopic organisms* are meant those living units which are so small as to render the individual unrecognizable to the naked or unaided eye. As generally understood microbes are so small as to require the use of a good compound microscope to bring the individual or individuals to view. A good simple lens or simple microscope, having a magnifying power ranging from four to ten diameters, will reveal the position in space of some of the larger forms of microbes but their detailed structure is not recognizable under such limited magnification. Microbes in mass are generally visible to the naked eye, thus we recognize the blue green mold on bread, bacterial membrane on potato and vinegar, algæ in water and in soil, etc.

Some microbes, presumably belonging to the group of so-called bacteria, and perhaps also to the group protozoa, are supposed to be too small to be seen, even under the highest power of the compound microscope and are spoken of as "ultra-microscopic." This is, however, mere theoretical assumption as no one has thus far succeeded in demonstrating the existence of ultra-microscopic organisms.

Although the terms microbe and microbiology are here used in the true broad and comprehensive sense, the subject-matter of the present volume is very largely devoted to a brief summarizing discussion of those microbes

or micro-organisms, concerned in human economy, as those having to do with health and disease, directly and indirectly, thus including scientific (theoretical) and applied bacteriology in the older sense with its more modern subdivisions as medical bacteriology, food bacteriology, pharmaceutical bacteriology, dental bacteriology, soil bacteriology, veterinary bacteriology, dairying bacteriology, poultry bacteriology, agricultural bacteriology, etc., and also medical and sanitary parasitology, much of pathology, general zymology immunology and scientific (theoretical) as well as practical or applied, serology; and still more remotely the subject also includes the fundamentals of public health and hygiene, sanitation and preventive medicine in general.

There are certain important factors not directly pertaining to the science of microbiology as above outlined which nevertheless have more or less direct bearing upon the subject and which must be touched upon for the sake of completeness and of a fuller understanding, such as carriers of disease, secondary causes of disease, disinfectants and disinfection, food preservatives, etc.

Bacterium (plural, bacteria) is a misleading term, though firmly established in general usage. Furthermore, the term is used in a generic sense, and again applied to the group of organisms as a whole. This causes confusion. Therefore, the generic term *Bacterium* is now abandoned and the term *Bacillus* is used to include all of the micro-organisms which are rod-shaped although generic sub-divisions are being made of this now very large group.

Whereas the general morphology of microbes is apparently quite simple, the physiology and chemistry is extremely complex, and as yet not fully understood. The morphological simplicity is no doubt only apparent, and not real. Perhaps, with the greater perfection of the compound microscope, we may discover marked structural differences which thus far have escaped our notice.

1. Classification of Microbes

Microbes are the smallest of the known living organisms. It is wholly impossible to see the single individual, even the largest, with the naked eye. The rod-shaped microbes (bacilli) range from 0.5μ to 10μ in length. Some are so minute as to pass through the pores of the finest clay filters (the cause of foot and mouth disease). To study them, a good compound microscope is absolutely necessary, though, as stated in the historical review (Period II), Leeuwenhoek and others observed the larger forms under the simple microscope.

The systematic position of microbes has from time to time received much attention. The great majority of biologists now unhesitatingly class

them as plants, belonging to the group fungi. It cannot be denied, however, that their origin (phylogeny) is still shrouded in mystery. Some suggest that they are derived from degenerate algal forms, in common with most of the fungi, while others declare that they in all probability originated as microbes. A few of the philosophical biologists, as Ernst Haeckel, place them in a separate group, the Monera, which is supposed to form the connecting link between plants and animals.

Without entering into lengthy discussion, we shall, in conformity with the opinion of the majority, class them as plants, belonging to the lowest of the group fungi (the fungi includes rust, smuts, cup fungi, moulds, spot fungi, toad-stools, etc.), namely, the Schizomycetes or fission fungi, so-called because they multiply by fission or division. They are related to the yeasts, though somewhat lower in the scale of evolution. They are single-cellcd, each cell forming a complete living unit, though the several units may be variously arranged into chains or clusters, or groups known as zoogloea.

The scientific grouping of microbes is as yet very unsatisfactory because so little is known of their ultimate morphology, their physiology and chemistry. Some have attempted to classify them as to form, others as to occurrence, as to action, etc. Thus, we have:

a. Micrococci or Coccaceæ.—Globular or non-elongated microbes.

b. Bacilli or Bacteriaceæ.—Cells more or less elongated. Rod-shaped microbes.

c. Spirillæ or Spirillaceæ.—Cells elongated and more or less spirally twisted. Or, we may have:

a. Bacteria of earth.

b. Bacteria of air.

c. Bacteria of water.

Or, again:

a. Chromogenic.

b. Zymogenic.

c. Pathogenic, etc.

These artificial groupings could be extended indefinitely, but such systems of classification would be as unsatisfactory as they are unscientific. The best system makes use of all of the known facts of bacteriology. Several such systems have been proposed from time to time, but the new discoveries along bacteriological lines makes it necessary to change them in the course of two or three years. Migula, Fischer, Eisenberg and others have proposed general systems, and a host of investigators have submitted more limited group systems. The following classification will serve to convey some idea as to the structural characteristics of the more important groups:

1. The Classification by Migula

BACTERIA OR MICROBES

(Schizomycetes or Fission Fungi)

I. *Family* COCCACEÆ.—Micrococci. Cells globular or not elongated. Division in two or three directions of space. Spore formation rare.

1. *Micrococcus.*—Cells spherical or biscuit-shaped. Division in one direction of space. With or without flagellæ. A large genus, represented by numerous species, pathogenic and non-pathogenic, chromogenic, zymogenic, etc.

2. *Streptococcus.*—Generic limitation not clearly defined. Often merely chain forms of above, resulting from cohesion of cells dividing in one direction of space.

3. *Sarcina.*—Division in three directions of space. Cells often in fours (Tetracoccus)—as for example, the sarcina of the stomach. With or without flagellæ.

II. *Family* BACTERIACEÆ.—Bacilli. Cells more or less elongated, cylindrical, straight; some are somewhat curved or irregular in outline. With or without flagellæ. Endospore formation. Transverse septation.

1. *Bacillus.*—Variable in size and length of cell. Numerous flagellæ. Endospore formation common. A very large group, to which belong many of the most important microbes. Includes the old genus *Bacterium.*

2. *Pseudomonas.*—Said to have only polar flagellæ. Doubtful genus, by many relegated to the group bacillus.

III. *Family* SPIRILLACEÆ.—Spirillæ. Cells elongated and spirally twisted. Transverse septation. Body fixed, with polar flagellæ.

1. *Spirillum.*—Numerous polar flagellæ. Large group.

2. *Microspira.*—Few polar flagellæ. A group Spirosoma is said to be without flagellæ.

IV. *Family* SPIROCHETACEÆ.—Spirocheta. Long, single-celled, flexible, spirally twisted threads without flagellæ. One genus—*Spirocheta.* (Some authorities place these organisms in the animal kingdom with the Protozoa.)

V. *Family* MYCOBACTERIACEÆ.—Filamentous organisms, perhaps forming a connecting link between bacteria proper and the lower filamentous fungi. Cells filamentous but not enclosed in a sheath. To this family belong the groups *Mycobacterium* and *Actinomyces* (ray fungus). No flagellæ have been observed. Mostly transverse septation. Gonidial (spore) formation has been observed.

VI. *Family* CHLAMYDOBACTERIACEÆ.—Resembling above family, but the cell filaments are enclosed in a sheath. The following not very clearly

defined groups are recognized: *Cladothrix*, *Crenothrix*, *Phragmidiothrix*, and *Thiothrix*.

VII. *Family* BEGGIATOACEÆ.—Beggiatoa. Family characters not clearly defined. Motile, though no flagellæ have been observed. *Beggiatoa* is the most important genus.

Recently (1917) the Society of American Bacteriologists appointed a committee on bacterial nomenclature with instructions to look into the matter of bacterial classification and to propose a system which would in a way represent the biological and phylogenetic relationships of the principle groups. The following classification is offered by this committee, hoping that it may serve as the basis for further efforts along this line.

2. Suggested Outline of Bacterial Classification

THE CLASS SCHIZOMYCETES

Minute, one-celled, chlorophyll-free, colorless, rarely violet-red or green-colored plants, which typically multiply by dividing in one, two or three directions of space, the cells thus formed sometimes remaining united into filamentous, flat, or cubical aggregates. Filamentous species often surrounded by a common sheath. Capsule or sheath composed in the main of protein matter. The cell plasma generally homogeneous without a nucleus. Sexual reproduction absent. In many species resting bodies are produced, either endospores or gonidia. Cells may be motile by means of flagella.

A. ORDER MYXOBACTERIALES.[1]—Cells united during the vegetative stage into a pseudoplasmodium which passes over into a highly-developed cyst-producing resting stage.

B. ORDER THIOBACTERIALES.[1]—Cells free or united in elongated filaments. Water forms, not easily cultivable. Life energy derived mainly from oxidative processes. Cells typically containing either granules of free sulphur or bacterio-purpurin, or both, usually growing best in the presence of hydrogen sulphide.

C. ORDER CHLAMYDOBACTERIALES.[1]—Cells normally united in elongated filaments. Sulphur and bacterio-purpurin are absent. Iron often present and usually a well-marked sheath.

D. ORDER EUBACTERIALES.—Ordo nov. Synonyms: Bacterina Perty 1852 in part; Eubacteria Schroeter 1886; Eubacteriaceæ A. J. Smith 1902.

The order Eubacteriales includes the forms usually termed the true bacteria, that is, those forms which are considered least differentiated and least specialized. The cell metabolism is not primarily bound up with

[1] These first three orders are included briefly to give the complete setting of the fourth, the *Eubacteriales*, with which we are primarily concerned.

hydrogen sulphide or other sulfur compounds, the cells in consequence containing neither sulfur granules nor bacterio-purpurin. The cells apparently do not possess a well-organized or well-differentiated nucleus. The cells are usually minute and spherical, rod-shaped or spiral in shape, in most genera not producing true filaments; the filaments when formed not sheathed, and frequently branching, thus being differentiated from the iron bacteria. The cells may occur singly, in chains or other groupings. The cells may be motile by means of flagella, or non-motile; they are never *notably* flexuous. Cell multiplication occurs always by transverse, never by longitudinal fission. Some genera produce endospores, particularly the rod-shaped types. More or less branching of cells and filaments may occur, reaching its maximum expression in the genera *Nocardia* and *Actinomyces* which may show typical mycelium formation, intergrading with the molds. Chlorophyll is absent, though the cells may be pigmented. The cells may be united into gelatinous masses, but never form motile pseudoplasmodia nor develop a highly specialized cyst-producing fruiting stage, such as is characteristic of the *Myxobacteriales*.

I. *Family* NITROBACTERIACEÆ.—Fam. nov. Organisms usually rod-shaped (sometimes spherical in *Nitrosomonas* and possibly in *Azotobacter*). Cells motile or non-motile; when motile with polar, never peritrichous, flagella. Endospores never formed. Obligate aërobes, capable of securing growth energy by the direct oxidation of carbon, hydrogen or nitrogen or of simply compounds of these. Non-parasitic (usually water or earth forms).

1. *Hydrogenomonas*. Jensen, 1909.—Monotrichic short rods capable of growing in the absence of organic matter, and securing growth energy by the oxidation of hydrogen (forming water). Kaserer (1905) who first described the organism states that his species will also grow well on a variety of organic substances.

The type species is *Hydrogenomonas pantotropha* (Kaserer) Jensen. Nikleuski (1910) described two additional species, *H. vitrea* and *H. flava*.

2. *Methanomonas*. Jensen, 1909.—Monotrichic short rods capable of growing in the absence of organic matter and securing growth energy by the oxidation of methane (forming carbon dioxide and hydrogen). The type species is *Methanomonas methanica* (Söhngen) Jensen.

3. *Carboxydomonas*. Jensen, 1909.—Autotrophic rod-shaped cells capable of securing growth energy by the oxidation of carbon monoxide (forming carbon dioxide). The type species, *Carboxydomonas oligocarbophila* (Beijerinck and van Delden) Jensen, is described as non-motile.

4. *Mycoderma*. Persoon, 1822 emended.—Synonyms: *Ulvina* Kuetzing 1837; *Umbina* Naegeli 1849; *Bacteriopsis?* Trevisan 1885; *Gliacoccus:* Maggi 1886; *Acetobacter* Eurhmann 1905; *Acetimonas* Jensen 1909.

Cells rod-shaped, frequently in chains, non-motile. Cells grow usually on the surface of alcoholic solutions, securing growth energy by the oxidation of alcohol to acetic acid. Also capable of utilizing certain other carbonaceous compounds, as sugar and acetic acid. Elongated, filamentous, club-shaped, swollen and even branched cells common and quite characteristic.

The type species is *Mycoderma aceti* Thompson?

5. *Nitrosomonas.* Winogradsky, 1892.—Includes *Nitrosococcus* Winogradsky 1892.

Cells rod-shaped, or spherical, motile or non-motile, if motile with polar flagella. Capable of securing growth energy by the oxidation of ammonia to nitrates. Growth on media containing organic substances scanty or absent.

The type species is *Nitrosomonas europæa* Winogradsky.

6. *Nitrobacter.* Winogradsky? 1892.—Synonym: *Nitrosobacterium?* Rullmann 1897.

Cells rod-shaped, non-motile, not growing readily on organic media or in the presence of ammonia. Cells capable of securing growth energy by the oxidation of nitrites to nitrates.

Winogradsky names no species, although he described one. It might be termed *Nitrobacter Winogradskyi* and made the type species.

7. *Azotobacter.* Beijerinck, 1901.—Synonyms: *Parachromatium* Beijerinck 1903; *Azotomonas* Jensen 1909.

Relatively large rods, or even cocci, sometimes almost yeast-like in appearance, dependent primarily for growth energy upon the oxidation of carbohydrates. Motile or non-motile; when motile, with tuft of polar flagella. Obligate aërobes usually growing in a film upon the surface of the culture medium. Capable of fixing atmospheric nitrogen when grown in solutions containing carbohydrates and deficient in combined nitrogen. The best-known free-living nitrogen-fixing bacteria of the soil.

The type species is *Azotobacter chroöcoccum* Beijerinck.

8. *Rhizobium.* Frank, 1889.—Synonyms: *Phytomyxa* Schroeter 1886; *Cladochytrium* Vuillemin 1888; *Rhizobacterium* Kirchner 1895; *Pseudorhizobium* Hartleb 1900; *Rhizomonas* Jensen 1909. (See also p. 104.)

Comment. *Phytomyxa* Schroeter has priority over *Rhizobium*, but because of the confusion which would arise from the substitution of the older correct name for the better known term Rhizobium, the committee recommends the adoption of the latter.

Minute rods, motile when young by means of polar flagella. Involution forms abundant and characteristic when grown under suitable conditions. Obligate aërobes, capable of fixing atmospheric nitrogen when grown in the presence of carbohydrates in the absence of com-

pounds of nitrogen. Produce nodules upon the roots of leguminous plants.[1]

The type species is *Rhizobium leguminosarum* Frank.

II. *Family* MYCOBACTERIACEÆ Chester, 1897.—Cells usually elongated, frequently filamentous and with a decided tendency to the development of branches, in some genera giving rise to the formation of a definite branched mycelium. Cells frequently show swellings, clubbed or irregular shapes. Endospores not produced, but conidia developed in some genera. Usually Gram-positive. Non-motile. Many species are parasitic in animals or plants. Complex proteins usually required. As a rule strongly aërobic (except for some species of Actinomyces and the genera *Fusiformis* and *Leptotrichia*), and oxidative. Growth on culture media often slow; some genera show mold-like colonies.

1. *Actinomyces*. Harz, 1877.—Synonyms: *Streptothrix* Cohn 1875, not *Streptothrix Corda* 1839; *Discomyces* Rivolta and Micellone 1878; *Micromyces* Gruber 1891, not *Micromyces* Dangeard 1888; *Oöspora* Sauvageau and Radais 1892; not *Oöspora* Wallroth 1833; *Cohnistreptothrix* Pinoy 1913.

Organism growing in form of a much-branched mycelium, which may break up into segments that function as conidia. Usually parasitic, with clubbed ends of radiating threads conspicuous in lesions in animal body. No *earial* hyphæ or conidia. Some species are microaërophilic or anaërobic. Non-motile.

The type species is *Actinomyces bovis* Harz.

2. *Nocardia*. Trevisan, 1889.—Synonyms: *Actinomyces* of many authors; *Streptothrix* of many authors; *Thermoactinomyces* Tsilinsky 1899.

Branched filaments, resembling a mycelium, readily breaking up into segments, usually saprophytic soil forms. Differs primarily from Actinomyces in the development of aërial hyphæ and conidia. Usually aërobic. Many are pigment formers. Colonies as a rule mold-like on culture media.

3. *Mycobacterium*. Lehmann and Neumann, 1896.—Synonyms: *Sclerothrix* Metschnikoff 1888, not *Sclerothrix* Kuetzing 1849; *Coccothrix* Lutz 1886; *Mycomonas* Jensen 1909.

Slender rods which are stained with difficulty, but when once stained are acid-fast. Cells sometimes show swollen, clavate or cuneate forms, and occasionally even branched filaments. Non-motile, Gram-positive. No endospores. Growth on media slow. Aërobic. Several species pathogenic to animals.

[1] True nodule forming bacteria occur in the shrub *Ceanothus integerrimus* and probably other non-leguminous plants.—Author.

The type species is *Mycobacterium tuberculosis* (Koch) Lehmann and Neumann.

4. *Corynebacterium*. Lehmann and Neumann, 1896.—Synonyms: *Corynemonas* Jensen 1909; *Corynethrix* Bingert 1901.

Slender, often slightly curved, rods with tendency to club formation, branching cells occasionally seen in old cultures. Barred irregular staining. Not acid-fast. Gram-positive. Non-motile. Aërobic. No endospores. Some pathogenic species produce a powerful exotoxin. Characteristic snapping motion is exhibited when cells divide.

The type species is *Corynebacterium diphtheriæ* (Loeffler) Lehmann and Neumann.

5. *Fusiformis*. Hoelling, 1910.—Synonym: *Mantegazzaea* Vuillemin 1913, not *Mantegazzaea* Trevisan 1879.

Obligate parasites. Cells usually elongate and fusiform, staining somewhat irregularly. Filaments sometimes formed; non-branching. Non-motile. No spores. Growth in laboratory media feeble.

The type species (?) is *Fusiformis termitidis* Hoelling.

6. *Leptotrichia*. Trevisan, 1879 emended.—Synonyms: *Leptothrix* Robin 1847, not *Leptothrix* Kuetzing 1843; *Rasmussenia* Trevisan 1889.

Thick, long straight or curved threads, frequently clubbed at one end and tapering to the other. Gram-positive when young. Threads fragment into short, thick rods. Anaërobic or facultative. Non-motile. Filaments sometimes granular; non-branching. No aërial hyphæ or conidia. Parasites or facultative parasites.

The type species is *Leptotrichia buccalis* (Robin) Trevisan.

III. *Family* PSEUDOMONADACEÆ.—Short rods, usually motile. Flagella single, polar. Gram-negative. Not obligate aërobes. Many species active ammonifiers. Many species produce water-soluble pigments or green fluorescence; yellow pigment common. Some species are photogenic. Soil and water bacteria, with many plant parasites.

1. *Pseudomonas*. Migula, 1894.—Synonyms: *Bactrillum* Fischer 1895; *Arthrobactrillum* Fischer 1895; *Eupseudomonas* Migula 1895; *Bactrinius* Kendall 1902; *Bactrillius* Kendall 1902; *Bacterium* Ehrenberg emended E. F. Smith 1905; *Denitromonas* Jensen 1909; *Liquidomonas* Jensen 1909.

Rod-shaped, short, usually motile by means of polar flagella or rarely non-motile. Aërobic and facultative. Frequently gelatin liquefiers and active ammonifiers. No endospores. Gram stain variable, though usually negative. Fermentation of carbohydrates as a rule not active. Frequently producing a water-soluble pigment which diffuses through the medium as green, blue, purple, brown, etc. In some cases a non-diffusible yellow pigment is formed. Many yellow species are plant parasites.

IV. *Family* SPIRILLACEÆ Migula, 1894.—Cells elongate, more or

less spirally curved. Cell division always transverse, never longitudinal. Cells non-flexuous. Usually without endospores. As a rule motile by means of polar flagella, sometimes non-motile. Typically water forms, though some species are intestinal parasites.

1. *Vibrio Miller*, 1773 emended E. F. Smith, 1905.—Synonyms: *Pacinia* Trevisan 1885; *Microspira* Schroeter 1886; *Pseudospira* DeToni and Trevisan 1889; *Liquidovibrio* Jensen 1909; *Solidovibrio* Jensen 1909; *Photobacterium?* Beijerinck 1889.

Cells short bent rods, rigid, single or united into spirals. Motile by means of a single (rarely two or three) polar flagellum, which is usually relatively short. Many species liquefy gelatin and are active ammonifiers. Aërobic and facultative. No endospores. Usually Gram-negative. Water forms, a few parasites.

The type species is *Vibrio choleræ* (Koch) Buchner.

2. *Spirillum.* Ehrenberg, 1930 emended Migula, 1894.—Synonyms: Spirobacillus? Metschnikoff 1889; Spirosoma Migula 1894; Sporospirillum? Jensen 1909.

Cells rigid rods of various thicknesses, length, and pitch of the spiral, forming either long screws or portions of a turn. Cells motile by means of a tuft of polar flagella (5 to 20) which are mostly half circular, rarely wavy-bent. These flagella occur on one or both poles; their number varies greatly and is difficult to determine, since in stained preparations several are often united into a common strand. Endospore formation has been reported in some species. Habitat; water or putrid infusions.

V. *Family* COCCACEÆ Zopf, 1884 emended Migula, 1894.—Synonyms; *Sphaërobacteria* Cohn 1872; *Coccogenæ* Trevisan 1885; *Coccacei* Schroeter 1886; *Coccobacteria* Schroeter 1886.

Cells in their free conditions, spherical; during division somewhat elliptical. Division in one, two or three planes. If the cells remain in contact after division they are frequently flattened in the plane of divisicn. Motility rare. Endospores absent. Metabolism complex, usually involving the utilization of amino-acids or carbohydrates.

Tribe 1. STREPTOCOCCEÆ Trevisan.—Parasites (thriving only or best on or in the animal body). Grow well under anaërobic conditions. Many forms grow with difficulty on media, none very abundantly. Planes of fission usually parallel, producing pairs or short or long chains, never packets. Generally stain by Gram. Produce acid but no gas in glucose and lactose broth. Pigment, if any, white or orange.

1. *Neisseria.* Trevisan, 1885.—Synonyms: *Diplococcus* Weichselbaum 1886 in part; *Gonococcus?* Neisser? 1879; *Merismopedia* Zopf 1885, not *Merismopedia* Meyen 1839.

Strict parasites, failing to grow or growing very poorly on artificial

media. Cells normally in pairs of flattened cells. Gram-negative. Fermentative powers low. Growth fairly abundant on serum media, usually whitish or yellowish.

The type species is *Neisseria gonorrhœæ* Trevisan.

2. *Streptococcus*. Rosenbach, 1884, emended Winslow and Rogers, 1905.—Synonyms: *Sphærococcus* Marpmann 1885, not *Sphærococcus* Agardh 1821; *Perroncitoa* Trevisan 1889; Babesia? Trevisan 1889; *Schuetzia* Trevisan 1889; *Lactococcus* Beijerinck 1901; *Hypnococcus* Bettencourt et al. 1904; *Myxokokkus* Gonnermann 1907, not *Myxococcus* Thaxter 1892; *Melococcus?* Amiradzibi 1907; *Diplostreptococcus* Lingelsheim 1912.

Chiefly parasites. Cells normally in short or long chains (under unfavorable conditions, sometimes in pairs and small groups, never in large packets). Generally stain by Gram. Capsules and zoöglea often formed. On agar streak, effused translucent growth, often with isolated colonies. In stab culture, little surface growth. Sugars fermented with formation of large amount of acid. Generally fail to liquefy gelatin or reduce nitrates.

Type species is *Streptococcus pyogenes* Rosenbach.

3. *Staphylococcus*. Rosenbach, 1884.—Synonyms: *Micrococcus* Cohn 1872 em. Migula 1894; *Botryomyces* Bollinger 1888; *Botryococcus* Kitt 1888, not *Botryococcus* Kuetzing 1849; *Galactococcus* Guillebeau; Bollingera Trevisan 1889; Gaffkya Trevisan 1885; *Pyococcus* Ludwig 1892; *Carphococcus* Hohl 1902; *Aurococcus* Winslow and Rogers 1906, *Indolococcus* Jensen 1909; *Liquidococcus* Jensen 1909; *Peptonococcus* Jensen 1909; *Enterococcus?* (Thiercelin) Rougentzoff 1914.

Parasites. Cells in groups and short chains, very rarely in packets. Generally stain by Gram. On agar streak good growth, of orange color. Sugars fermented with formation of moderate amount of acid. Gelatin often liquefied very actively.

Type species is *Staphylococcus aureus* Rosenbach.

4. *Albococcus*. Winslow and Rogers, 1905.—Differs from *Staphylococcus* in forming more abundant surface growth of porcelain white color, and in fact that liquefaction of gelatin when present is less vigorous.

Tribe 2. Micrococceæ. Trevisan.—Facultative parasites or saprophytes. Thrive best under aërobic conditions. Grow well on artificial media, producing abundant surface growths. Planes of fission often at right angles; cell aggregates in groups, packets or zoöglea masses. Generally decolorize by Gram. Pigment yellow or red.

5. *Micrococcus*. Cohn, 1872, emended Winslow and Rogers, 1905.—Synonyms: *Microsphæra* Cohn 1872, not *Microsphæra* Leveille 1851; *Pediococcus* Balcke 1884; *Merista* Van Tieghem 1884, not *Merista* (Banks and Soland) Cunningham 1839; *Planococcus* Migula 1894; *Urococcus*

Miquel 1879, not *Urococcus* Kuetzing 1849; *Pedioplana* Wolff 1907; *Tetradiplococcus?* Bartoszewicz and Schwarzwasser 1908; *Solidococcus* Jensen 1909; *Planomerista* Vuillemin 1913.

Facultative parasites or saprophytes. Cells in plates or irregular masses (never in long chains or packets). Generally decolorize by Gram. Growth on agar abundant, with formation of yellow pigment. Glucose broth slightly acid, lactose broth generally neutral. Gelatin frequently liquefied, but not rapidly.

The type species is *Micrococcus luteus* (Schroeter) Cohn.

6. *Sarcina.* Goodsir, 1842, emended Winslow and Rogers, 1905.—Synonyms: *Urosarcina* Miquel 1879; *Planosarcina* Migula 1849; *Lactosarcina* Beijerinck 1908; *Sporosarcina?* Jensen 1909.

Sarcina differs from *Micrococcus* solely in fact that cell division occurs under avorable conditions in three planes, forming regular packets.

The type species is *Sarcina ventriculi* Goodsir.

7. *Rhodococcus.* Flügge, 1891, emended Winslow and Rogers, 1906.—Synonyms: Not *Rhodococcus* Molisch 1907.

Saprophytes. Cells in groups or regu'ar packets. Generally decolorize by Gram. Growth on agar abundant with formation of red pigment. Glucose broth lightly acid, lactose broth neutral. Gelatin rarely liquefied. Nitrates generally reduced to nitrites.

VI *Family* BACTERIACEÆ Cohn, 1872, emended.—Rod-shaped cells without endospores. Gram-negative. Flagella when present peritrichic. Metabolism complex, amino-acids being utilized, and generally carbohydrates.

1. *Bacterium.* Ehrenberg, 1838, emended Jensen, 1909.—Synonyms: *Actinobacter* Duclaux 1882 in part; *Klebsiella* Trevisan 1885 in part; *G iscrobacterium* Malerba and Sanna Salaris 1888; *Aërobacter* Beijerinck 1900; *Salmonella* Lignières 1900; *Denitrobacterium* Jensen 1909.

Notile or non-motile rods, staining evenly. Easily cultivable. Animal pathogens or saprophytes. Often chromogenic. Many forms actively decompose carbohydrates.

The type species is *Bacterium coli* Escherich.

2. *Erwinia.* Nov. gen.—Plant pathogens, Growth usually whitish, often slimy. Indol generally not produced. Acid usually formed in certain carbohydrate media, but as a rule no gas.

3. *Pasteurel a.* Trevisan, 1887.—Synonyms: *Octopsis?* Trevisan, 1885; *Coccobacillus* Gamaleia 1888, not *Coccobacillus* Leube 1885.

Short rods, sing'e or rarely in chains, usually showing distinct polar staining. Non-motile. Gram-negative. Without spores. Aërobic and facultative. Powers of carbohydrate fermentation slight; no gas produced.

Gelatin not liquefied. Parasitic, frequently pathogenic, producing plague in man and hemorrhagic septicemia in the lower animals.

The type species is *Pasteurella choleræ-gallinarum* (Flügge) Trevisan.

4. *Hemophilus.* Gen. nov.—Synonyms: *Pyobacillus?* Koppanyi 1907; *Diplobacillus* Morax 1896, not *Diplobacillus* Weichselbaum 1887.

Minute rod-shaped cells, non-motile, without spores, strict parasites, growing best (or only) in the presence of hemoglobin, and in general requiring blood serum or ascitic fluid. Gram-negative.

The type species is *Hemophilus Influenzæ* (Pfeiffer).

VII. *Family* LACTOBACILLACEÆ. Fam. nov.—Rods, often long and slender, Gram-positive, non-motile, without endospores. Usually produce acid from carbohydrates, as a rule lactic. When gas is formed, it is CO_2 without H_2. The organisms are usually somewhat thermophilic. As a rule microaërophilic; surface growth on media poor.

1. *Lactobacillus.* Beijerinck, 1901.—Synonyms: *Dispora?* Kern 1882; *Saccharobacillus?* van Laer 1889; *Streptobacillus* Rest and Khoury 1902; *Brachybacterium* Trioli-Petersson 1903; *Caseobacterium* Jensen 1909.

Generic characters those of the family.

The type species is *Lactobacillus caucasicus* (Kern?) Beijerinck.

VIII. *Family* BACILLACEÆ.—Rods producing endospores, usually Gram-positive. Flagella when present peritrichic. Actively decompose protein media through the agency of enzymes.

1. *Bacillus.* Cohn, 1872.—Synonyms: *Bactrella?* Morren 1830; *Metallacter?* Perty 1852; *Bactridium* Davaine 1868 in part; *Urobacillus* Miquel 1879; *Pollendera* Trevisan 1884; *Zopfiella* Trevisan 1885; *Streptobacter* Schroeter 1886; *Cornilia* Trevisan 1889; in part; *Bacterium* Ehrenberg, emended Migula 1894 in part; *Bactridium* Fischer 1895, not *Bactridium* Wallroth 1832; *Bactrinium* Fischer 1895; *Bactrillum* Fischer 1895; *Endobacterium* Lehmann and Neumann 1896; *Astasia* Meyer 1898; *Fenobacter* Beijerinck 1900; *Bacterius* Kendall 1902 in part; *Aplanobacter* E. F. Smith 1905 in part; *Semiclostridium* Maassen 1905; *Plennobakterium* Gonnermann 1907; *Myxobacillus* Gonnermann 1907; *Thermobacillus* Jensen 1909; *Serratia* Vuillemin 1913 in part, not *Serratia* Bizio 1823.

Aërobic forms. Mostly saprophytes. Liquefy gelatin. Often occut in long threads and form rhizoid colonies. Form of rod usually nor greatly changed at sporulation.

The type species is *Bacillus subtilis* Cohn.

2. *Clostridium.* Prazmowski, 1880.—Synonyms; *Amylobacter* Trecul 1865; *Cornilia* Trevisan 1889 in part; *Granulobacter Beijerinck* 1893; *Clostrilum* Fischer 1895; *Clostrinium* Fischer 1895; *Paracloster* Fischer

1895; *Semiclostridium* Maassen 1905; *Botulobacillus* Jensen 1909; *Butryibacillus* Jensen 1909; *Cellulobacillus* Jensen 1909; *Putribacillus* Jensen 1909.

Anaërobes. Often parasitic. Rods frequently enlarged at sporulation, producing clostridium or plectridium forms.

The type species is *Clostridium butyricum* Prazmowski.

Organisms Intermediate Between Bacteria and Protozoa

Spirochætaceæ. Swellengrebel, 1907.—Free living or parasitic spirilliform organisms with or without flagella, with undulating or rigid spiral twists. Reproduction by transverse division and by "coccoid bodies," the equivalent of spores.

Four genera are recognized as follows:

1. *Spirochæta.* Ehrenberg.—Non-parasitic, with flexible undulating body and with or without flagelliform tapering ends. Common in sewage and foul waters.

The type species is *Spirochæta plicatilis* Ehrenberg.

2. *Cristispira.* Gross.—Giant forms with undulating body and peculiar flattened ridge erroneously called an "undulating membrane" which runs the length of the body. Parasitic in molluscs.

The type species is *Cristispira balbianii* Certes, from the crystalline style of the oyster.

3. *Saprospira.* Gross.—Non-parasitic forms similar to Cristispira, but without the flattened ridge or "crista" which is, if present, here replaced by a straight columella or thickening of the periplast.

The type species is *Saprospira grandis* Gross.

4. *Treponema.* Schaudinn.—Parasitic and frequently pathogenic forms with undulating or rigid spirilliform body. Without crista or columella. With or without flagelliform tapering ends.

The type species is *Treponema pallidum* Schaudinn.

1. Artificial Key to the Orders of the Schizomycetes

Cells united during the vegetative stage into a pseudoplasmodium . . . . A. Myxobacteriales

Cells not forming a pseudoplasmodium

Cells free or united in elongated filaments, often with a well-defined sheath. Conidia frequently formed. Free sulphur, iron or bacteriopurpurin often present.

Cells typically containing granules of sulphur or bacterio-purpurin or both . . . . B. Thiobacteriales

Sulphur and bacterio-purpurin absent; iron often present . . . . C. Chlamydobacteriales

Cells never in sheathed filaments. Conidia only in the mycelial. Mycobacteriaceæ. Flagella often present. Free iron, sulphur, or bacterio-purpurin never present . . . . D. Eubacteriales

2. Artificial Key to the Families of the Eubacteriales

Cells spiral with polar flagella . . . . IV. Spirillaceæ
Not as above
Cells spherical; rarely, if ever, motile; spores never produced; never securing growth energy from nitrogen or ammonia . . V. Coccaceæ
Not as above
Cells short rod-shaped with a single rarely two polar flagellum; usually forming green or yellow pigment . . . . III. Pseudomonadaceæ
Not wholly as above
Spores formed . . . . VIII. Bacillaceæ
Spores never formed
Metabolism simple, securing growth energy form carbon, hydrogen or their simple compounds; flagella, if present, polar . . . . I. Nitrobacteriaceæ
Metabolism complex, dependent upon more complex carbohydrate and protein substances; flagella, if present, peritrichic. Cells clubbed, fusiform, filamentous, branching or mycelial; those not distinctly so are either acid-fast or show barred irregular staining . . . . II. Mycobacteriaceæ
Not as above
Gram-positive; non-motile . . . . VII. Lactobacillaceæ
Gram-negative; often motile. . . . . VI. Bacteriaceæ

3. Artificial Key to the Genera of the Eubacteriales

I. Nitrobacteriaceæ
Fixing nitrogen or oxidizing its compounds
Fixing nitrogen
Cells large; in soil . . . . 7. Azotobacter
Rods minute; in roots of leguminous plants . . . . 8. Rhizobium
Oxidizing nitrogen compounds
Oxidizing ammonia . . . . 5. Nitrosomonas
Oxidizing nitrites . . . . 6. Nitrobacter
Not as above
Oxidizing carbon compounds
Oxidizing alcohol; branching forms common . . . 4. Mycoderma
Not as above, using simpler carbon compounds
Oxidizing CO . . . . 3. Carboxydomonas
Oxidizing CH_4. . . . . 2. Methanomonas

II. Mycobacteriaceæ
Slender rods, staining with difficulty and acid-fast 3. Mycobacterium
Not as above
Mycelium and conidia formed
With aërial hyphæ and conidia; usually saprophytic soil organisms . . . . 2. Nocardia
Hyphæ and conidia not aërial; usually parasitic in animals . . . . 1. Actinomyces
Not as above; cells rod-like, usually somewhat curved, clubbed, fusiform, or even branched, but never mycelial.
Thick, long threads, fragmenting into short thick rods . . . . Leptotrichic
Not as above
Cells usually elongate and fusiform; filaments, if formed, not branching; staining somewhat irregularly . . 5. Fusiformis
Cells slightly curved, clubbed, or in old cultures even branching; not filamentous; showing definitely barred staining . . . . 4. Corynebacterium

III. Pseudomonadaceæ
Generic characters mainly those of family . . . 1. Pseudomonas

IV. Spirillaceæ
Flagellum single (rarely 2 or 3) . . . . 1. Vibrio
Flagella tufted (5–20) . . . . 2. Spirillum
V. Coccaceæ
Abundant re-pigmented growth on agar . . . . . 7. Rhodococcus
Not as above
Gram-negative
Normally in paris of flattened cells; growth on plain agar scanty, never bright yellow . . . . 1. Neisseria
Normally in plates, packets, or irregular masses, growth on plain agar abundant, pigment definitely yellow.
Cells in regular packets . . . . 6. Sarcina
Cells not in regular packets . . . . 5. Micrococcus
Gram-positive (Exceptions rare and not easily confused with above genera).
Cells normally in chains, sometimes in paris (especially in acid environment) never in large irregular masses. Gelatine rarely liquefied.
Growth on plain agar usually translucent, never heavy, never yellow or orange . . . . 2. Streptococcus
Cells normally in groups or masses (occasionally in plates in

Albococcus?); chains short and irregular, if present. Gelatine often liquefied.

Agar growth abundant, white to orange

Pigment orange (rarely lacking) gelatine often liquefied actively . . . . 3. Staphylococcus

Whitish to porcelain white; liquefaction less vigorous . . . . 4. Albococcus

VI. Bacteriaceæ

Plant pathogens . . . . 2. Erwinia

Not as above; saprophytes or in animal habitats (intestines, tissues, etc.).

Usually motile and exhibiting active fermentative powers; typically parasitic in intestines of man and higher animals; growing well on ordinary media . . . . 1. Bacterium

Not wholly as above

Growing only in presence of hemoglobin, ascitic fluid or serum . . . . 4. Hemophilus

Growth on media scanty, but less sensitive than the above; short rods with tendency to bipolar stain . . . . 3. Pasteurella

VII. Lactobacillaceæ

Generic characters mainly those of family . . . 1. Lactobacillus

VIII. Bacillaceæ

Aërobic, usually saprophytic, cells not greatly enlarged (if at all) at sporulation . . . . 1. Bacillus

Anaerobic, often sprophytic, cells frequently enlarged at sporulation . . . . 2. Clostridium

3. General Morphology of Microbes

As already stated, the morphology of microbes is simple. They consist of a single cell composed of cell-wall and cell-contents. The cell-wall consists of cellulose, and is very thin; stains readily with the various bacterial stains. The chief cell-contents is the cytoplasmic or protoplasmic living base commonly designated as the nucleoplasm, which is of a granular nature, and by some is supposed to be a nucleus in a divided state. A nucleus proper does not exist, or, rather, has not been demonstrated. The cytoplasm, as a rule, stains quite readily. Distributed through the cytoplasm may be found various substances, elaborated by cytoplasmic activity. Polar granules (metachromes or Babes-Ernest granules) have been observed. Sulphur, fat, pigment, chlorophyll, etc., may be found.

The cell-walls of many species undergo a gelatinous change. This change may affect the outer layers only, or it may involve the entire thickness of the wall, forming the gelatinous substances noticeable in bacterial cultures and in other substances (stringy cultures, stringy milk, etc.). This gelatinous substance also causes the individual organisms to cling to each other, thus causing the formation of the peculiar zooglea masses in in natural as well as in artificial culture media.

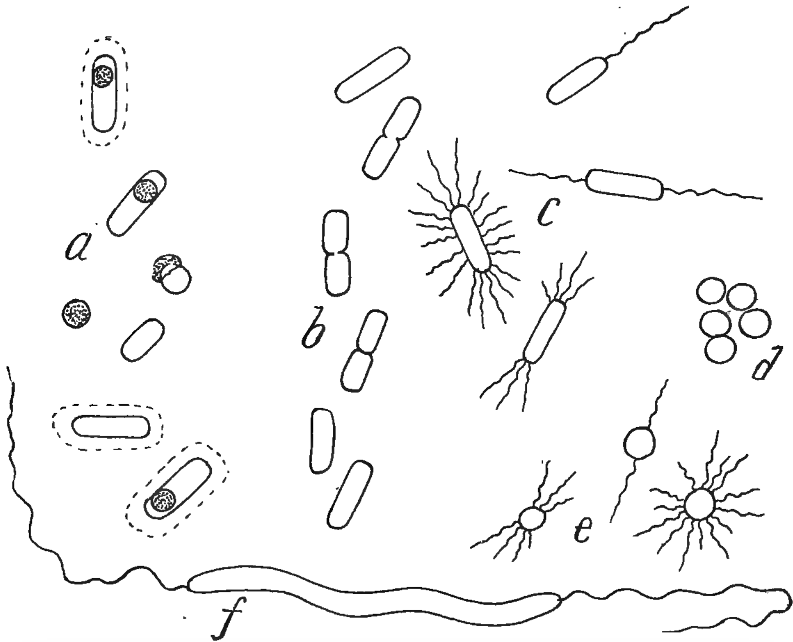

FIG. 4.—Illustrating the general morphology of microbes. *a*, showing general structure of a bacillus, endospore formation, and development of new bacillus from a spore; *b*, showing manner of transverse septation; *c*, arrangement of flagellæ, single unipolar, single bipolar; and multiple, polar and general; *d*, cocci; *e*, flagellæ of cocci; *f*, spirillum with single polar cilia.

The cilia or flagellæ are very delicate threads, supposed to extend from the cell-plasm, through the cell-wall, into the surrounding medium. The delicate threads are probably cytoplasmic in nature, and by their rapid vibratory motion enable the microbe to move about within liquid media. Some microbes are apparently without flagellæ, nor is it definitely determined that all motile microbes have flagellæ. The attempt to make generic distinctions based upon the absence or presence of few or many flagellæ, upon the existence of polar or non-polar flagellæ, etc., is unsatisfactory. Special staining methods are necessary to demonstrate the presence or absence of flagellæ.

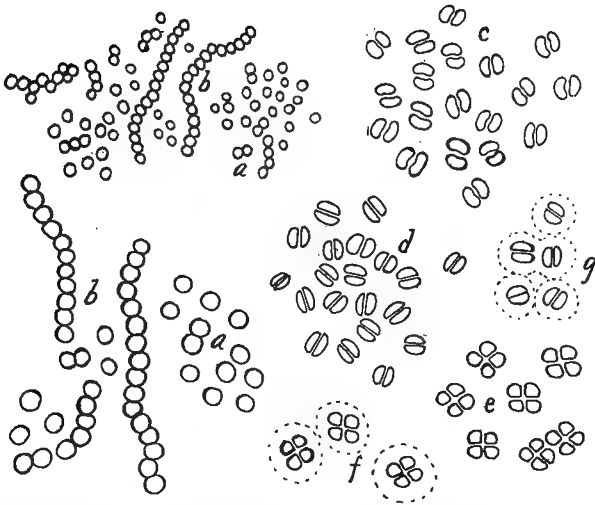

Fig. 5.—Illustrating the general morphology of Coccaceæ. *a*, *b*, micrococci (*a*) differing in size, showing chain formation or streptococci (*b*); *c*, diplococcus; *d*, diplococcus; *e*, tetracoccus; *f*, gelatinized tetracoccus; *g*, gelatinized diplococcus.

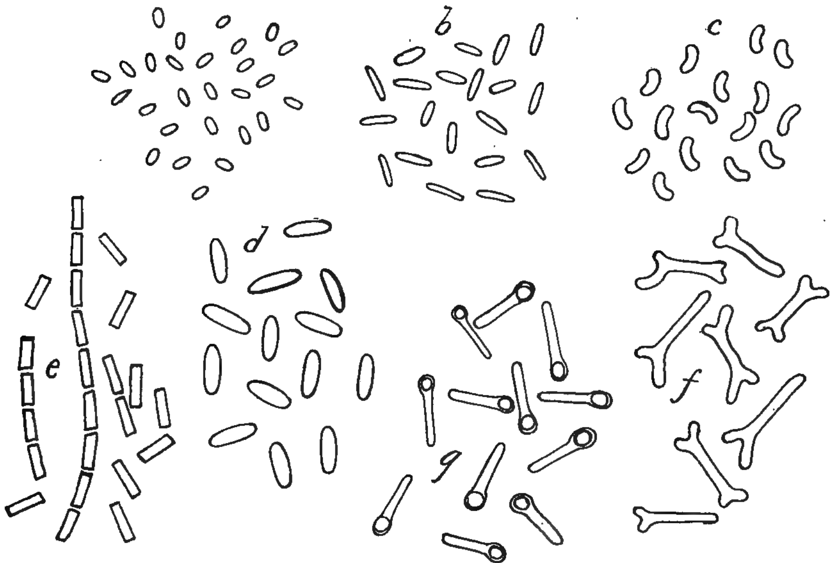

Fig. 6.—General morphology of Bacteriaceæ. *a*, *b*, *c*, *d*, bacilli differing in size and form; *c*, shows curved bacilli like those of Asiatic cholera; *e*, hay bacillus (*B. subtilis*); *f*, Y-shaped or branched bacilli, as of clover root nodules; *g*, drum-stick (Trommelachläger) bacilli, as of tetanus—form due to the enlarged endospores.

The rate of motion of bacteria has been measured. The cholera bacillus moves at the rate of 18 cm. per hour. The typhoid bacillus is slower

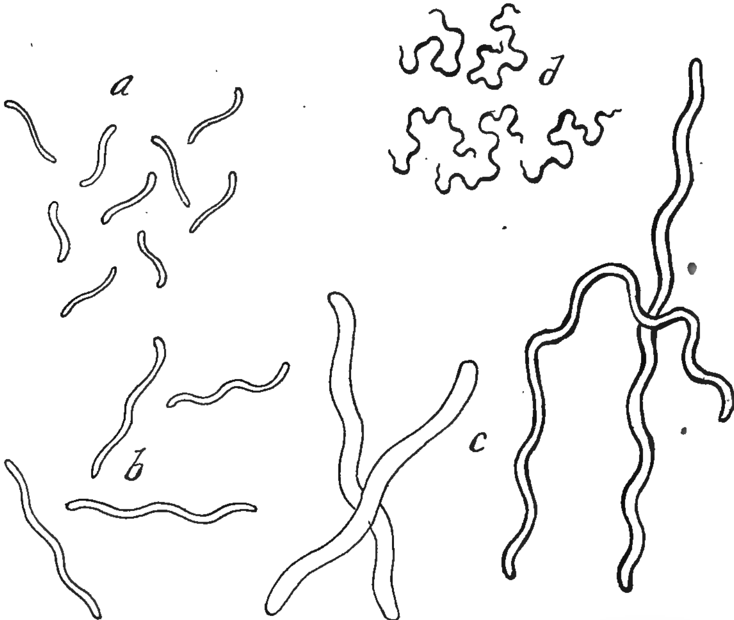

Fig. 7.—General morphology of the Spirillaceæ. *a*, S-shaped or single spiral; *b*, double spiral; *c*, multiple spirals; *d*, slender threads; *a* and *b* have fixed bodies, motion being caused by flagellæ; *c* and *d*, bodies flexible, motion not due to flagellæ.

moving a distance of 4 mm. in one hour. The rate of motion in one and the same species is, however, variable, being comparatively rapid at one

Fig. 8.—Illustrating polymorphism or pleomorphism. Involution forms of the bacillus of Asiatic cholera. (*Williams.*)

time under certain conditions of food supply, warmth, etc., and at other times comparatively slow.

When the bacteria approach the end of the life cycle, or when the conditions for growth and septation become unfavorable, spore formation may take place. However, not all species of bacteria form spores (endospores). For example, most of the pathogens do not form spores. Each cell forms one spore only, there being apparently no exception to this rule. In most cases the spore occupies a position nearer one end of the cell, more rarely it occurs in a median position. As to form the spore is generally somewhat oval in the direction of the long axis of the cell. It may be more or less irregular in outline, as in *Bacillus botulinus*, the cause of botulism. The cell which contains a median spore causing a bulging of the cell is called a *clostridium*. If it causes a terminal bulging it is called

Fig. 9.—Illustrating polymorphism or pleomorphism. *a* to *d*, inclusive, represent different forms of the same organism—the Diphtheria bacillus. (See also Figs. 46–50 inclusive.)

a *plectridium*, also drum stick bacillus (Trommelschläger bacillus). The spore is formed from the cell plasm, and differs from it in its higher refractive index and its peculiar resistance to the action of stains. As soon as spore formation is complete, the rest of the cytoplasm dies, the cell-wall disintegrates, and the spore is thus set free. Spores have a remarkable resisting power to high temperatures and other unfavorable conditions. In a dry atmosphere they may lie dormant for a long time, even several years. Boiling from one to two hours does not kill some of them (spores of hay bacillus). As soon as the spores are placed in suitable media (adequate warmth, moisture, and food supply) they develop into new individuals, which continue to septate until spore formation again takes place.

The classification given above, into families and genera, and Figs. 2 to 10, inclusive, will serve to give a fairly good idea of the general structural characteristics of microbes.

4. General Physiology of Microbes

Microbes, in common with living things generally, spring from pre-existing parents, take in and assimilate food, grow and multiply, and finally die. The rate of growth and of multiplication (septation or division) varies somewhat, depending on temperature, moisture, and food supply. The average life of one individual (from division to division) is perhaps thirty minutes. Under favorable condition the period is much shortened. This life period of the individual cell must not be confounded with the life cycle of the individuals resulting from a single cell or parent. It is known that under uniform conditions of temperature, moisture, food supply, and the environment generally, the progenations from a single parent cell

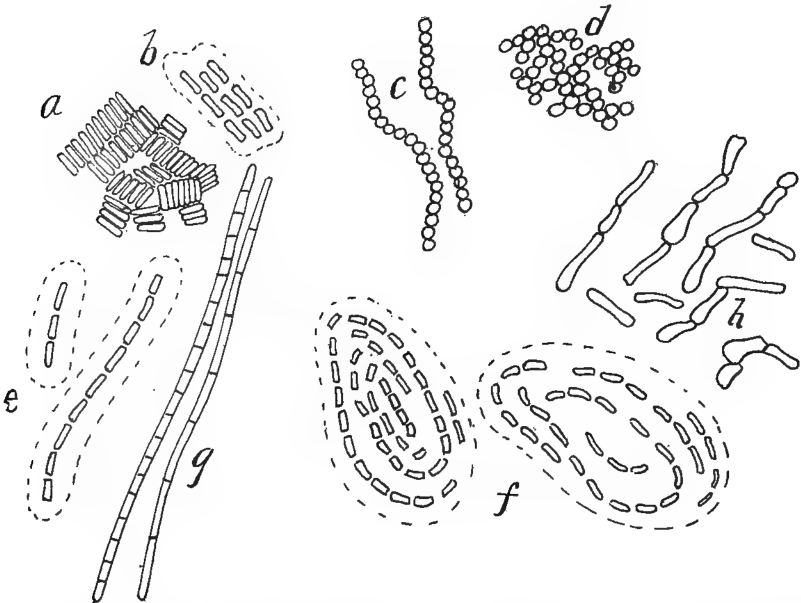

FIG. 10.—Illustrating zooglea formation. *a*, bacillar aggregates resulting from cohesion; *b*, aggregates resulting from cohesion of bacilli with gelatinized cell-walls; *c*, streptococcus formation resulting from the septation of a coccus form; *d*, cohering cocci forms; *e*, bacilli united end to end (resulting from septation), enclosed in a gelatinous coat; *f*, bacillar thread enclosed in gelatin; *g*, mycobacterial form; *h*, irregular cell forms, as ***Mycoderma aceti***.

show an increasing rate of septation, a stationary period, followed by a gradual decline, ending in total cessation of all septation, and in death. These life cycles have not yet been carefully determined; in fact, they are but little understood. It is highly probable that the cycles of existence play a very important part in the course and development of diseases of bacterial origin.

Whereas the period from one septation to another septation is very short, the life cycle referred to is often quite long, perhaps months and, under certain conditions lasting for years. The period of the life cycle can be modified artificially by food supply, chemicals, etc.

Investigators have succeeded in prolonging the life cycle of *Paramecium*. Normally *P. caudatum* dies out in about 175 generations; but by applying alcohol (1–5000 to 1–10,000) the cycle has been increased to 860 generations. Very dilute solutions of strychnine gave similar results. If the life cycle or vital impulse of these simple organisms can be prolonged it is probable that similar effects can be produced in higher organisms. Numerous investigators have from time to time sought after agents which might inhibit the senile changes in cells and circulatory system (arteriosclerosis) but thus far without conclusive results.

Microbes feed upon organic substances generally. Those which feed upon dead organic substances are said to be saprophytic; those feeding upon living substances are said to be parasitic. If they can live on dead organic substances only, they are obligatively saprophytic; if they can feed on both dead and living organic substances, they are facultatively saprophytic, or, *vice versa*, facultatively parasitic. The great majority of microbic parasites are facultatively so, as is evidenced by the fact that they can be grown in artificial culture media. Many of the microbic saprophytes will develop on living substances under certain conditions, thus showing that they are facultatively parasitic. It is no doubt true that no known microbic parasite actually feeds upon the living substances of the various hosts, since the cytoplasm is in all instances dead before it is taken up and assimilated by the microbe. It would therefore be more correct to say that parasitic microbes are biologically associated with living organisms, while the saprophytes are biologically associated with dead organic substances, and that they all feed upon and assimilate dead organic substances. In certain mutualistic symbioses (as in the root nodules of the Leguminosæ) the biological relationship of microbe and host plant is very intimate, but there is no actual interchange of living material.

All microbes require moisture and warmth (comparatively speaking) for their development, although they are enabled to withstand greater extremes of heat and cold than other organisms. The temperature of liquid air (about −270°F.) does not kill them at once, and the spores may be boiled for some time without destroying their germinating power. Cold (freezing temperature) promptly checks growth and septation, and so does dryness and excessive warmth, although life may not be destroyed. The majority of microbes develop most actively at a temperature of 25°C., a few species develop more actively at a lower temperature (20°C.), and a few others at a higher temperature (38°C.). Those which develop at a

temperature ranging from 0°C. to 20°C. are said to be cold loving (psychrophile), from 10° to 45°C., mesophile, from 40° to 70° C., thermophile. Thermophile species are found in decaying vegetable matters, whereas psychrophile species are found in cold water and cold soils.

Bacterial life processes result in the formation of many substances, some of which are of the greatest importance. It is impossible to estimate properly the enormous tasks performed by these minute organisms, nor shall we at this time make any attempt to set forth the great good and the apparent great harm done by them. We need only state that without rotting microbes soil formation would be impossible, and without soil, higher plant and animal life, as we now know them, would be impossible. Without plant food digesting microbes crop growing would be impossible. The saltpeter deposits in South America and the iron deposits of the Mesabi range of Minnesota are said to be the result of bacterial action. We make extensive practical use of microbes in medical practice, in the dairying industry, etc.

We will mention only a few substances of undoubted microbic origin. Ptomaines and toxalbumins are well-known poisons elaborated by saprophytic microbes which feed on meats and other organic substances, causing the familiar putrefactive changes. Pahogenic microbes elaborate toxins to which are due the manifestations of the disease. Acetic acid, lactic acid, and butyric acid are elaborated by *Bacillus aceticus*, *B. acidi lactici*, and *B. butyricus*, respectively. Some species liberate odoriferous substances, others gases, coloring substances, phosphorescence, etc. The phosphorescence observed on the ocean is supposed to be due to bacteria (*Bacillus phosphorescens indicus*). Phosphorescent bacteria occur in dead fish and in meat. Old cultures in animal nutrient media and in the presence of sodium salts are phosphorescent in the dark, sufficiently so, to have suggested making bacterial lamps and signal lights.

It has been suggested that certain diseases, of which the causes are at present unknown (as yellow fever, measles), may be due to organisms so small as to be invisible (ultra micro-organisms). It is known that the virus of yellow fever will pass through the most compact clay or porcelain filter. Attempts have been made to demonstrate the presence of ultra micro-organisms by special photomicrographic methods, aided by special illuminating devices (the ultra microscope of Siedentopf and Szigmondy) but without success. Furthermore, no one has succeeded in culturing such theoretically surmised organisms in artificial media, which would certainly render them visible *en masse*. It may, however, be possible that some ultra-organisms are obligative parasites hence will not develop in artificial media.

The biological (symbiotic) relationship of different species of bacteria

to each other and to their host are, in many instances at least, not well understood. For example, it is not clear what biological relationship the different species of bacteria in a mixed infection bear to each other. In the case of the root nodule organisms of the Leguminosæ it is known that there is a mutually beneficial (mutualistic symbiosis, mutualism) relationship between microbe and host but it is not obligatively so, since the symbionts can exist independently of each other. In most diseases due to microbic invasion there is one species of bacterium which acts as the primary cause. It is known that tuberculosis, especially the pneumonic form, usually shows a mixed infection, and it is probable that the associated organisms as bacteria and higher fungi act as predisposing causes, preparing the tissues so as to yield more readily to the invasion of the primary cause, the *Bacillus tuberculosis*. Such an association may be designated compound symbiosis, in which the relationship of the invading organisms (secondary and primary) is mutualistic and the relationship of these to the host is antagonistic. It is known that certain microbic diseases predispose to other microbic invasions, thus we may say that these organisms are mutualistically disposed toward each other.

Since it is possible to cultivate most disease germs in and upon artificial culture media (hence dead organic substances) it is evident that they are only facultatively parasitic.

In many instances the biological association of bacteria and higher plants and animals is loosely mutualistic, as the bacteria upon roots and rootlets of all plants and the bacteria lining the intestinal tract of animals. The hay bacillus (*Bacillus subtilis*) is a constant associate with the Gramineæ and serves an important function, assimilating or binding for the use of the host plant, the free nitrogen of the air. Certain soil organisms (*Bacillus megatherium*, *B. ellenbachiensis*, *B. mesentericus*, *B. pyocyaneus*, *B. prodigiosus*, the Azotobacter group, *Clostridium pastorianum*, certain moulds as *Aspergillus niger* and *Penicillium glaucum*) are capable of assimilating the free nitrogen of the air thus enriching the soil for the benefit of higher plants.

CHAPTER V

RANGE AND DISTRIBUTION OF MICROBES

Microbes are omnipresent over the surface of the earth. In number and in bulk they exceed all other organisms (plants and animals) put together. They form a large percentage of the bulk of the soil. They occur in the air, in water, in snow, in hail, in raindrops, in and upon plants, in and upon animals. All substances with which we come in contact are likely to hold microbes. Our clothing teems with them. They are in the air we breathe, in the food we eat, and in the liquids we drink. The floating dust particles of the air carry microbes; the particles of organic matter in water harbor microbes; they are found on wood, on cloth, on paper, on metal, glass, and rock surfaces, in fact on all exposed surfaces. The hands, the hair, the entire body surface of man and of the lower animals contain or hold microbes. They line all mucous membranes. The mouth cavity is a veritable bacteriological laboratory. The entire intestinal tract teems with millions upon millions of these minute beings.

Each animal and each plant has a microbic flora peculiar to itself. Each portion of the plant or animal, again, has distinctive bacterial groups. The microbic flora of the intestinal tract of the dog is different from that of the pig, or cat, or fowl, or man. Certain species predominate in the mouth cavity, others in the stomach, still others in the small intestine, in large intestine, etc.

Microbes are found on the highest mountain peaks and in the deepest valleys. It is, however, true that the higher atmospheric strata contain fewer microbes than the lower strata. The deeper layers of soil contain fewer microbes than the upper. The atmosphere of the country contains fewer microbes than that of the cities and towns. Since sunlight and absence of moisture are natural enemies of microbes, we may expect to find microbes more abundant in dark, damp, and moist places and areas. Microbes are always more abundant in cellars, basements, dark hall-ways, and alleys than they are in attics, sunlit living rooms, and along broad boulevards and highways. As suggested in the Chapter on the Origin of Bacteria, cosmic dust or telluric and interstellar dust, no doubt carry microscopic organisms.

Good drinking water, whether from hydrant, spring, or well, contains only a comparatively few microbes, from fifty to one hundred per cc., or even less. Stagnant, foul water teems with microbes, besides other organ-

isms, such as protozoa. So-called pure milk contains comparatively more microbes than pure water. The average good milk contains as many as 30,000 microbes per cc. Filthy milk may contain millions of microbes per cc. From 100,000 to 3,000,000 microbes per cc. is not uncommon in some milk which careless dairymen declare to be "good." Soups, broths, etc., boiled squash, potatoes, meats, and cooked organic substances generally, if allowed to stand for a day or two, contain many living microbes. In the course of two or three days, if the weather is warm, these substances teem with microbes and are rendered wholly unfit for human consumption because of rotting microbes which develop highly poisonous ptomaines and toxins.

Microbes do not grow and multiply in antiseptic substances, such as strong solutions of acids, of alkalies, of salts, etc. Used and dirty cups, drinking vessels, milk bottles, dishes, cooking utensils, knives, spoons and forks, hold numerous microbes. The public drinking cup has been the source of numerous disease infections. Disease is carried by the tools of the careless dentist and by the clothing, the apparatus and the clinical thermometer of the indifferent and careless physician. The hand-shaking and kissing habits spread disease. These facts are generally known and indicate the wide dissemination of the different kinds of microbes.

From the foregoing it becomes clear that microbes are present almost everywhere, and that it is impossible to escape them. It is the aim of the science of bacteriology to distinguish between good and bad microbes, between those which are desirable and those which are undesirable, between useful and harmful microbes. It is not the aim of the science of bacteriology to destroy them all, or to devise ways and means to escape from all of them. In fact, we owe our very existence to these very minute organisms, as has already been explained.

Under certain conditions bacteria multiply very rapidly. Such substances as meat, milk, and organic foods of all kinds, if exposed to moisture, warmth and removed from sunlight, soon swarm with microbes. Certain non-pathogenic microbes, as the root nodule bacteria (of the Leguminosæ), multiply very rapidly within the tissue cells. Others multiply upon the exterior of roots and of root hairs, where they no doubt serve a useful purpose to the plant. In bacterial diseases of plants and animals the microbes multiply very rapidly and form large aggregates, as a rule. To pathological conditions accompanied by extensive and general bacterial or microbic invasion, we apply the term bacteremia. In some diseases the microbic invasion remains localized and yet there are pronounced general or systemic effects, due to the absorption, into the system, of the toxins liberated by the microbes. To such conditions we apply the term toxemia. Toxemia may, however, also occur in bacteremia.

Microbes do not multiply in the air itself, rather upon the organic dust particles present, provided warmth and moisture are adequate.

Since microbes multiply rapidly, perhaps one septation in from twenty to thirty minutes, it is evident that the rate of numerical increase, under favorable conditions, is very great. Allowing thirty minutes for each septation, there would be a colony of 2,097,152 microbes in ten hours, developed from a single cell, or about 75,000,000,000,000 cells in twenty-four hours. However, under natural conditions septation never proceeds in such uniform ratio. All manner of checks to septation come into play sooner or later which may finally bring about complete cessation of septation and sporulation.

CHAPTER VI

BACTERIOLOGICAL TECHNIC

As may readily be supposed, the minuteness and wide distribution of microbes call for special methods of study and examination. Even the largest forms are far below the ken of unaided vision. Their general dissemination through organic substances calls for special methods for the separation and isolation of individuals or single bacterial cells. The difficulties of some phases of laboratory technic are further increased by the resistance of spores to various agents and substances which are readily fatal to higher organisms. The methods of examination are also greatly complicated by the marked polymorphism of many species.

Bacteriological technic comprises the use of glassware, compound microscope, and other apparatus, a thorough knowledge of sterilization and disinfection, the preparation and use of culture media, the making of micro-

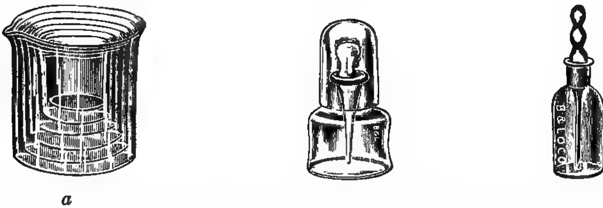

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FIG. 11.—*a*, Nest of beakers and reagent bottles. The smaller and medium size beakers are more desirable for bacteriological work. The reagent bottles are for Canada balsam, stains, clearing fluid, etc.

bic cultures, and the study of cultures. Methods vary greatly. The following represents a brief summary of general methods which are noted for simplicity and which have proven very satisfactory after years of testing.

1. Cleaning the Glassware

All glassware, such as test-tubes, flasks, beakers, Petri dishes, pipettes, shells, bottles, etc., which is to be used in bacteriological work must be *clean;* that is, free from all extraneous organic as well as inorganic matter. To accomplish this, it is necessary to use an abundance of pure water, hot as well as cold, aided by sand, paper shreds, brushes, towels, alcohol, acids, soap, sodic and potassic hydroxides, and whatever else may be necessary. Boil, wash, rinse, and wipe within and without repeatedly until it looks,

and is, absolutely clean. The following solution will be found useful as a cleansing agent for old as well as new glassware:

Potassium Dichromate,	6 parts.
Sulphuric Acid,	30 parts.
Water,	40 parts.

Of course, the sulphuric acid must be added little by little with constant stirring, in order to avoid excessive heat development. Soak the glassware

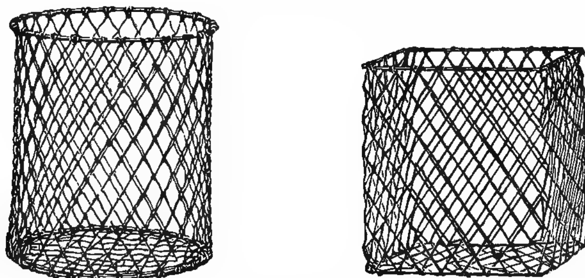


FIG. 12.—Wire baskets for holding test-tubes. Cylindrical form and square form. Each basket holds about fifty test-tubes. The wire is galvanized to prevent rusting The round wire baskets should be used.

in this solution for some time, several hours or more, and rinse, wash, drain and wipe thoroughly afterward. The sole object to be attained is *cleanliness* in the true sense of the word. The glassware must be clean bacteriologically and chemically; that is, it must be free from microbes and chemical substances.

2. Plugging Containers with Cotton

After the thorough cleansing above outlined, the test-tubes and flasks are plugged with a good quality of non-absorbent commercial cotton. The dry cotton plug forms most efficient germ filter. All microbes are caught and held in the meshes of the cotton, and yet the air is permitted to pass through into the tube or flask.

Open a roll of cotton, find the free end, and lay it out on the work table. Take the test-tube in the left hand; remove a goodly tuft of cotton with right hand, using thumb and first and second fingers. Place this over the mouth of the tube or flask, and push it down to a distance of ½ to ¾ inch by means of a solid glass rod rounded (by heat) at the ends. The rod must not be too thick, as it will then not permit enough cotton to enter the opening nor yet too thin, as it will then be forced through the cotton. The plug must not be too tight, as that would interfere with subsequent manipulations nor, yet too loose, for obvious reasons. Enough cotton should project above the opening to permit of ready grasping between the fingers in the later operations.

Plugging may also be done with fingers alone, but this is tedious and non-professional. A far better method is to use a pair of fairly large blunt-pointed pincers. Remove the cotton from the roll by means of the pincers and insert it into the test-tube with the pincers.

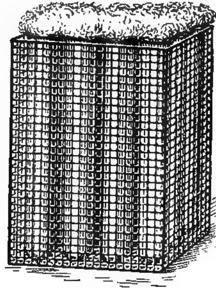


FIG. 13.—Wire basket filled with test-tubes plugged with cotton. A little cotton should be placed in the bottom of the basket to lessen the danger of breaking the test-tubes. (*Williams.*)

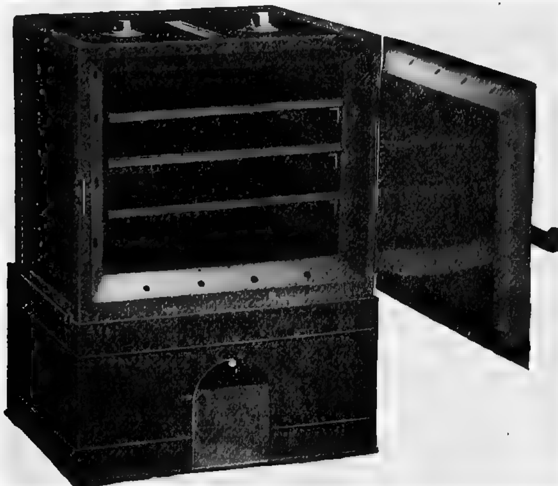


FIG. 14.—A hot air sterilizer. These sterilizers are double-walled, on stand, with perforations at top for thermometers. Ordinary baking ovens which can be secured from hardware dealers will serve the purpose.

Whatever method is used, remove the amount of cotton required to plug one tube or flask at one time. Do not attempt to plug with several small pieces. If an excess of cotton projects above the opening, pluck it away with the fingers; do not cut it away with scissors. Plug the tubes as uniformly as possible.

3. Filling Test-tubes with Culture Media

The rule is to pour the culture media hot, although this is not absolutely essential. For example, if the media are liquid in the cool or cold state, as bouillon, serum, milk, etc., they may be poured cold. A good rule is to pour a desired amount of the media just as soon as they are prepared, whether they are still hot or merely warm or cold. Of course, gelatin and agar media must be poured hot or must be liquefied before they can be poured.

Fill a small to medium-sized beaker about two-thirds full of the culture medium. Grasp a plugged tube near the upper end, holding it between thumb and first two fingers of the left hand. Remove the cotton plug by

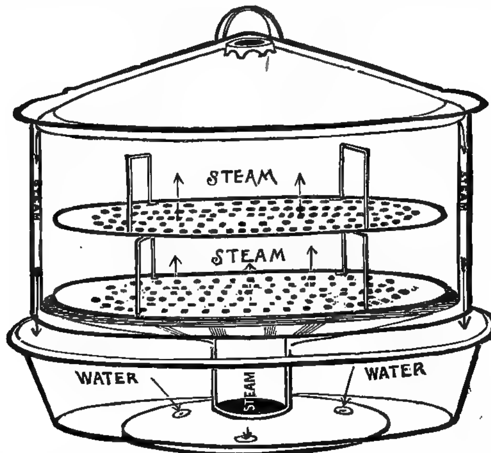


Fig. 15.—Diagrammatic sectional view of Arnold steam sterilizer illustrating the principle of steam formation, circulation and condensation.

means of the first and second, second and third, or third and fourth fingers of the right hand, grasping the free portion of the plug with the back of the fingers toward the cotton. Holding the tube slightly inclined on a level with the mouth, take beaker with medium in right hand (at the same time holding the cotton plug as described), see that the beak rests lightly upon and projects slightly over the edge of the tube, and pour, at the same time shifting the eyes to the lower end of the tube to watch the filling process. Fill tubes one-third full. Set down the beaker and replace the cotton plug. Place the filled tubes in special wicker baskets, with a little cotton at the bottom to prevent breaking. Some practice is necessary in order to pour so that none of the liquid comes in contact with the upper third of the tube. This must be avoided, in order to prevent the cotton plug from sticking. Tubes may also be filled from funnel with rubber hose, stop-

cock, and glass nib attachment. Occasionally it is desirable to place exact amounts of culture media in the tubes, in which case a graduate, a burette, a pipette, or other convenient measuring device may be used.

4. Sterilization of Culture Media

All culture media in tubes as above set forth, and the portions remaining after the desired number of tubes are filled, must be considered as being contaminated with living microbes and their spores. These microbes and spores are killed by the sterilizing process. For all ordinary purposes the discontinuous or fractional method answers the purpose admirably. Place the test-tubes, flasks, and other cotton-plugged containers with culture media, in a steam sterilizer (Arnold steam sterilizer, either board of health or cylindrical form; or kitchen vegetable cooker or steamer). The test-tubes are placed in wire baskets (rectangular or cylindrical). These several containers with culture media are exposed to live steam for about thirty minutes, whereupon the flame is turned out, and if convenient the containers are allowed to remain in the sterilizer. Caution must be observed to guard against condensed steam running into the several containers. The better way is to remove the containers and place them in an incubator kept at a temperature of 20° C. In twenty-four hours, or thereabouts, steam is again applied for thirty minutes. This is repeated a third time on the second day after the first sterilization. The first sterilization presumably kills most of the vegetative cells. During the first interval of twenty-four hours most of the spores present develop into vegetative cells, which are killed at the second sterilization. Should any survive the second steaming, they are sure to be killed during the third sterilization. During this time the cotton plugs have not been removed. The media thus fractionally or discontinuously sterilized are now ready for use in making microbic cultures, or they may be set aside for an indefinite period of time.

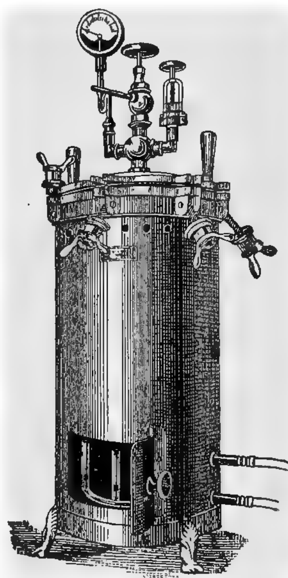


FIG. 16.—Autoclave for using steam under pressure for purposes of sterilization. Many different forms and sizes of autoclaves are on the market. Some of them to be used with gas heat, others with electricity. The enormous autoclaves used by the large canneries will hold several tons. Enormous sterilizers on the order of the autoclave are used at the national quarantine stations, as at Angel Island, San Francisco, and at New Orleans.

It is, of course, evident that in the above process of sterilization the temperature does not exceed 100° C., and it may be less in certain portions of the sterilizer, steamer, or cooker, say, 95° to 97° C. Certain kinds of sterilizations are done by steam under pressure. The apparatus used for this purpose is known as autoclave. It consists of a strong steam cylinder with a screwed-down top, safety valve, steam gauge, and thermometer. The articles (media, etc.) to be sterilized are placed inside, the top is securely fastened down, steam is generated until the thermometer registers, say, 120° C. The temperature is kept up to that degree for about 10 to 20 minutes, which is sufficient to destroy all life, including spores. For certain purposes the autoclave is not applicable. Blood serum, gelatin media, and all media containing carbohydrates, undergo certain chemical changes when the temperature is raised above 100° C., or even if kept at 100° C. for a long time or for a short time, if oft repeated. The autoclave is convenient for sterilizing discarded cultures, test-tubes, and glassware generally, and such media as beef broth and agar.

In many instances it is desirable to sterilize at a temperature lower than 100° C. Albumen and blood serum, for instance, will coagulate at that temperature. Again, it is desired to kill the microbes without destroying the toxins which they form, as in the manufacture of bacterial vaccines. In the sterilization (pasteurization) of milk, a lower temperature is employed. In the sterilization of these and other substances the temperature ranges from 50° to 85° C. The discontinuous method is employed, differing from the method already described in that the period of exposure is much prolonged, about one hour. The number of daily exposures ranges from one to six. For example, milk exposed to a temperature of 60° to 70° C. for one hour is considered sufficiently sterilized, whereas blood serum is subjected to hourly exposures of a temperature of 60° C. for six successive days before it is pronounced completely sterilized.

5. Preparation of Culture Media

The pharmacist should give especial attention to the preparation of bacterial culture media, as in this he may be of service to the physician. The busy general practitioner who is not equipped with a suitable bacteriological laboratory, or who does not have time to prepare culture media, would no doubt consider it a very decided advantage should the pharmacist offer to assist him. This will be more fully set forth in the last chapter.

In brief, it may be stated that microbes feed upon the same substances that we feed upon. In the presence of adequate warmth and moisture they attack all organic substances. This being the case, it may readily be assumed that there are many substances or media which can be used

as food for bacteria. Such is the case, and the number of media which have been used is legion. Almost any organic substance may be used, provided it is not antiseptic in its properties.

Culture media are liquid or solid, simple or compound. In the case of liquid or liquefiable solid media, the following physical properties are desired, in so far as it is possible to attain them:

a. Culture media should be perfectly clear. There should be no sediment, no opacity or flocculent suspension, and no floating matter. In the case of broths, extracts generally, gelatin media, and blood serum, these requirements are easily attained. Perfectly clear agar is difficult to obtain. Milk is normally opaque.

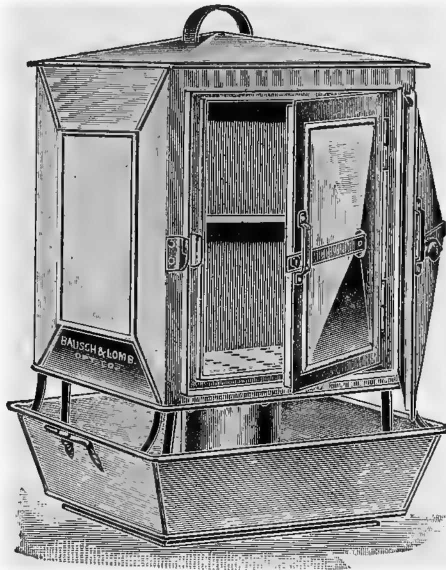


FIG. 17.—Arnold Steam Sterilizer. Boston Board of Health Form. This sterilizer is square, and constructed with a side-door all in accordance with the recommendation of the Boston Board of Health. Its large size makes it well suited to the requirements of Board of Health laboratories, and it has been found to be very serviceable and convenient. It is made of copper throughout, following the same principles as employed in the construction of the other sterilizers.

b. Media should be neutral or very slightly alkaline to litmus, which is equivalent to a slightly acid reaction to phenolphthalein, at a temperature of about 20° C. Most microbes develop best in media of such reaction.

c. They must be free from living microbes and their spores, and from other organisms. This requirement is attained by sterilization as already described. Culture media contaminated with living organisms are not usable in bacteriological work.

The essential requirements given under *a*, *b*, and *c* are obtained by filtration, neutralization, and sterilization, as will be more fully explained. Non-liquefiable solid media, as potato, bread, squash, etc., must be clean, free from living microbes and other organisms, and there should be a comparatively smooth exposed inoculating surface. These requirements are attained by washing and otherwise cleansing, disinfecting, rinsing, and heat sterilization (dry heat, steam or hot-water bath).

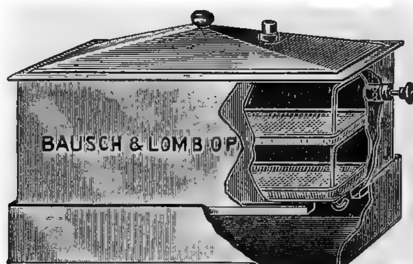


FIG. 18.—Arnold Steam and Hot-Air Sterilizer for Surgical Instruments. This sterilizer is a combination and portable sterilizer, so designed that instruments may be both sterilized and then dried by hot air, if desired. About 100° C. can be attained with the hot air by simply turning the valve shown in the illustration, which turns the steam as it escapes from the chamber into the base.

The following are the more important media:

A. *Nutrient Bouillon.*—

Beef Extract (Armour's, Liebig's, etc.),	3 gm.
Peptone,	10 gm.
Salt,	5 gm.
Distilled Water,	1000 cc.

Mix ingredients and boil for a few minutes. Filter through filter paper. This bouillon may be modified by adding glycerin (6 per cent.), and sugars, as dextrose, saccharose, or lactose (1 per cent.).

B. *Loeffler's Blood Serum.*—Very largely used in making diagnostic diphtheria bacillus cultures. In many cities this medium, with sterilized cotton swabs, in sterilized test-tubes, is furnished free to physicians by the board of health. In cities and towns where this is not done, the pharmacist should be prepared to furnish the materials to the physicians. The medium consists of —

Bouillon with 1 per cent. Glucose,	1 part.
Blood Serum,	3 parts.

The bouillon is prepared as above described, with 1 per cent. of glucose added. The blood serum can be obtained from calf, sheep, ox, or cow, through the butcher or at the abattoir. Collect the blood in a clean,

sterile jar or flask, closed with cotton plug. Place on ice for twenty-four to forty-eight hours, during which time coagulation has taken place; the serum may then be siphoned off. The proper sterilization of Loeffler's serum requires care. After the bouillon and serum are mixed, pour into test-tubes and coagulate in a Koch serum coagulator at a temperature of 80° C. Any form of sterilizer may, however, be used. The essentials are that the temperature should be raised very gradually and must be kept below the boiling-point, and the tubes should be slanted at a degree which will bring the medium close to the cotton plug, making what are commonly called tube slants. After the medium is coagulated in the tubes it is sterilized fractionally on three successive days (one hour each day) at a temperature of 80° C. These tube slants are now ready for the physician.

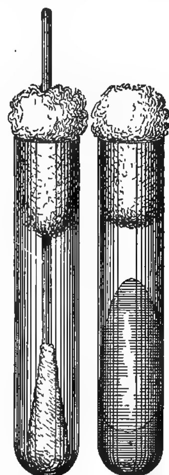


FIG. 19.—Culture tube and swab tube used by physicians in the diagnosis of diphtheria. The swab tube should be long enough to have the entire length of swab inside, not projecting as shown in the figure. (*Williams.*)

To prevent evaporation of the medium in the test-tubes, cover the cotton plug and upper end of tube with tin foil fastened with thread, and dip into melted paraffin several times. Tubes thus sealed can be kept for a year or more without any considerable shrinking of the medium. Dip the tin foil in a 1 : 2000 corrosive sublimate solution before capping on tubes.

A simpler way is to use rubber caps which are especially made to fit over the end of the test-tube and the cotton plug. These rubber caps must be sterilized before applying them, for which purpose the 1–2000 corrosive sublimate solution will be found satisfactory. Rubber stoppers may also be used but they are more expensive and inferior to the rubber cap or the tin foil with coat of paraffin.

C. *Liquid Blood Serum.*—Obtained as for Loeffler's serum. Sterilize fractionally at a temperature of from 56° to 58° C. for one hour on each of six days. The serum will be liquid and clear.

D. *Milk.*—Secure fresh milk directly from cow, or, if in cities, demand certified milk. Keep on ice, in a covered jar, for twenty-four hours. Siphon off the middle portion, rejecting cream and sediment. Sterilize like Loeffler's blood serum. Litmus milk is prepared by adding 1 per cent. of azolitmin before sterilizing. This indicator will show whether or not acids are formed by the microbes which may be cultivated in the milk. Only pure milk will answer the purpose. Milk to which preservatives (formaldehyd, salicylic acid, borax, boric acid) have been added must not be used.

E. *Peptone Solution.*—The medium is employed to test for the development of indol by certain bacteria. It consists of

Peptone,	10 gm.
Salt,	5 gm.
Distilled Water,	1000 cc.

Boil, filter, and sterilize as for bouillon. The bacteriological indol test is of great importance in medical practice, and the chances are that physicians will require this medium. However, sugar-free beef broth is also used for this test; in fact, it is generally preferred. Beef contains a small amount of muscle sugar, which must first be removed.

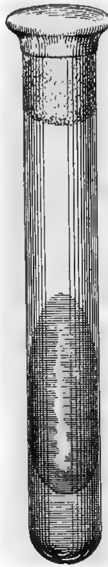


FIG. 20.

FIG. 21.

FIG. 20.—Test-tube cultures. *a*, Stab culture. This tube is closed with a rubber stopper to prevent drying of medium; *b*, streak or smear culture on slant, tube closed with rubber cap. (*Williams.*)

FIG. 21.—The ordinary rice cooker. A most valuable apparatus in preparing culture media and for sterilizing test-tubes and other objects.

F. *Sugar-free Bouillon.*—Grind the fat-free beef through a meat grinder; add water, and inoculate at once with a pure culture of *Bacillus coli communis*, and allow to incubate for twelve to fifteen hours at 38° C., then boil, filter, add peptone and salt, and prepare like bouillon; or, inoculate nutrient bouillon with the colon bacillus and prepare as above. However, before using the medium it should be tested for indol, as it has been proved that *B. coli communis* may form indol in beef extract. The indol test in bacterial cultures is made by adding two drops of concentrated

sulphuric acid and one drop of a 0.01 per cent. sodium nitrite solution to a four-day peptone-broth culture. If a pink color appears at the end of one-half hour it indicates the presence of indol.

G. *Beef Broth.*—This medium is now not as extensively used as formerly. It is more difficult to prepare, and shows no advantages over the bouillon already described.

Ground or Chopped Lean Beef,	500 gm.
Peptone,	10 gm.
Salt,	5 gm.
Distilled Water,	1000 cc.

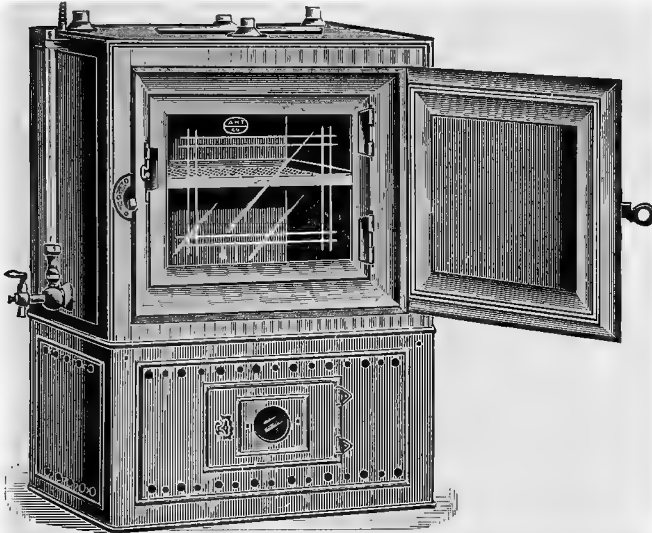


FIG. 22.—This is a copper double-walled incubator covered with non-conducting material and provided with a water gauge, tubulations for thermometer and thermostat, a ventilating strip, enclosed base and inner glass door. The incubating chamber is 24 cm. high, 30 cm. wide and 24 cm. deep.

Add the water to the minced meat, shake frequently, and keep on ice for twenty-four hours, then strain forcibly through cloth, or press out in a hand press. Add the salt to the liquid, boil, make up to 1000 cc., and add the peptone. Titrate to reaction of + 1.0 per cent., filter, and sterilize. It will be apparent that the cold water meat infusion contains merely the meat salts, meat sugar, and acids, and a certain proportion of the albumens. The albumens are coagulated and removed in the filtering process, so that nothing remains of the meat but the salts, acids, and the trace of muscle sugar. Nearly the whole of the meat proper is wasted. It is apparent, therefore, that the meat extract bouillon answers all the purposes of the beef broth.

H. *Gelatin Medium.*—

Beef Extract,	3 gm.
Gelatin,	100 gm.
Salt,	5 gm.
Peptone,	10 gm.
Distilled Water,	1000 cc.

Mix ingredients in a rice cooker and boil for one-half hour, stirring frequently; titrate to +1.0 per cent. and filter. This forms a very efficient culture medium for most bacteria, and is clear and remains solid at ordinary temperatures. It must be borne in mind, however, that frequent or prolonged heating tends to liquefy gelatin permanently.

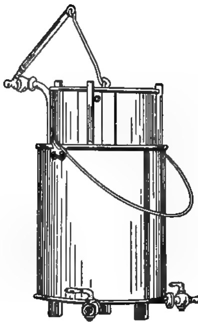


FIG. 23.

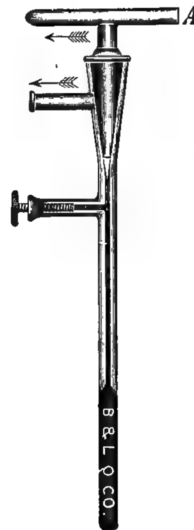


FIG. 24.

FIG. 23.—Murrill's Gas Pressure Regulator. This apparatus in its most improved form is to be used in connection with a thermostat for the maintenance of a constant temperature. The use of this regulator relieves the thermostat of the necessity of caring for the wide variation which is apt to occur in the gas pressure, and with it the temperature may be held constant to within 0.1° C.

FIG. 24.—Reichert thermo-regulator or thermostat used with incubator and other apparatus requiring a uniform degree of temperature. May be used in conjunction with the gas pressure regulator.

I. *Agar Medium.*—Agar is a seaweed found on the Japanese coast. It forms an important article of diet among the Japanese and Chinese. The medium consists of

Beef Extract,	3 gm.
Agar,	15 gm.
Salt,	5 gm.
Peptone,	10 gm.
Distilled Water,	1000 cc.

Prepare like the gelatin medium. Titrate to +1.0 per cent. Agar is difficult to filter, and the medium is never quite clear. The agar medium liquefies at a higher temperature than gelatin, and does not tend to remain liquid, no matter how often or how long it may be heated.

J. *Agar-gelatin Medium.*—This has the advantage of both media, and is now much used in general bacteriological work.

Agar,	8 gm.
Gelatin,	40 gm.
Salt,	5 gm.
Peptone,	10 gm.
Distilled Water,	1000 cc.

Mix, boil in rice cooker, stir; titrate to +1.0 per cent. filter, and sterilize as for other media.

The above includes the more important culture media used in bacteriological work. Others can be prepared as ocasion requires. It is not necessary to make up the full amounts indicated if it is evident that smaller quantities will suffice. The student should prepare all of the media in small amounts (one-quarter the quantities given) several times, in order to get the necessary experience and practice.

6. General Directions for the Preparation of Culture Media

Book information alone is not sufficient. Experience must be added. Also, brief, concise explanations are far more valuable than lengthy descriptions of unessential details. Those possessed of good judgment do not require lengthy explanations, and lengthy explanations would certainly be wasted on those who lack good judgment. This does not imply, however, that it is unnecessary to adhere strictly to established methods. The novice must follow closely the methods formulated by those who have devoted many years to some one particular mode of procedure, as it is wholly unlikely that he can improve upon them. Furthermore, when a physician calls for Loeffler's blood serum, for example, he wishes to be assured that the medium has been prepared according to the standard method. Any substitution or deviation, no matter how slight, may bring about wholly negative or erroneous results and conclusions. With this in mind the following suggestions are added:

A. *Selection of Ingredients.*—Great care must be observed in the selection of the ingredients used in the preparation of culture media. Meats used must be from healthy animals, and there must be absolute certainty that no preservative has been added. Buy the meat personally from the nearest reliable butcher who keeps fresh meats only. Remove as

much of the fat as possible. The so-called round steak of beef is usually employed.

Use only the best gelatin; the so-called best French gelatin is usually employed, although much of the "French gelatin" comes from Berlin, Chicago, Omaha, or other places equally remote from France. Do not attempt to use old friable gelatin.

The milk requirements have already been referred to. The milk must be fresh, placed on ice at once, and sterilized within twenty-four hours after it is taken from the cow. If the milk is obtained from an unknown dealer, test it for the presence of added water, preservatives, and other foreign matter.

Agar does not deteriorate readily, and may be kept in good condition for a long time. Other highly gelatinous seaweeds may be used, although this is not permissible in the preparation of any of the standard culture media .

Serum, egg albumen, peptone, various indicators, etc., must be pure. Too much caution cannot be observed in this regard. Secure the blood for serum personally whenever possible, from healthy animals. Use egg albumen from fresh eggs, not from cold-storage eggs. Dried egg albumen may be used. Before doing so, it should be examined microscopically and if it contains excessive bacteria, 100,000,000 or more per gram, it should not be used. Much of the dried egg and dried egg albumin of the market is highly contaminated by bacteria. Peptone and other chemicals should be secured from reliable dealers.

B. *Suggestions on the Preparation of Culture Media.*—First of all, some experience is necessary before a neat article can be prepared. Do not expect to prepare a medium which meets all of the requirements the very first time. In preparing gelatin media, remember that these are injured by excessive heating, and in preparing agar media, remember that they are very difficult to filter. Both must be filtered hot, using hot-water funnels; or the ordinary filtering device can be used by keeping the unfiltered portion hot and pouring into the funnel from time to time. Cover funnel with filter paper to keep out dust, and keep in the heat as much as possible. In so far as possible filter all media through filter paper (one thickness, properly folded), but it is practically impossible (for reasons of time) to pass agar through filter paper. This medium is usually filtered through cotton upon which a neatly folded and perforated sheet of filter paper has been placed. Puncture the filter paper several times with a small knife blade. Filtering through cotton is quick, but the media are much less clear than when filtered through filter paper. The filtering process may also be hastened by means of pressure (suction); connect funnel with aspirator bottle and pump, but see to it that the connections

with the hydrant are properly made and that the flow is properly regulated, in order to guard against any back pressure, which may cause the receiver to fill with hydrant water. This accident is best avoided by interpolating a flask or bottle. Agar may also be clarified by precipitation. Pour the hot agar into an ordinary percolator used by pharmacists. The dirt particles and other impurities will gradually settle to the bottom. When cool, take out the solid medium and cut away the lower portion containing the sediment.

C. *Titration of Culture Media.*—As already stated, most bacteria grow best in neutral or very slightly alkaline (to litmus) media, and since most media are quite decidedly acid in reaction, it is desirable to alkalinize. This is done by means of normal sodium hydroxide solution. In order to understand the method of procedure clearly, it is necessary to make certain explanations.

A normal (N/1)solution of any substance contains as many grams per liter of the substance as there are units in its molecular weight, if the substance contains one atom of replaceable hydrogen. If it contains two atoms of replaceable hydrogen, the number of grams used equals the molecular weight divided by two, and so on. According to this, a normal solution of sodium hydroxide contains 40 gm. of sodium hydroxide in a liter. Exact normal solutions are, however, not prepared by weight. Crystallized oxalic acid is used as the basis for making normal solutions. This acid has a molecular weight (including a molecule of water of crystallization) of 126, and, since it is dibasic, 63 gm. per liter are taken. Any normal acid solution will exactly neutralize an equal volume of normal alkaline solution. To make a normal sodium hydroxide solution, add about 14 gm. of pure caustic soda to one liter of distilled water. Determine the amount of this solution required to just neutralize 1 cc. of normal oxalic acid solution. This volume contains the quantity of sodium hydroxide which should be present in 1 cc. of normal solution, and from this we may calculate the volume of distilled water to be added in order that 1 cc. of sodium hydroxide solution will neutralize 1 cc. of normal oxalic acid solution. Having a normal solution of sodium hydroxide, it is now possible to prepare a normal solution of hydrochloric acid, etc. A tenth- (N/10), twentieth- (N/20), fiftieth- (N/50) normal solution is a normal solution diluted ten, twenty, and fifty times.

An acid reaction is indicated by +, and an alkaline by −. The degree of acidity of any culture medium in preparation may be indicated by the amount of normal sodium hydroxide solution required to render it neutral to phenolphthalein. Neutralization by titration is done as follows: Place 5 cc. of the medium to be neutralized in a dish, add 45 cc. of distilled water, stir, and bring to a boil. Add 1 cc. of phenolphthalein solution

(0.5 per cent. of phenolphthalein in 50 per cent. alcohol). Add enough of twentieth-normal sodium hydroxide solution (in a burette), with constant stirring, to give a faint but distinct pink color. Read the amount of twentieth-normal sodium hydroxide necessary to neutralize the 5 cc. of medium and from this calculate the amount of normal sodium hydroxide solution necessary to neutralize the entire quantity of culture medium. Now boil the medium and again titrate, when it will be found that there is a slight acid reaction. A third titration is rarely necessary.

Another method is to take 10 cc. of the culture medium, add a few drops of the phenolphthalein solution. From a burette add, drop by drop, with constant stirring, a normal sodium hydroxide solution (0.4 per cent.) until a faint pink color appears, which indicates the beginning of the alkaline reaction. Repeat this with two more samples. Note the amount of sodium hydroxide solution required in each case, and take the average and calculate the amount required for the entire quantity of medium. If, for example, the average was 1 cc. for each 10 cc. of medium, then 1000 cc. of bouillon would require 100 cc. of the sodium hydroxide solution; a concentrated solution being used, in order to avoid the dilution of the medium with the water of the caustic-soda solution. Flocculency of the medium usually indicates excessive alkalinity.

The old, crude, rough-and-ready method is to add, from a beaker, drop by drop, a tenth-normal sodium hydroxide solution, with constant stirring, until red litmus paper just begins to turn blue. In practice it is found that when a culture medium is neutral or slightly alkaline to litmus it is still acid to phenolphthalein. In fact, it is claimed that most bacteria develop best in a medium having a reaction indicated by +1 or +0.5 that is, it is sufficiently acid to phenolphthalein to require 1 per cent. or 0.5 per cent. of normal sodium hydroxide solution to render it neutral to phenolphthalein.

D. *Suggestions on the Preparation of Culture Media for Physicians.*—First of all, the pharmacist must have the necessary laboratory equipment and necessary skill and experience to prepare culture media. He should explain to a few representative physicians that he is ready to prepare such media as the busy physician may require. The physicians will in all probability indicate that media are likely to be needed in the course of their practice. Allow yourself to be guided by these several suggestions and prepare the media accordingly.

Make sure that the culture media are clear. There must be no sediment and no flocculency. Not infrequently the medium fails to become sufficiently clear, even though every precaution has been taken. In such cases clarification may be tried, rather than to discard it. Add the white of an egg, thoroughly beaten, to a liter of the medium in the liquid state

and at a temperature below the coagulating point for albumen, mix thoroughly; boil for ten minutes, and filter. The coagulating albumen takes up the impurities which remain upon the filter with the albumen, while the medium comes through perfectly clear. Media which have become infected with bacteria as the result of inadequate sterilization should be discarded. Do not attempt to clarify them. They may become clear, but they are nevertheless objectionable because of the substances which the bacteria may have liberated and which might interfere with the development of the bacteria to be grown in it subsequently.

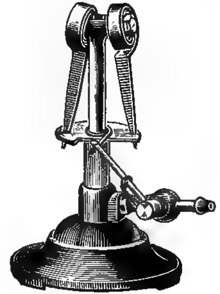


Fig. 25.—Koch safety burner. Should the flame be blown out, an automatic devise shuts off the gas.

Most of the tubes with solid media (Loeffler's serum, gelatin, agar, and gelatine-agar) should be slants. The slanting surface offers certain advantages in making diagnostic bacterial cultures. The usual, non-slanting tubes, for deep stab cultures, should, however, also be held in readiness. Keep all tubes in suitable containers, in a dry, cool, clean place. To guard against infection by mold and other organisms, it is well to cap all tubes with the rubber caps or the

Fig. 26.—Hot water funnel with stand and ring gas burner.

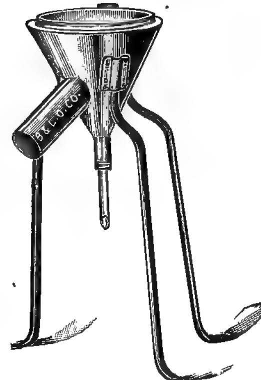


Fig. 27.—Hot water funnel with stand.

tin foil dipped in corrosive sublimate and paraffin, as already suggested. In case of liquid media, the rubber stoppers or the rubber caps are much preferred, or the hot paraffin may be painted over the tin foil and upper

end of tube by means of a small brush. Apply two or three coats. Thus protected, there is no danger of outside infection.

The chances are that the physician who calls for tube culture media will also require the use of an incubator. This the pharmacist should

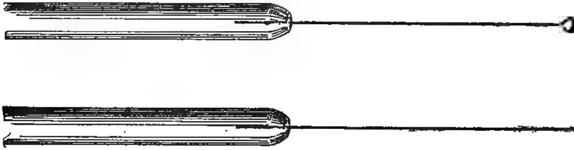


Fig. 28.—Glass rods with platinum wire, straight and loop, for inoculating culture tubes, Petri plates, etc.—(*Williams.*)

have in readiness. The usual copper double-walled water-jacket incubator, with thermo-regulator, kept at a temperature of about 37° C., will serve the purpose.

The swab to be supplied with each tube of slanted Loeffler's serum consists of a piece of wire or of pine wood four inches long, around the

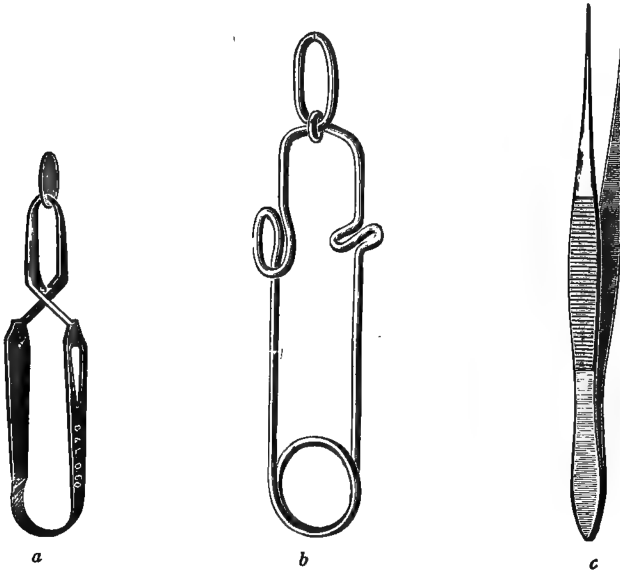


Fig. 29.—Cover-glass pincers. *a* and *b* are self-clamping but the pressure is often enough to break thin covers.

lower end of which a pledget of absorbent cotton has been wound and firmly tied by means of thread. This is placed in a test-tube, which is then plugged with cotton and sterilized in the dry sterilizer (one hour at a temperature of 150° C.). The physician wipes the cotton end of the swab over

the suspected throat area, and then lightly rubs it over the surface of the serum tube slant. The swab is returned to the tube, the cotton plug is restored and then returned to the board of health to be destroyed in stove or furnace fire, or destroyed by the attending physician in case there is no board of health to receive it.

7. Making Bacterial Cultures

This branch of the science of bacteriology is of comparatively little importance to the pharmacist. While it is desirable to know what bacterial cultures are and how to make some of them, it is wholly unlikely that the pharmacist will be called upon to do extensive work along this line. This is the work of those who make bacteriology a specialty. Such bacterial cultures as are likely to come to the notice of pharmacists will most generally be prepared by physicians, health officers, and other specialists in bacteriology. The pharmaceutical bacteriologist *may* be called upon to make bacterial examinations of drinking water, of milk, of ice cream, and other food materials; of syrups, liquors, aquæ, tinctures, fluidextracts, infusions, etc., and he should, if possessed of some skill and adequate laboratory facilities, be able to do so.

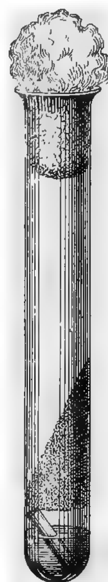


FIG. 30.—Cotton plugged tube with a potato slant resting on a bit of glass rod to keep the potato out of the water in the bottom of the tube. (*Williams.*)

The prime object in growing bacteria in artificial culture media is to make possible their further more careful and more extended study. The study of bacteria in their natural or normal surroundings is all-important, but is not complete without the artificial culturing.

As a rule, bacteria are biologically associated with other organisms, and it is unusual to find pure cultures in nature or in natural media. An open sore may contain several or many species and varieties of bacteria, in addition to the pus germs. The intestinal tract of the cholera patient contains bacteria other than the comma bacillus of Koch. The tubercular bronchials always show a mixed infection. The diphtheric membrane contains some foreign germs, etc. Some infections, particularly those of internal tissues or organs, as lymphatic glands for example, may present practically pure cultures. However, no matter how mixed an infection may be, there is always a predominating type present, or, to state it more correctly, it is the greater development of the predominating type which determines the diagnostic characteristics of the infection.

It must also be borne in mind that bacteria behave differently when

taken out of their natural environment and placed in artificial culture media. It does not at all follow that, in the case of a mixed infection, the predominating and diagnostic microbe will remain the predominating type when said mixed infection is transferred to some artificial culture medium. In fact, the predominating microbe may develop

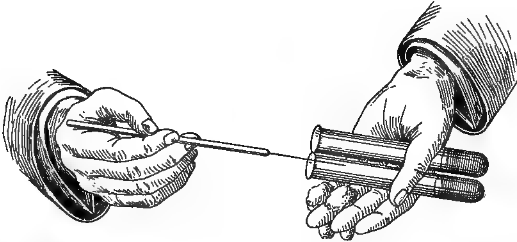


FIG. 31.—Manner of holding tubes when making subcultures. The cotton plugs, removed from the two tubes, should be held in hand holding the platinum rod, as explained in the text. (In this figure the cotton plugs are held in the hand holding the test tubes, which is wrong.) (*Williams.*)

very slowly or with great difficulty, if at all, in the artificial culture media; whereas one or more of the associated microbes may thrive remarkably well, soon entirely overshadowing the former. These and

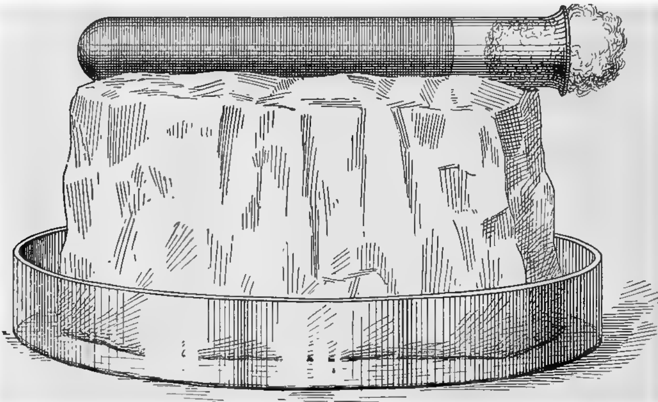


FIG. 32.—Making an Esmarch roll-tube culture. A lump of ice is placed in a dish and the inoculated tube is placed horizontally in a groove in the ice and revolved until the medium is well set. The groove may be made with test-tube full of hot water. (*Williams.*)

other conditions occasion some of the great difficulties encountered in determining the primary causes of some microbic and protozoic diseases and infections.

A. *Test-tube Cultures.*—Inoculate several test-tubes, containing nutrient gelatin or agar gelatin, with any material which is known to be bacterially infected. This is done by touching the infected material

with the tip of a heat-sterilized (by holding in flame of Bunsen burner until red hot) platinum needle (prepared by fusing a platinum wire, 1½ inches long, into the end of a glass rod, six to seven inches long), then removing the cotton plug from the test-tube, and pushing the needle, carrying the microbes, into the culture medium down to the very bottom of the tube. Replace the cotton plug at once, pass the needle into the flame of the Bunsen burner until red hot, to sterilize it, and lay aside for the next tube inoculation. This is known as a deep stab tube inoculation. In this manner inoculate some five or six tubes. Also make streak inoculation on tube slants by simply passing the infected platinum needle over the middle of the tube slant surface, from lower end toward the top, observing the instructions regarding the cotton plug and needle sterilization, with each tube inoculation. Number the tubes serially, and in a special notebook make entry of all desirable data pertaining to each inoculation, making such entries under each tube number. Place tubes vertically in a suitable holder, as tumbler, beaker, wire basket, etc., and set aside in incubator or in some container to which you alone have access.

FIG. 33.—Kitasato filter for filtering hypodermic solutions, culture media, sera, water, etc. The material to be filtered is placed in the globose container and forced through the clay (infusorial earth) tube (Berkefeld filter bougie) by connecting the receiver with a vacuum pump. All parts of the filter must, of course, be sterilized by heat before and after using. (*Williams.*)

In warm weather the first bacterial growths may appear at the end of thirty-six hours. In cold or cool weather nothing may appear for two, three, and even four to five days. Note the nature of the bacterial growth in a deep stab inoculation and in the streak inoculation, as to

a. *Growth*—scanty, moderate, abundant; slow, rapid.

b. *Form of growth*—outline clearly defined, spreading, rugose, beaded, etc.

c. *As to surface*—flat, raised, concave, convex.

d. *Color*—translucent, glistening, waxy, transparent, opaque, light, chalky white, grayish-white, dark red, green, blue, yellow, lemon color, purple, etc.

e. *Odor*—comparative description.

f. *Consistency*—viscid, slimy, stringy, membranous, friable or brittle, dry, watery, etc.

g. *Changes in medium*—gelatin liquefied, gelatin not liquefied; colored, as grayed, browned, reddened, blued, etc. In case indicators are used, any color changes should be noted.

h. *Deep stab culture*—where is growth most active? If at bottom, it indicates anaërobic tendencies. If limited to top of medium, it indicates decidedly aërobic tendencies. (Most bacteria are decidedly aërobic; that is, they require free oxygen to thrive.)

The test-tube cultures do not necessarily represent pure cultures, and the student cannot know whether the growths in the test-tubes represent the predominating bacterial flora in the substance from which the inoculations were made. The chief object in making the above

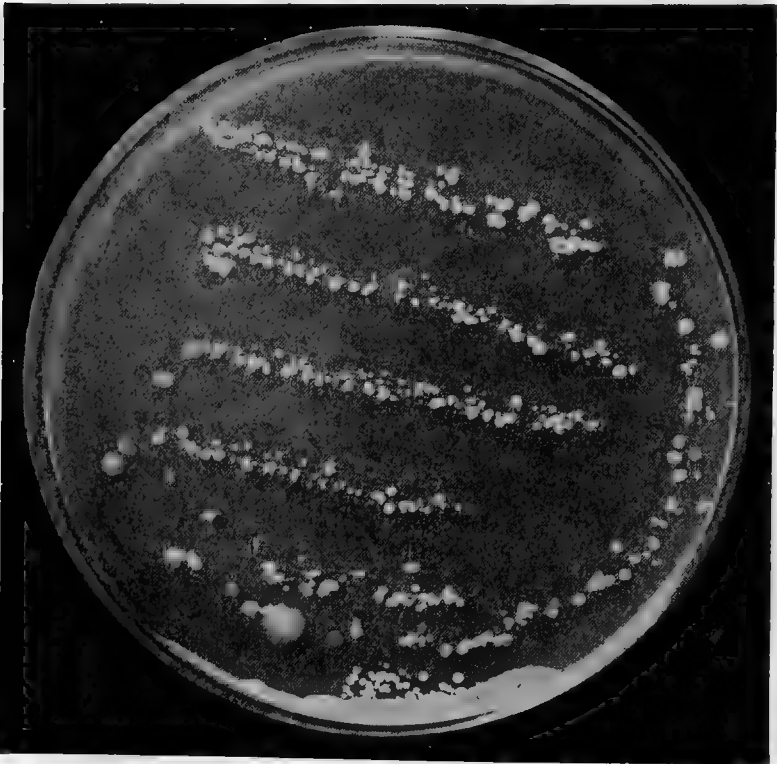


FIG. 34.—Streak culture on agar in a Petri dish. (*Delafield* and *Prudden*.)

cultures is to enable the student to get practice in this preliminary work, particularly as to making the cultural observations above indicated.

The student should now make transfers (sub-cultures) from the first tube cultures into second tubes, and note whether or not the characteristics originally noted are continued or repeated. If the transfer cultures are the same as the originals, it is an indication that the first cultures were pure (representing one species or variety), which is generally the case, though it must be borne in mind that one and the same

species of microbe may undergo considerable change in extended culturing, as indicated in the changed culture characters. In fact, some of the changes are so extreme as to confuse even the most expert bacteriologists.

B. *Isolating Bacteria by the Plate Method.*—In order to separate or isolate the several species and varieties of bacteria in any contaminated substance, it is only necessary to dilute the inoculating material sufficiently. For this purpose there is necessary, sterilized Petri dishes containing heat-sterilized gelatin or other solid media through which the bacteria from the contaminated substance are disseminated in numbers so small that the colonies from each and every microbe present may be visible to the naked eye (or aided by the microscope). This is done as follows:

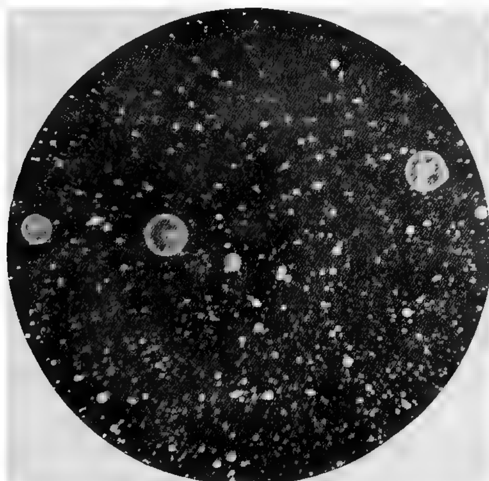


FIG. 35.—Appearance of colonies on gelatin in a Petri dish. Differences in size of colonies may indicate different species. Differences in color also indicating different species, cannot be shown in the figure. (*Williams.*)

To obtain isolation cultures of air bacteria it is only necessary to expose the Petri dish (with a layer of gelatin or agar-gelatin medium, sterilized) for about two minutes, immediately closing the dish and setting it aside to await developments. Making isolation cultures from contaminated solids or liquids is not quite so simple. Proceed as follows: Liquefy the gelatin in four or five test-tubes and keep them at a temperature of not more than 30° C., just high enough to keep the contents liquid; set them in a beaker filled with warm water (30° C.) until needed. Number the tubes from 1 to 5.

Dip a platinum loop (bend the end of a straight needle into a small loop) into the infected liquid, as bouillon, milk, water, tea, syrup, tincture, fluidextract, etc., etc., and pass one loopful into tube No. 1 (sterilize

loop and return to its proper place). Rotate tube (replugged with the cotton and held vertically) rapidly between the hands for twenty seconds, to mix contents. By means of the platinum loop take two loopfuls (one loopful may serve) from tube No. 1 (which you have just inoculated and rotated) and pass them into tube No. 2. Plug both tubes, set aside tube No. 1, and rapidly rotate tube No. 2. Take two loopfuls from tube No. 2 and transfer to tube No. 3, and proceed as before. Now pour contents of tube No. 1 into a sterile Petri dish, also numbered 1; contents of tube 2 into Petri dish 2; and tube 3 into Petri dish 3. Wait until the media in the Petri dishes are solidified, and then set aside at the room temperature to await developments. In the course of two or three days it will perhaps be found that very many minute specks are visible in dish No. 1, some one hundred or more may appear in dish No. 2, and perhaps not more than ten or twenty in dish No. 3. Observe carefully the several growths in dishes 2 and 3. Each visible growth indicates the development from a single microbe. Are the several growths all alike, or do they differ? Differences in color and in outline of growths indicate different species of bacteria. The several different kinds of bacteria may now be transferred to test-tubes by means of the straight platinum needle or the loop, and the observations may thus be extended. Transfers can be made to different kinds of media, as agar, gelatin, agar-gelatin, beef broth, milk, prepared potato, etc.

C. *Making Bacterial Counts.*—In order to determine the number of bacteria in any given substance, the same procedure as was just described is fol-

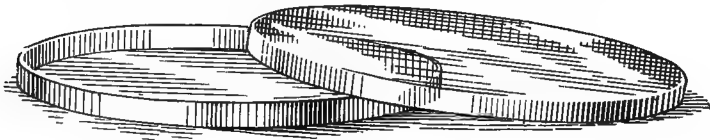


Fig. 36.—Petri dish. These dishes are among the essentials in the bacteriological laboratory. (*Williams.*)

lowed, with the difference that a definite amount of the thoroughly mixed contaminated substance is added to a definite amount of culture medium in the test-tubes in which the dilution mixtures are made. For example, we will suppose that it is desired to determine the number of bacteria (per cc.) in milk: Thoroughly mix the sample of milk by shaking it in the container. Take 0.1, 0.2, 0.5, or 1 cc. of the milk (by means of a sterilized graduated pipette) and add it to 9 cc. of the liquefied culture medium in tube No. 1; 1 cc. of tube No. 1 to tube No. 2, also with 9 cc. of medium; 1 cc. of tube No. 2 to tube No. 3 (with 9 cc. of medium), following the other directions as already given. Plate out as already explained, and watch developments.

In Petri dish No. 1 the number of bacterial growths (colonies) will no doubt be so great as to make counting impossible. Petri dish No. 2 may contain 360 colonies, and dish No. 3 may contain not more than 40. An average is obtained by repeating the test (using the same milk sample) a number of times. In the above milk sample the average may be 42,000 microbes per cc. If the bacterial content is high, it is necessary to extend the dilution four and even five times. Usually boiled distilled water is used with which to make desired dilutions (1–10, 1–100, 1–1,000, 1–10,000, etc).

If it is desired to determine the number of bacteria per gram of dry soil, it will be necessary to carefully weigh a small quantity (1 gm., more or less) of average soil, triturate the entire sample with say, 100 cc. of sterile distilled water, and from this make the dilution cultures as above described, using 1 cc. or less of the soil triturate. To compute the number of bacteria per gram of *dry* soil, it will now be necessary to determine the moisture percentage in a sample of soil taken from the same place as the sample which was used in making the triturate. The solution is simple. We will suppose the triturate sample weighed 0.856 gm. and the number of bacteria found was 3,000,000; and the percentage of moisture was 10. From these data it would be found that 1 gm. of dry soil will contain 3,855,011 microbes.

The above is sufficient to make clear how one might proceed to determine the number of microbes in and upon old pills, tablets, powders; on one ivory vaccine tip, in one glycerinated vaccine tube, in 1 cc. of bacterial vaccine, antitoxin, syrup, tincture, fluidextract, camphor water, distilled water, sewage, drinking water, etc. Naturally, great caution and care must be observed to avoid errors and faulty conclusions. In fact, no one should attempt such work in actual practice until after considerable preliminary laboratory experience.

It is not practicable nor is it necessary to give fuller information regarding bacterial cultures. We have not touched upon the various methods for determining whether or not the microbes under investigation are essentially aërobic or essentially anaërobic; the manner of determining the thermal death-point; relationship of rate of growth to temperature, etc. We have said nothing of the use of indicators added to culture media, as litmus, rosolic acid, and phenolphthalein, nor have we explained the special use of special culture media in determining the nature and identity of bacteria. These and many other details we must omit, merely stating that, should it become desirable to make such investigations, the necessary information must be secured elsewhere, as in some standard laboratory guide in bacteriological technic.

The following outline of special methods will serve as a guide in making bacteriological examinations of soils, air, pharmaceuticals, liquids, etc.

D. *Culturing Soil Bacteria.*—Soil is a mixture of dead and decayed organic matter, sand and living organisms and their spores. Near the surface the soil contains large numbers of bacteria, from 10,000 to 10,000,-000 per gram, and more. In fact the fertility of the soil is practically proportional to the number of bacteria present. Most species of soil bacteria are harmless to man though the bacilli of tetanus (lockjaw), of typhoid fever, of malignant edema, of anthrax, and of pus formation may be present. The tetanus germ is quite common in garden soils and the anthrax germ is apt to occur in cattle pens, pastures and other places frequented by cattle. Other soil bacteria are decidedly useful as will be more fully explained elsewhere.

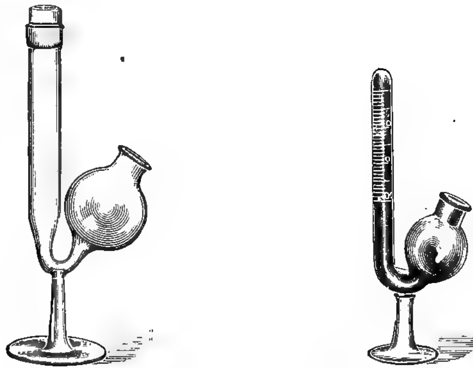


FIG. 37.—Graduated fermentation tube. These tubes are required for gas determination with colon bacillus and other gas-forming micro-organisms.

Some soil bacteria (the nitrifiers) do not grow on the usual media while others thrive exceedingly well in such media. Anaërobic forms must be cultured in the absence of air or oxygen.

The root nodule bacteria of the leguminosæ can be grown readily on gelatin or agar. The tubercles or nodules must be thoroughly cleansed and repeatedly washed in boiled distilled water, then rinsed for ten seconds in a 1–1000 corrosive sublimate solution, and finally thoroughly rinsed (three minutes) in boiled distilled water. Crush several of the sterilized nodules in a sterile watch crystal, by means of a sterile glass rod and from this make the dilution plate cultures and set aside at room temperature. Colonies of small motile bacteria (*Rhizobium mutabile*) will appear in about four days.

To test the soil bacterially, select thoroughly mixed samples and plate out as already suggested, using every precaution to prevent the introduction of extraneous germs. Cultures can also be made from internal plant tissues by following, in general, the directions given under root nodule

bacteria, excepting that after the washing and rinsing, the root, instead of being crushed, is cut or broken across and the inoculation material is taken from the inner tissue by means of a platinum needle or scalpel.

E. *Bacteria of the Air.*—Air currents carry the germ-laden dust and dirt particles. The number and kind of air bacteria depends upon environment, climatic conditions, moisture, sunlight, etc. The air currents are the main factors in germ dissemination. Spores and dry (though not dead) bacilli may be carried many miles. Air microbes are derived from the soil surface and from the objects surrounded by the air. Bacteria are exhaled with the breath (as in talking, sneezing, coughing) and are carried and distributed from and by animals, plants and clothing.

The air may carry organisms derived from the soil, from water and from other substances contaminated by organisms. The dirt and dust particles wafted about by air currents may have lodged upon them the germs of tuberculosis, the pus formers, the streptococcus group, rarely also the anthrax bacillus, the tetanus bacillus and the bacillus of malignant œdema; beside the spores of higher fungi, yeast cells and even the larvæ of intestinal parasites, etc.

Air microbes may be studied by exposing a Petri dish containing sterilized agar or gelatin, for two minutes or longer. The number of colonies that will appear will depend upon the locality, season, air moisture, etc. To determine the number of microbes in a given volume of air the Sedgwick-Tucker aërobioscope is used, though similarly constructed apparatus may be made by any fairly skillful student. The aërobioscope consists of a glass cylinder as shown in the illustration. The open ends are plugged with cotton. Granulated sugar is loosely packed into the narrow end and all is then sterilized in a hot-air sterilizer (not over 120° C.). Pass a given quantity of air through the aërobioscope by attaching an aspirator bottle to the narrow end and allowing a given volume of water to run out of the bottle. The volume of air drawn through equals the volume of water run from the bottle. Of course the cotton plug is removed from the larger end of tube while the water is running. The bacilli and spores are caught in the sugar, while the air passes through. Replace cotton plug and shake the sugar into the larger end of tube. Remove cotton plug again and pour in about 10 to 15 cc. of liquefied (40° C., not hot) gelatin. Roll the tube held horizontally. The gelatin dissolves the sugar and mixes with it. Roll on ice to hasten the hardening of the gelatin. Set aside in incubator, at room temperature (20° C., about). The number of colonies which appear indicates approximately the number of microbes in the volume of air aspirated. Let us suppose that the number of colonies was 125, the volume of air aspirated 10 liters, from which we would get 1250 bacteria per cubic meter of air.

F. *Bacteria of Liquid Substances.*—The bacteria of water, milk, tinctures, fluid extracts, aquæ, aërated waters, mineral waters, distilled water, broth, and liquids generally, can be studied quantitatively in a comparatively simple manner. By means of a sterile 1 cc. graduated pipette, run 0.1 cc. to 0.5 cc. of the liquid into the center of a sterilized petri dish, pour upon this enough (about 10 cc.) melted (sterile) agar or gelatin and mix by tilting the dish slightly from side to side. Set aside for the medium to harden and incubate at the room temperature, or at 37° C., as may be required. This method is satisfactory if the number of bacilli present is comparatively small. If very abundant, dilutions must be made in the manner already described.

The following general suggestions should be observed in making bacteriological determinations of liquids:

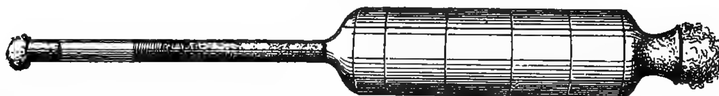


FIG. 38.—Aerobioscope after Sedgwiek-Tucker, plugged with cotton. The larger end in which the culturing is done is ruled to facilitate the counting of colonies.

a. Containers for samples (other than the original containers) must be sterile and closed with sterile corks or cotton plugs. If the samples are to be carried any distance they should be packed in ice. In no case is it wise to keep a sample longer than forty-eight hours before culturing it. If the sample is to be examined within two or three hours after collecting it, placing on ice is not absolutely necessary.

b. Every sample should be thoroughly mixed before making cultures. Shake well, about twenty times. This is very important.

c. All glassware, pipettes, etc., must be thoroughly sterilized by washing, rinsing, wiping, hot air or steam sterilization, etc.

d. The methods of making dilutions, the amounts to be planted or tubed, the culture media to be used, etc., etc., cannot be given in detail in a work of this kind. All will depend upon the kind of analysis to be made, the results to be attained, etc. The special methods to be employed in special cases must be looked up in suitable text-books and carefully followed. The following general suggestions are in order at this time.

e. Thus far there are no standards for the bacteriological testing of pharmaceuticals. Tinctures and fluidextracts should show only few colonies per cc., not over 30 to 60. Sera should show none. Well prepared and properly ripened small-pox vaccine should show only a few colonies, from 20 to 500, per ivory point or per tube.

f. The colon bacillus should not be present in specified volumes of drinking water, of milk and pharmaceuticals. If present in such volumes, it indicates excessive sewage or other objectionable contamination. The colon bacillus is motile in young broth cultures, forms no spores, is gas- (dextrose broth cultures in fermentation tube) and indol-forming, reduces nitrates to nitrites, does not liquefy gelatin and is not stained by Gram's method.

g. Syrups of all kinds, unless very carefully prepared and carefully kept to prevent fermentation, are apt to show numerous bacteria, yeasts and molds. Any syrup showing signs of yeast fermentation (gas bubbles, vinous odor) or moldiness, it not fit for use and should be rejected. The attempt to render it usable by boiling, is unsatisfactory, furthermore the changes produced by the organisms are always objectionable and cannot be rectified by heating or by other methods of sterilization.

h. Recent investigations have shown that many of the marketed (bottled) mineral waters contain numerous bacteria, from 10,000 to 300,000,000 and more per cc. In some cases colon bacilli have been found. These findings prove that in many instances the methods of bottling must be careless or otherwise unsatisfactory. Undoubtedly the contamination is in some instances due to reused and inadequately cleaned and sterilized containers and in other instances to impure and inadequately sterilized mineral water. A popular opinion prevails that the chemicals in the mineral waters are sufficiently germicidal to destroy bacteria but this is not the case.

G. *Bacteria in Canned Fruits.*—The work recently demanded by the pure food laws (federal and state) has shown that such food substances as canned fruits of all kinds, including jams, jellies, preserves, catsups, tomato pastes, etc., are frequently highly contaminated with yeast cells, molds and their spores, and other higher fungi, and bacteria. It is, however evident that the food products named may be kept quite free from such contamination as may be seen from the examination of canned food products prepared by the careful housewife. That manufacturers may approximate the home condition is demonstrated by the fact that factory products are found on the market, which are quite free from contamination.

Since wholesome ripe fruit contains yeast cells, bacteria and mold in very small numbers only, and since most of these organisms are removed in the various steps of the processing, as washing, peeling, steaming, etc., it is evident that the finished factory product should, like the home-made product, contain these organisms in negligibly small numbers only, provided, of course, that wholesome fruit is used. However, most of the factory samples thus far examined have shown numerous dead yeast cells,

mould spores, mould hyphæ, and bacteria, indicating the use of fruit, fruit pulp, fruit juices, fruit refuse, etc., which was decomposed or undergoing fermentation or decomposition prior to or at the time of manufacture. The organisms named prevail in varying amounts in different products. Yeast organisms are apt to predominate in jellies, fruit juices and fruit

FIG. 39.—Thoma-Zeiss hemacytometer. Complete equipment for blood counting. This is very convenient for making bacterial counts in catsups, jams, jellies and other vegetable foods and also in animal food substances.

pulp; bacteria in catsups and pastes; and molds in certain fruits as strawberries, blackberries and raspberries.

The presence of numerous dead yeast cells (1,000,000 to 50,000,000 per cc.) is evidence that the material was undergoing alcoholic fermentation

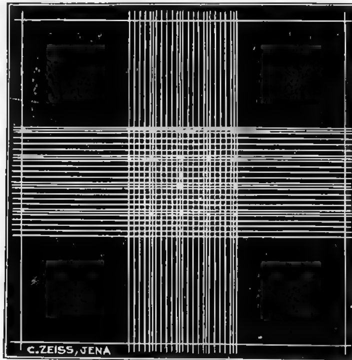


FIG. 40.—Zappert ruling of the Thoma-Zeiss hemacytometer. This form of ruling is especially convenient for making bacterial counts and counts of fat globules in milk.—(*Carl Zeiss.*)

just prior to or at the time of manufacture. Tomato pastes have been found on the market showing over 35,000,000,000 bacteria per cc. besides numerous yeast cells and considerable mold. The bacterial content of catsups is apt to run high, from 50,000,000 to 500,000,000 and more per cc. Not including the vinegar bacteria, which are introduced into catsups and

pastes, such high bacterial content is generally due to bacterial development during or after manufacture. The presence of mold organisms and their spores (other than Penicillium) indicates the use of mold-infested fruit. Penicillium, which is entirely saprophytic in habit, may develop after manufacture, particularly on the surface of inadequately sterilized fruit products in containers not entirely filled.

"Swelling" of cans containing fruit products is generally due to yeast development, though it may also be due to bacterial activity, and indicates inadequate sterilization of either the container or of the fruit or both. Examination will show the presence of living yeast cells, or bacteria, perhaps air bubbles, and the characteristic vinous odor of yeast may be noted.

Based upon such conditions as can be made to prevail in carefully operated factories, the following may be given as the limits of the number of organisms permissible in the fruit products under discussion.

a. Yeast cells, either living or dead, not to exceed 1,000,000 per cc.

b. Mold spores not to exceed 1,000,000 per cc.

c. Hyphal clusters and hyphal fragments not to exceed 10,000 per cc.; or not over 25 per cent. of separate and distinct fields of view under the compound microscope should show hyphal clusters or hyphal fragments.

d. Bacteria (either living or dead but not including vinegar bacteria in products to which vinegar is added)not to exceed 25,000,000 per cc.

The above figures apply only to fruit products supposedly made from comparatively fresh fruits and fresh fruit juices. The yeast, bacterial and spore counts are made with a Thoma-Zeiss hemacytometer (Turck ruling) using a No. 5 (1/5 in.) objective with No. 2 (1 in.) ocular.

H. *Quantitative and Qualitative Bacteriological Testing.*—The following will serve as a general outline of bacteriological analyses which may be made in food and drug laboratories. The substances which require such bacteriological examination include catsups, tomato pastes, vinegars, water supplies, mineral waters, milk, ice creams, any and all substances which are suspected to be sewage contaminated, etc., etc.

The sequence of processes here given bear a progressive relationship. Whether process II is carried out will depend upon the findings under I and whether III shall be undertaken will depend upon the findings under II. The essential facts to be ascertained are whether or not there is possible sewage contamination as indicated by the presence of the colon bacillus, sewage streptococci and possibly the typhoid bacillus. The typhoid agglutinating tests are apt to prove unsatisfactory. In most instances this test will be unnecessary as the presence of the colon bacillus is evidence that the food, drug or drink is excessively contaminated with sewage and is hence unfit for human use.

I. *Direct Count.*—For this purpose the Thoma-Zeiss hemacytometer

with Turck ruling[1] is used (No. 2 ocular with 1/5 in. objective) which can be secured from any bacteriological supply house. The instructions for using it can be obtained from the dealer, though the measuring values indicated on the hemacytometer are sufficient to indicate the manner of making the counts. The rulings generally used for bacterial countings are 1/400 sq. mm. × 1/10 mm. deep, making an area of 1/4000 cu. mm., or reduced to decimal fractions, 0.04 sq. mm. × 0.1 mm. deep = 0.004 cu. mm. We will suppose that the average of 20 counts shows 5 bacilli, then 1 cu. mm. would contain 20,000 bacilli or 20,000,000 in 1 cc.

The direct count is, in many instances, very unsatisfactory for several reasons. Particles other than micro-organisms may be mistaken for bacilli or cocci and, furthermore, it cannot be known for a certainty that the organisms are dead or alive. If they are present in great abundance (10,000,000 to 100,000,000 and more per cc.), ordinary smear preparations may be stained, using methyl blue or Hoffmann's violet. Dead bacilli, that is those which have been dead for some time, do not take the stain well, due to the fact that the cellplasm is disintegrated.

Tomato pastes, anchovy pastes, catsups, some mineral waters and imilar preparations, may contain bacteria in such numbers that dilutions are desirable or necessary to make counting possible. Dilutions of 1–10, 1–100 will, as a rule, be sufficient. Weigh or measure one part (1 gm. or 1 cc.) of the substance, add it to 9 or 99 parts filtered distilled water and mix thoroughly by shaking.

If the direct count shows bacilli in great numbers or if for any reason sewage contamination is suspected, and also to determine the number of living bacilli and spores suspected, proceed as follows:

II. *Plate Culture Counts.*—Make one set of plate cultures, using lactose litmus agar,[2] and incubate at 20° C. Make a second set of plate cultures, also upon lactose litmus agar, and incubate at 38° C. The usual dilution methods are followed when necessary, using preferably 0.1 cc. quantities for the plates. This temperature differential test is considered of great importance. Colon bacilli and other micro-organisms, whose natural habitat is the intestinal canal, will develop actively at the higher temperature (38° C.), whereas the usual air, soil and water bacteria develop best at the lower temperature (20° C.). If the high temperature colonies approximate the low temperature colonies, sewage contamination may be suspected. If in addition many of the high temperature lactose litmus agar colonies show pink or light vermilion, the sewage contamination is practically proven. The colon bacillus, as well as sewage streptococci,

[1] To render the ruled lines visible rub a very soft pencil over the ruled area.

[2] Add 1 per cent. of lactose to the usual agar medium and enough tincture of litmus to give it a lilac tinge.

give pink colonies, the latter being the brighter, more vermilion in coloration, due to the formation of acid (in the fermenting lactose). Examine the pink colonies under the microscope. The colon microbe is rod-shaped, rather thick, non-sporing, and shows motility in recent broth cultures, whereas the streptococci are smaller and are not rod-shaped. High temperature colonies as compared with low temperature colonies should not exceed 1:100 or 1:25. If the proportion is 1:4 or less, sewage contamination is very likely. After 36 hours the pink colonies may turn blue, due to the development of ammonia and amines.

Naturally the high temperature colonies must be studied within twenty-four to thirty hours whereas the low temperature cultures require much more time, two to four days.

If the temperature and color differential tests indicate sewage contamination, then the following additional tests should be carried out.

III. *Indol Reaction and Gas Formula.*—The indol reaction has already been explained. The gas formula is determined as follows: To sets of four graduated fermentation tubes containing glucose bouillon and lactose bouillon, add 0.1, 0.2, 0.5, and 10 cc. of the suspected liquid and incubate at 38° for 48 hours. If gas formation is observed the presence of colon bacilli may be suspected. If the 0.1 cc. tubes show gas formation then the presence of colon bacilli may be assumed. Fill the bulb of a tube, showing gas formation, with a 2-per cent. solution of sodic hydrate, hold thumb tightly over the opening and mix contents by tilting back and forth carefully. The portion of gas absorbed is CO_2 whereas the unabsorbed portion is supposedly hydrogen. The colon bacillus shows a gas formation of 1/3 hydrogen. Of course the total volume of gas is recorded before the sodic hydrate is added.

The gas formula with a positive indol reaction is practically conclusive as far as the presence of the colon bacillus is concerned. Add to this the other tests and we have conclusive evidence of sewage contamination.

The colon bacillus, the bacilli of the hog cholera group and others, have the power of reducing neutral red; producing a greenish-yellow fluorescence. For this reaction use glucose bouillon to which has been added 1 per cent. of a 0.5 per cent. solution of neutral red. In examining milk, the pus cell and leucocyte count is considered important; centrifugalize 10 cc. of milk for five minutes, pour off supernatant milk and mix sediment with 0.5 cc. normal salt solution and make counts of pus cells and leucocytes per cc. from the amount (0.5 cc.). Abundant pus cells and leucocytes indicate abscess or other pathological condition of milk ducts or glands. This test is, however, of little significance excepting in the hands of authorities on diseases of cows. It is stated that as many as 100,000 leucocytes per cc. may occur in apparently healthy animals.

Gelatin-liquefying organisms may be looked upon with suspicion when found in milk, water and other liquid-food substances intended for human consumption, as has already been explained.

It should be borne in mind that the colon bacillus is one of a group of some fifteen or more species and varieties of closely related micro-organisms which resemble each other in the following particulars:

1. Do not form spores.
2. Do not liquefy gelatin.
3. Produce acid in milk and cause milk coagulation.
4. Produce acid and gas in glucose and lactose media.
5. Produce acid and gas in bile-salt-glucose broth.
6. Grow well at temperatures ranging from 38° to 42° C.

In differentiating the colon bacillus, remember that this organism is rod-shaped (2 to 3μ long by 0.5 to 0.6μ wide), is motile, produces indol, gives rise to pink colonies on lactose (or glucose) litmus agar and reduces neutral red glucose (or lactose) agar with a greenish-yellow fluorescence.

It should also be remembered that sewage is a highly complex substance and contains micro-organisms in great variety and in great abundance. Among the organisms present are species of *Spirillum*, *Vibrio*, *Proteus* and *Beggiatoa* in addition to the bacilli and streptococci already mentioned. The typhoid bacillus does not thrive well in sewage. The number of bacteria present in crude or ordinary sewage (domestic, city, hospital, mixed, etc.) ranges from 1,000,000 to 100,000,000 and more per cc. The work of these organisms is to break down and render soluble and assimilable (for plants) the organic matter composing the sewage, thus assisting the work of rotting bacteria generally.

The following is a tabulation of the bacteriological testing that should be made of foods (including pastes, catsups, milk, ice creams, water supplies, mineral waters, alcoholic beverages, etc.) that may show an excess of bacterial growth or which may be sewage contaminated:

BACTERIOLOGICAL EXAMINATION

I. Direct Count.

   1. Bacilli per cc. . . . .
   2. Cocci, per cc. . . . .

II. Plate and Tube Cultures. (Lactose-litmus-agar.)

   1. Temperature differential test.
      a. (20° C.) Colonies per cc. . . . .
      b. (38° C.) Colonies per cc. . . . .
   2. Color differential test.
      a. Pink colonies per cc . . . .
      c. Not pink colonies per cc . . . .

3. Colorless gelatin liquefying colonies per cc.
4. Neutral red reduction, + or −.
5. Indol reaction, + or −.
6. Gram stain behavior, + or −.
7. Gas (hydrogen) formula.

III. Agglutinating tests for Typhoid Germs.

8. Staining Bacteria

Staining consists of the infiltration of the cell-substance with solutions of various coloring materials obtained for the most part from the group of coal-tar derivatives known as the aniline dyes. As is generally known, different cells and different portions of one and the same cell react differently with the various dyes used. This peculiar behavior brings out contrasts in appearances which aid very materially in determining the morphological characters. The prime object, therefore, in using stains is to aid in the study of cell morphology. Different bacteria react differently with the several stains used. Some species take certain stains very readily, while they are quite indifferent to other stains. The vegetative cell stains much more readily than do the spores. In fact, spores are stained with great difficulty; however, after they are once thoroughly stained they hold the color persistently.

The dyes which may be used in bacteriologic work are of many kinds, differing as to color and as to staining powers with different cells, cell-contents, and cell-parts. They are usually classified as acid or basic. Eosin, acid fuchsin, and picric acid are acid stains, and are said to be diffuse in their effects, having no special affinity for any special cell structure. fuchsin, methylene blue, and gentian violet are basic, and appear to have special attraction for bacteria and for plasmic and nuclear substances of cells generally, for which reasons they are most generally employed as bacterial stains. Fuchsin is, in fact, about the only efficient stain for endospores, while gentian violet and methylene blue are excellent stains for the bacterial cell-wall.

It is known that certain substances possess the property of preparing the bacterial cells in such a way as to induce them to take up the dye more readily, thus intensifying the stain, as aniline oil and carbolic acid. Such substances are called mordants, and may be used separately or added directly to the stain itself.

Certain liquids or solutions remove the stain from the bacterial cell more or less readily, as water and alcohol, but more particularly solutions of acids. Such substances are quite generally employed for removing any excess of stain from the bacterial cell or from the matrix in which the bac-

teria are fixed or embedded. Acidulated (with HCl) alcohol is most commonly employed. Ordinarily, rinsing in a small stream of water is sufficient. Some bacteria resist the decolorizing process with acids more strongly than others, and are said to be acid fast or acid proof, as, for example, the bacilli of leprosy and of tuberculosis, while the great majority of species give up the stain very readily. It is a fact that one and the same species of microbe reacts variably with one and the same stain, depending upon a variety of causes. Moderate heat hastens and intensifies the staining.

For ordinary purposes a single stain only is used, but sometimes structural differences are more clearly shown by what is known as double or contrast staining. Take, for example, a spore-bearing microbe, as that of anthrax. The spores may be stained by means of carbol fuchsin; the entire cell, excepting the spore, can be completely decolorized in acidulated alcohol, and then methylene blue or gentian violet applied as the contrast stain. We then have a blue cell-wall with a red spore. However, the beginner is apt to be disappointed in his attempts at double staining.

The pharmacist will have comparatively little to do as far as the actual staining of bacteria is concerned. He should, however, be able to prepare the more important stains, mordants, and other solutions which may be required by the city or health board bacteriologist or the physician, and we shall therefore give the more commonly employed preparations.

A. *Stock Solutions.*—Make saturated solutions of the basic dyes (fuchsin, gentian violet, and methylene blue) in 95 per cent. alcohol. Keep these in glass-stoppered bottles in a cool, dark place, ready for use in preparing the stains. The stock solutions should in all instances be filtered before using. Secure the dyes from reliable dealers and in small quantities. Do not make up large quantities of stock solutions or stains proper, as they gradually deteriorate, particularly if exposed to light.

B. *Mordants.*—The principal substances used are aniline, carbolic acid, tannic acid, glacial acetic acid, ferrous sulphate, sodium hydroxide solution, chromic acid, and a few others. Those in general use are the two first named. The others have a more limited use in special cases.

1. *Aniline Water*

Aniline,	2 cc.
Distilled Water,	98 cc.

Shake frequently, and finally filter several times through filter paper. It should be perfectly clear. This preparation deteriorates rapidly. Make up small amounts and keep in a dark place. It becomes worthless, even when observing all precautions, in a few weeks.

2. Carbolic Acid Solution

Carbolic Acid,	20 cc.
Distilled Water,	100 cc.

Filter. This mordant is rarely used by itself.

C. *Stains.*—We give here the more important stains, approximately in the order of preferred use.

1. Loeffler's Methylene Blue

Stock Solution (saturated) Methylene Blue,	30 cc.
1:10,000 Sol. KHO in Dist. Water,	100 cc.

Mix, shake filter. This stain is much used as a general bacterial stain and in the examination of blood, pus, etc.

2. Aniline Gentian-Violet

Aniline Water,	75 cc.
Stock Solution Gentian-Violet,	25 cc.

Mix, shake, filter. This is an excellent bacterial stain.

3. Carbol-Fuchsin

Stock Solution of Basic Fuchsin,	10 cc.
5 per cent. Sol. Carbolic Acid,	100 cc.

Mix, shake, filter. This is one of the most useful stains with the so-called acid-proof microbes. It is also a spore stain, and is the most commonly employed stain used in contrast or double staining. It is a comparatively slow stain, but is quite permanent.

4. Gram's Stain

Gram's stain is used for diagnostic purposes, and is perhaps the best known stain in the entire field of bacteriological technic. Its value depends upon the fact that certain microbes, when stained and afterward treated with a solution of iodine and washed in alcohol, give up the stain. Such microbes are known as Gram-negative, whereas those which do not give up the stain are said to be Gram-positive.

The method of using this stain is somewhat complicated, requires care, and, with a beginner, often yields disappointing results. Keeping in mind the following will minimize the disappointments:

a. Long-continued (one year or more) subcultures frequently lose the Gram-stain behavior.

b. Old cultures, that is, those which have been growing in the same medium for several days or more, as a rule do not stain characteristically. With such cultures the results are often neither negative nor positive, just enough to be confusing and perplexing.

c. The solutions used must be fresh. The gentian-aniline solution, as well as the iodine solution, deteriorates quite rapidly.

d. Do not overstain, and do not decolorize too long. Stop decolorizing as soon as no more violet color comes away.

In the Gram method two solutions are used, namely:

1. Aniline gentian-violet, and
2. Gram's iodine solution.

Iodine,	1 gm.
Potassium Iodide,	2 gm.
Distilled Water,	300 cc.

The method, briefly outlined, is as follows:

a. Spread the bacteria evenly and thinly over the cover-glass (the usual smear preparation). Stain with the aniline gentian-violet for from two to five minutes. Warming will hasten and intensify the staining. Wash in water to remove excess of stain.

b. Drop on the iodine solution and allow it to act for about one minute or until the preparation assumes a coffee-brown color. It may be desirable to apply the iodine a second time.

c. Wash off the excess of iodine in water and then decolorize by dropping on 95 per cent. alcohol. Tip the slide and allow alcohol to run over the preparation; continue until the violet color ceases to stream away.

d. Finally rinse in water and examine in water. If desired, dry and mount permanently in Canada balsam or some other suitable mounting medium.

e. A contrast stain, such as eosin, fuchsin, safranin, or Bismarck brown, may be used, following (c).

Keeping in mind the difficulties already referred to in using the Gram method, and the additional possible source of error due to the fact that one and the same microbe will stain but feebly at one time and very intensely at another time, we now name the principal organisms which are Gram-positive or Gram-negative.

Bacteria and other Organisms Stained by the Gram Methcd

Staphylococcus pyogenes aureus.
Staphylococcus pyogenes albus.
Streptococcus pyogenes.
Micrococcus tetragenus.
Micrococcus lanceolatus.
Bacillus diphtheriæ.
Bacillus tuberculosis.
Bacillus of anthrax.
Bacillus of tetanus.
Bacillus of leprosy.
Bacillus aërogenes capsulatus.
Oidium albicans.
Actinomyces (of Actinomycosis).

Bacteria not Stained by the Gram Method

Diplococcus of meningitis (intracellular).
Diplococcus of gonorrhea.
Micrococcus melitensis.
Bacillus of chancroids (Ducrey's).
Bacillus of dysentery (Shiga's).
Bacillus of typhoid fever.
Bacillus of bubonic plague.
Bacillus of influenza.
Bacillus coli communis.
Bacillus pyocyaneus.
Bacillus of Friedlander.
Bacillus proteus.
Bacillus mallei (glanders).
Spirillum of Asiatic cholera.
Spirillum of relapsing fever.

5. *Pappenheim's Stain*

Sat. Aqueous Sol. Methyl Green,	50 cc.
Sat. Aqueous Sol. Pyronine,	15 cc.

Mix and filter. This is much used for staining bacteria in pus and other pathological secretions. The bacteria are stained a bright red, while the cell nuclei are blue to purple.

6. *Smith's Stain*

Stock Sol. Basic Fuchsin,		10 cc.
Methyl Alcohol,		
Formaldehyde,	each	10 cc.
Distilled Water,	to make	100 cc.

Mix and filter. Let stand for twenty-four hours before using. Renew in three weeks. The stain is much used to distinguish between bacteria and nuclear substances. Allow the stain to act for from two to ten minutes.

7. *Flagella Staining*

Care is necessary in staining flagellæ. Numerous methods have been recommended, but Pitfield's method, as modified by Muir, is perhaps the best and at the same time comparatively simple. The following solutions are required:

a. Mordant

Tannic Acid (10 per cent. Aq. Sol.),	10 cc.
Sat. Aq. Sol. Mercuric Chlor.,	5 cc.
Sat. Aq. Sol. Alum,	5 cc.
Carbol-Fuchsin,	5 cc.

Mix, shake, filter or centrifuge. This soluton does not keep longer than one week.

b. Stain

Sat. Aq. Sol. Alum,	10 cc.
Stock Sol. Gentian-Violet,	2 cc,

Mix, filter. Carbol-fuchsin may be used instead of gentian-violet. This stain will not keep longer than a few days.

The method is as follows:

1. Drop on mordant. Leave for one minute, with gentle heat.
2. Rinse in water for two minutes.
3. Dry carefully at slight warmth.
4. Stain for one minute with gentle heat.
5. Wash, dry, and mount in Canada balsam.

In making the cover-glass preparation, take a loopful from a young aqueous subculture of some motile bacillus and touch it on the carefully cleaned cover and allow the drop to spread by rotating and tilting the cover. Do not use the loop more than is necessary. Flagellæ are very delicate and easily destroyed. Dry very carefully, and do not pass through flame more than three times.

8. *Spore Staining*

As already stated, spores (endospores) of microbes stain with great difficulty, for which reason a contrast is effected negatively; that is, the rest of the cell is quickly stained, leaving the unstained, highly refractive spore to appear like a bit of glass within the colored frame. This is in many ways the most satisfactory way of demonstrating the presence of spores. The spores may, however, be stained by the usual acid-fast or acid-proof methods, care being observed in decolorizing. Stain with hot carbol-fuchsin for a few minutes, wash, and decolorize quickly with 3 per cent. hydrochloric acid in 95 per cent. alcohol, and then use a contrast stain, as gentian-violet or methylene blue. The red spores will then appear in the violet or blue frame.

9. *Capsule Staining*

The gelatinous capsule of microbes is also stained with great difficulty, and requires special methods and experience to yield anything like satisfactory results. The methods of Welch and Hiss are quite satisfactory. The capsule is, however, generally visible without any staining because of the light contrast that naturally exists. Certain substances, as glacial acetic acid (Welch method), cause the capsule to enlarge and take up the stain more readily. Certain staining methods bring out the capsule of certain microbes, as, for example, the Gram method as applied to pneumonia sputum.

The Muir method is perhaps the best for capsule staining. It is as follows:

1. Stain in carbol-fuchsin for one-half minute, with gentle heat.
2. Wash lightly in alcohol (95 per cent.).
3. Wash well in water.
4. Flood with mordant of

Sat. Aq. Sol. Mercuric Chlor.,	2 cc.
Tannic Acid (20 per cent. Aq. Sol.),	2 cc.
Sat. Aq. Sol. Potassium Alum,	5 cc.

5. Wash in water.
6. Wash in 95 per cent. alcohol, one minute.
7. Wash in water.
8. Stain with methylene blue for one-half minute.
9. Decolorize somewhat and let dry.
10. Clear in xylene, and mount in Canada balsam.

There are numerous other special stains and special staining methods, which need not be mentioned here. Should the pharmacist be called upon to prepare any of these, he will find full particulars in any standard work on medical bacteriology.

9. Studying Bacteria

The complete study of any one species of microbe with a view to determining its identity is a long and tedious process. It involves a study of the organism in its natural element and in artificial culture media, and its behavior in animal inoculation tests, etc. Special apparatus, experimental animals (as rats, mice, guinea-pigs, dogs, etc.), and technical experience and skill are necessary. Just what kind of observations are involved in such study is indicated in the complete method as outlined by the Society of American Bacteriologists (Jan., 1908), which is hereby submitted for the benefit of those who may wish to acquaint themselves with such details. The glossary of terms should be carefully considered first of all. The decimal system for indicating groups relationships of microbes (Table I) is most unique and is very convenient for active workers. Those interested will find the desired explanations of the methods and reagents mentioned, in any of the larger works on medical bacteriology and on bacteriological technology. It is not at all likely that the pharmacist will ever have occasion to make use of the special methods cited. He should nevertheless acquaint himself with them sufficiently to comprehend their application in the study of pathogenic bacteria.

Our bacteria nomenclature is in some confusion, and unless the methods of naming bacteria are corrected, the confusion is certain to become much greater. The trouble lies in the failure to define group or generic delimita-

tions. The present generic terms, "bacillus" and "micrococcus," include too many species. We have a confusing and almost incomprehensible array of synonyms, of which those applied to *Rhizobium mutabile* may serve as an example. The different names that have been given to this organism may be arranged as follows:

Pasteuraceæ, Laurent.
Bacteria, Woronin, 1866.
Bakteroiden, Brunchorst and Frank, 1885.
Microsymbiont, Atkinson, 1893.
Spores or gemmules, Ward and Ericksson.
Bacillus radicicola, Beyerinck, 1888.
Cladochytrium leguminosarum, Vuellemin.
Phytomyxa leguminosarum, Schroeter.
Schinzia leguminosarum, Woronin.
Rhizobium leguminosarum, Frank, 1890
Rhizobium Frankii, (in part) Schneider, 1892.
Rhizobium mutabile, Schneider, 1902.
Pseudomonas radicicola, Moore, 1905.
Rhizobium leguminosarum. The Com. 1917.

The above synonomy is also interesting because it indicates a most remarkable difference of opinion regarding the nature and identity of this root-nodule organism. Further, as the result of the wholly inadequate group delimitations we have such name-monstrosities as *Granulobacillus saccharobutyricus mobilis nonliquifaciens*, and *Micrococcus acidi paralactici liquifaciens Halensi*. Reform in nomenclature is very desirable, and it must come through a careful definition of generic groups based on physiological characters, rather than upon largely morphological characters, as is done now.

It is advised that the pharmacist refrain from experimenting with pathogenic organisms, excepting in so far as he may act in coöperation with practicing physician or health officers. When experimenting with pathogenic organisms the greatest caution is necessary to guard against autoinoculation and the spreading of disease. It should be made a rule to treat every microbe studied as though it were virulently pathogenic, capable of spreading an epidemic. Never expose a colony (plate culture, tube culture, etc.) in such a way as to permit the escape of the organisms into the air. Pour a disinfecting solution (5 per cent. carbolic acid) into cultures that are to be discontinued and then boil container and all, for thirty minutes, before washing and cleaning the glassware. Never forget to sterilize the platinum needle *before* and *after* making an inoculation or a culture transfer.

DESCRIPTIVE CHART—SOCIETY OF AMERICAN BACTERIOLOGISTS[1]

Glossary of Terms

Agar Hanging Block, a small block of nutrient agar cut from a poured plate, and placed on a cover-glass, the surface next the glass having been first touched with a loop from a young fluid culture or with a dilution from the same. It is examined upside down, the same as a hanging rock.

Ameboid, assuming various shapes like an ameba.

Amorphous, without visible differentiation in structure.

Arborescent, a branched, tree-like growth.

Beaded, in stab or stroke, disjointed or semi-confluent colonies along the line of inoculation.

Brief, a few days, a week.

Brittle, growth dry, friable under the platinum needle.

Bullate, growth rising in convex prominences, like a blistered surface.

Butyrous, growth of a butter-like consistency.

Chains, short chains, composed of 2 to 8 elements. Long chains, composed of more than 8 elements.

Ciliate, having fine hair-like extensions, like cilia.

Cloudy, said of fluid cultures which do not contain pseudozooglea.

Coagulation, the separation of casein from whey in milk. This may take place quickly or slowly, and as the result either of the formation of an acid or of a lab ferment.

Contoured, an irregular, smoothly, undulating surface, like that of a relief map.

Convex, surface the segment of a circle, but flattened.

Coprophyl, dung bacteria.

Coriaceous, growth tough, leathery, not yielding to the platinum needle.

Crateriform, round, depressed, due to the liquefaction of the medium.

Cretaceous, growth opaque and white, chalky.

Curled, composed of parallel chains in wavy strands, as in anthrax colonies.

Diastasic action, same as *Diastatic*, conversion of starch into water-soluble substances by diastase.

Echinulate, in agar stroke a growth along the line of inoculation, with toothed or pointed margins; in sag cultures growth beset with pointed outgrowths.

Effuse, growth thin, veily, unusually spreading.

Entire, smooth, having a margin destitute of teeth or notches.

Erose, border irregularly toothed.

Filamentous, growth composed of long, irregularly placed or interwoven filaments.

Filiform, in stroke or stab cultures a uniform growth along line of inoculation.

Fimbriate, border fringed with slender processes, larger than filaments.

Floccose, growth composed of short curved chains, variously oriented.

Flocculent, said of fluids which contain pseudozooglea, *i.e.*, small adherent masses of bacteria of various shapes and floating in the culture fluid.

Fluorescent, having one color by transmitted light and another by reflected light.

Gram's Stain, a method of differential bleaching after gentian-violet, methyl-violet, etc. The + mark is to be given only when the bacteria are deep blue or remain blue after counterstaining with Bismarck brown.

Grumose, clotted.

[1] Prepared by F. D. Chester, F. P. Gorham, Erwin F. Smith, Committee on Methods of Identification of Bacterial Species. Endorsed by the Society for general use at the annual meeting, January, 1908.

Infundibuliform, form of a funnel or inverted cone.
Iridescent, like mother-of-pearl. The effect of very thin films.
Lacerate, having the margin cut into irregular segments as if torn.
Lobate, border deeply undulate, producing lobes (see *Undulate*).
Long, many weeks or months.
Maximum Temperature, temperature above which growth does not take place.
Medium, several weeks.
Membranous, growth thin, coherent, like a membrane.
Minimum Temperature, temperature below which growth does not take place.
Mycelioid, colonies having the radiately filamentous appearance of mould colonies.
Napiform, liquefaction with the form of a turnip.
Nitrogen Requirements, the necessary nitrogenous food. This is determined by adding to *nitrogen-free* media the nitrogen compound to be tested.
Opalescent, resembling the color of an opal.
Optimum Temperature, temperature at which growth is most rapid.
Pellicle, in fluid bacterial growth either forming a continuous or an interrupted sheet over the fluid.
Peptonized, said of curds dissolved by trypsin.
Persistent, many weeks, or months.
Pseudozoogleæ, clumps of bacteria, not dissolving readily in water, arising from imperfect separation, or more or less fusion of the components, but not having the degree of compactness and gelatinization seen in zooglea.
Pulvinate, in the form of a cushion, decidely convex.
Punctiform, very minute colonies, at the limit of natural vision.
Rapid, developing in twenty-four to forty-eight hours.
Raised, growth thick, with abrupt or terraced edges.
Repand, wrinkled.
Rhizoid, growth of an irregular branched or root-like character, as in *B. mycoides*.
Ring, same as *Rim*, growth at the upper margin of a liquid culture, adhering more or less closely to the glass.
Saccate, liquefaction the shape of an elongated sac, tubular, cylindrical.
Scum, floating islands of bacteria, an interrupted pellicle or bacterial membrane.
Slow, requiring five or six days or more for development.
Short, applied to time, a few days, a week.
Sporangia, cells containing endospores.
Spreading, growth extending much beyond the line of inoculation, *i.e.*, several millimeters or more.
Stratiform, liquefying to the walls of the tube at the top and then proceeding downward horizontally.
Thermal Death-point, the degree of heat required to kill young fluid cultures of an organism exposed for ten minutes (in thin-walled test-tubes of a diameter not exceeding 20 mm.) in the thermal water-bath. The water must be kept agitated so that the temperature shall be uniform during the exposure.
Transient, a few days.
Turbid, cloudy with flocculent particles; cloudy flocculence.
Umbonate, having a button-like, raised center.
Undulate, border wavy with shallow sinuses.
Verrucose, growth wart-like, with wart-like prominences.
Vermiform-contoured, growth like a mass of worms, or intestinal coils.
Villous, growth beset with hair-like extensions.

Viscid, growth follows the needle when touched and withdrawn, sediment on shaking rises as a coherent swirl.

Zoogleæ, firm gelatinous masses of bacteria, one of the most typical examples of which is the *Streptococcus mesenteroides* of sugar vats (*Leuconostoc mesenterioides*), the bacterial chains being surrounded by an enormously thickened firm covering inside of which there may be one or many groups of the bacteria.

Notes

(1) For decimal system of group numbers see Table 1. This will be found useful as a quick method of showing close relationships inside the genus, but is not a sufficient characterization of any organism.

(2) The morphological characters shall be determined and described from growths obtained upon at least one solid medium (nutrient agar) and in at least one liquid medium (nutrient broth). Growths at 37° C. shall be in general not older than twenty-four to forty-eight hours, and growths at 20° C. not older than forty-eight to seventy-two hours. To secure uniformity in cultures, in all cases preliminary cultivation shall be practised as described in the revised Report of the Committee on Standard Methods of the Laboratory Section of the American Public Health Association, 1905.

(3) The observation of cultural and bio-chemical features shall cover a period of at least fifteen days and frequentlylonger, and shall be made according to the revised Standard Methods above referred to. All media shall be made according to the same Standard Methods.

(4) Gelatin stab cultures shall be held for six weeks to determine liquefaction.

(5) Ammonia and indol tests shall be made at end of tenth day, nitrate tests at end of fifth day.

(6) Titrate with $\frac{N}{20}$ NaOH, using phenolphthalein as an indicator: make titrations at same time from blank. The difference gives the amount of acid produced.

The titrations should be done after boiling to drive of any CO_2 present in the culture.

(7) Generic nomenclature shall begin with the year 1872 (Cohen's first important paper).

Species nomenclature shall begin with the year 1880 (Koch's discovery of the poured plate method for the separation of organisms).

(8) Chromogenesis shall be recorded in standard color terms.

TABLE 1

A Numerical System of Recording the Salient Characters of an Organism (Group Number)

100..............Endospores produced.
200..............Endospores not produced.
10..............Aërobic (Strict).
20..............Facultative anaërobic.
30..............Anaërobic (Strict).
1..............Gelatin liquefied.
2..............Gelatin not liquefied.
0.1..............Acid and gas from dextrose.
0.2..............Acid without gas from dextrose.
0.3..............No acid from dextrose.

0.4............No growth with dextrose.
.01...........Acid and gas from lactose.
.02...........Acid without gas from lactose.
.03...........No acid from lactose.
.04...........No growth with lactose.
.001..........Acid and gas from saccharose.
.002..........Acid without gas from saccharose.
.003..........No acid from saccharose.
.004..........No growths with saccharose.
.0001.........Nitrates reduced with evolution of gas.
.0002.........Nitrates not reduced.
.0003.........Nitrates reduced without gas formation.
.00001........Fluorescent.
.00002........Violet chromogens.
.00003........Blue chromogens.
.00004........Green chromogens.
.00005........Yellow chromogens.
.00006........Orange chromogens.
.00007........Red chromogens.
.00008........Brown chromogens.
.00009........Pink chromogens.
.00000........Non-chromogenic.
.000001.......Diastasic action on potato starch, strong.
.000002.......Diastasic action on potato starch, feeble.
.000003.......Diastasic action on potato starch, absent.
.0000001......Acid and gas from glycerin.
.0000002......Acid without gas from glycerin.
.0000003......No acid from glycerin.
.0000004......No growth with glycerin.

The genus according to the system of Migula is given its proper symbol which precedes the number thus: (7)

BACILLUS COLI (Esch.) Mig. becomes.........B. 222.111102
BACILLUS ALCALIGENES Petr. becomes........B. 212.333102
PSEUDOMONAS CAMPESTRIS (Pam.) Sm. becomes.................................Ps. 211.333151
BACTERIUM SUICIDA Mig. becomes.........Bact. 222.232103

DETAILED FEATURES

NOTE.—Underscore required terms. Observe notes and glossary of terms.

I. MORPHOLOGY (2)

1. *Vegetative Cells,* Medium used..............temp........age..........days
Form, *round, short rods, long rods, short chains, long chains, filaments, commas, short spirals, long spirals, clostridium, cuneate, clavate, curved.*
Limits of Size..........
Size of Majority.......
Ends, *rounded, truncate, concave.*

Agar Hanging-block {
Orientation (grouping)........
Chains (No. of elements)........
Short chains, *long chains*.
Orientation of chains, *parallel*, *irregular*.
}

2. *Sporangia*, medium used........temp.......age.......days
Form, *elliptical*, *short rods*, *spindled*, *clavate*, *drum-sticks*.
Limits of Size......Size of Majority.....

Agar Hanging-block {
Orientation (grouping).......
Chains (No. of elements).....
Orientation of Chains, *parallel*, *irregular*.
}

Location of Endospores, *central*, *polar*.

3. *Endospores*.
Form, *round*, *elliptical*, *elongated*.
Limits of Size........
Size of Majority.....
Wall, *thick*, *thin*.
Sporangium wall, *adherent*, *not adherent*.
Germination, *equatorial*, *oblique*, *polar*, *bipolar*, *by stretching*.

4. *Flagella*, No........Attachment, *polar*, *bipolar*, *peritrichiate*. How Stained.....
5. *Capsules*, present on..........
6. *Zoogleæ*, Pseudozooglea.
7. *Involution Forms*, on..........in..........days at.....°C.
8. *Staining Reactions*.
1:10 watery fuchsin, gentian-violet, carbol-fuchsin. Loeffler's alkaline methylene blue.
Special Stains
Gram..............................Glycogen............................
Fat...............................Acid-fast............................
Neisser.............................

II. CULTURAL FEATURES (3)

1. *Agar Stroke.*
Growth, *invisible*, *scanty*, *moderate*, *abundant*.
Form of growth, *filiform*, *echinulate*, *beaded*, *spreading*, *plumose*, *arborescent*, *rhizoid*.
Elevation of growth, *flat*, *effuse*, *raised*, *convex*.
Luster, *glistening*, *dull*, *cretaceous*.
Topography, *smooth*, *contoured*, *rugose*, *verrucose*.
Optical Characters, *opaque*, *translucent*, *opalescent*, *iridescent*.
Chromogenesis (8)..................
Odor, *absent*, *decided*, *resembling*..................
Consistency, *slimy*, *buytrous*, *viscid*, *membranous*, *coriaceous*, *brittle*.
Medium *grayed*, *browned*, *reddened*, *blued*, *greened*.

2. *Potato*.
Growth, *scanty*, *moderate*, *abundant*, *transient*, *peristent*.
Form of growth, *filiform*, *echinulate*, *beaded*, *spreading*, *plumose*, *arborescent*, *rhizoid*.
Elevation of growth, *flat*, *effuse*, *raised*, *convex*.
Luster, *glistening*, *dull*, *cretaceous*.
Topography, *smooth*, *contoured*, *rugose*, *verrucose*.
Chromogenesis (8)..................Pigment in water *insoluble*, *soluble;* other solvents..................

Odor, absent, decided, resembling..........
Consistency, *slimy, butyrous, viscid, membranous, coriaceous, brittle.*
Medium *grayed, browned, reddened, blued, greened.*

3. *Loeffler's Blood-serum.*
Stroke *invisible, scanty, moderate, abundant.* Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*
Elevation of growth, *flat, effuse, raised, convex.*
Luster, *glistening, dull, cretaceous.*
Topography, *smooth, contoured, rugose, verrucose.*
Chromogenesis (8).................
Medium *grayed, browned, reddened, blued, greened.*
Liquefaction begins in...........d, complete in...........d.

4. *Agar Stab.*
Growth, *uniform, best at top, best at bottom; surface growth scanty, abundant; restricted widespread.*
Line of puncture, *filiform, beaded, papillate, villous, plumose, arborescent: liquefaction.*

5. *Gelatin Stab.*
Growth, *uniform, best at top, best at bottom.*
Line of puncture, *filiform, beaded, papillate, villous, plumose, arborescent.*
Liquefaction, *crateriform, napiform, infundibuliform, saccate, stratiform;* begins in ....d, complete in...........d.
Medium *fluorescent, browned*..........

6. *Nutrient Broth.*
Surface growth, *ring, pellicle, occulent, membranous, none.*
Clouding, *slight, moderate, strong; transient, persistent; none; fluid turbid.*
Odor, *absent, decided, resembling.*
Sediment, *compact, occulent, granular, flaky, viscid on agitation, abundant, scant.*

7. *Milk.*
Clearing without coagulation.
Coagulation *prompt, delayed, absent.*
Extrusion of whey begins in.................days.
Coagulum *slowly peptonized, rapidly peptonized.*
Peptonization begins on......d, complete on......d.
Reaction, 1d......, 2d......, 4d......, 10d......, 20d......
Consistency, *slimy, viscid, unchanged.*
Medium *browned, reddened, blued, greened.*
Lab ferment, *present, absent.*

8. *Litmus Milk.*
Acid, alkaline, acid then alkaline, no change.
Prompt reduction, no reduction, partial slow reduction.

9. *Gelatin Colonies.*
Growth *slow, rapid.*
Form, *punctiform, round, irregular, ameboid, mycelioid, filamentous, rhizioid.*
Elevation, *flat, effuse, raised, convex, pulvinate, crateriform (liquefying).*
Edge, *entire, undulate, lobate, erose, lacerate, fimbriate, filamentous, floccose, curled.*
Liquefaction, *cup, saucer, spreading.*

10. *Agar Colonies.*
Growth *slow, rapid* (temperature...........).
Form, *punctiform, round, irregular, ameboid, mycieloid, filamentous, rhizoid.*

Surface *smooth, rough, concentrically ringed, radiate, striate*
Elevation, *flat, effuse, raised, convex, pulvinate, umbonate.*
Edge, *entire, undulate, lobate, erose, lacerate, fimbriate, floccose, curled.*
Internal structure, *amorphous, finely-, coarsely-granular, grumose, filamentous, floccose, curled.*

11. *Starch Jelly.*
Growth, *scanty, copious.*
Diastasic action, *absent, feeble, profound.*
Medium stained............

12. *Silicate Jelly (Fermi's Solution).*
Growth *copious, scanty, absent.*
Medium stained............

13. *Cohn's Solution.*
Growth *copious, scanty, absent.*
Medium *fluorescent, non-fluorescent.*

14. *Uschinsky's Solution.*
Growth *copious, scanty, absent.*
Fluid *viscid, not viscid.*

15. *Sodium Chloride in Bouillon.*
Per cent. inhibiting growth.

16. *Growth in Bouillon over Chloroform, unrestrained, feeble, absent.*

17. *Nitrogen.* Obtained from *peptone, asparagin, glycocoll, urea, ammonia salts, nitrogen.*

18. *Best media for long-continued growth*...

19. *Quick tests for differential purposes*...
....

III. PHYSICAL AND BIOCHEMICAL FEATURES.

1. Fermentation tubes containing peptone-water or sugar-free bouillon and	Dextrose	Saccharose	Lactose	Maltose	Glycerin	Mannit
Gas production, in per cent.						
$\left(\frac{H}{CO_2}\right)$						
Growth in closed arm						
Amount of acid produced 1d.						
Amount of acid produced 2d.						
Amount of acid produced 3d.						

2. *Ammonia production, feeble, moderate, strong, absent, masked by acids.*

3. *Nitrates in nitrate broth.*
Reduced, not reduced.

Presence of nitrites.................... ammonia........................
Presence of nitrates.................. free nitrogen......................

4. *Indol production, feeble, moderate, strong.*
5. *Toleration of Acids: Great, medium, slight.*
   Acids tested.
6. *Toleration of NaOH: Great, medium, slight.*
7. *Optimum reaction for* growth in bouillon, stated in terms of Fuller's scale..........
8. *Vitality on culture media: Brief, moderate, long.*
9. *Temperature relations:*
   Thermal death-point (10 minutes' exposure in nutrient broth when this is adapted to growth of organism).............. C.
   Optimum temperature for growth....... C.: or best growth at 15° C., 20° C., 25° C., 30° C., 37° C., 40° C., 50° C., 60° C.
   Maximum temperature for growth.................. C.
   Minimum temperature for growth................... C.
10. *Killed readily by drying:* resistant to drying.
11. *Per cent. killed by freezing* (salt and crushed ice or liquid air).................
12. *Sunlight:* Exposure on ice in thinly sown agar plates, one-half plate covered (times 15 minutes), *sensitive, not sensitive.*
    Per cent. killed...............
13. *Acids produced*................
14. *Alkalies produced*..............
15. *Alcohols*.....................
16. *Ferments: Pepsin, trypsin, diastase, invertase, pectase, cytase, tyrosinase, oxidase, peroxidase, lipase, catalase, glucase, galactase, lab, etc.*............................
    ....
17. *Crystals formed*..................................
18. *Effect of germicides:*

Substance	Method used	Minutes	Temperature	Killing quantity	Amt. required to restrain growth

IV. PATHOGENICITY.

1. *Pathogenic to animals.*
   Insects, crustaceans, fishes, reptiles, birds, mice, rats, guinea-pigs, rabbits, dogs, cats, sheep, goats, cattle, horses, monkeys, man.
2. *Pathogenic to Plants:*
   ....
   ....
   ....
3. *Toxins, soluble, endotoxins.*
4. *Non-toxin forming.*
5. *Immunity bactericidal.*
6. *Immunity non-bactericidal.*
7. *Loss of virulence on culture-media: Prompt, gradual, not observed* in........ months.

BRIEF CHARACTERIZATION

Mark + or O, and when two terms occur on a line erase the one which does not apply unless both apply.

Section	Group	Feature	Mark
(2) MORPHOLOGY		Diameter over 1 μ	
		Chains, filaments	
		Endospores	
		Capsules	
		Zooglea, Pseudozooglea	
		Motile	
		Involution forms	
		Gram's Stain	
(3) CULTURAL FEATURES	Broth	Cloudy, turbid	
		Ring	
		Pellicle	
		Sediment	
	Agar	Shining	
		Dull	
		Wrinkled	
		Chromogenic	
	Gel. Plate	Round	
		Proteus-like	
		Rhizoid	
		Filamentous	
		Curled	
	Gel. Stab.	Surface-growth	
		Needle-growth	
	Potato	Moderate, absent	
		Abundant	
		Discolored	
		Starch destroyed	
		Grows at 37° C.	
		Grows in Cohn's Sol	
		Grows in Uschinsky's Sol	
BIOCHEMICAL FEATURES	Liquefaction	Gelatin (4)	
		Blood-serum	
		Casein	
		Agar, mannan	
	Milk	Acid curd	
		Rennet curd	
		Casein peptonized	
		Indol (5)	
		Hydrogen suphide	
		Ammonia (5)	
		Nitrates reduced (5)	
		Fluorescent	
		Luminous	

DISTRIBUTION	Animal pathogen, epizoon	....
	Plant pathogen, epiphyte	....
	Soil	....
	Milk	....
	Fresh water	....
	Salt water	....
	Sewage	....
	Iron bacterium	....
	Sulphur bacterium	....

A. *Counting Plate Colonies.*—If the colonies in a Petri dish culture are few, not exceeding fifty to one hundred, they may readily be counted in full. If the colonies are quite numerous, the counting may be made easier by marking off (by means of a grease pencil or chalk) the bottom of the plate into two right angled cross-lines (quarter sectors) and these again into equal parts (⅛ sectors). Or one of the recommended special counting plates may be used. Either the square or circular plate will answer the purpose (see figures). When colonies are very numerous (200 and more) in a plate culture and quite uniformly distributed, it is not necessary to count them all. Count the colonies in a number of squares or sector areas (square centimeters) and multiply the average of twenty counts by the number of squares representing the entire surface area of the culture plate. As a rule the counting should be complete, however.

From the plate counts it is possible, by simple mathematics, to determine the number of microbes in the dilution cultures of water, milk, tinctures, fluidextracts, as has already been explained.

Studying Plate Colonies.—The plate colonies should be studied macroscopically and also with the aid of a pocket lens and under the low power of the compound microscope. Place the dish on the stage of the microscope and focus upon the colonies carefully by means of the coarse adjustment. Note color, outline and other characteristics of the colonies, etc., as already set forth under tube cultures and in the official methods of the Society of Bacteriologists.

B. *Making Tube-cultures* (Subcultures).—Inoculate test-tubes (containing gelatin, agar or other media) with such colonies as it is desired to study further. This is done as follows: Hold the test-tube to be inoculated in left hand. Take up the platinum needle (straight or loop) in the right hand and pass the entire needle and glass rod (excepting the end held) through the flame of a Bunsen burner several times; heat the needle to a glowing red for a few seconds and then allow it to cool a few seconds. Lift the cover of the Petri dish high enough to pass the needle under, touch end of the platinum needle (straight or loop) on colony desired; let the

dish cover drop into place again; remove the cotton plug from test-tube by grasping it between two fingers (back of fingers toward the test-tube); make the inoculation (deep stab, shallow stab, or streak); withdraw needle; replace cotton plug; hold needle in flame until glowing red. To prevent the sputtering of the material on the end of the needle, hold near flame until dry and then heat to redness. Singe free exposed end of the tube cotton plug in flame to kill and remove microbes and spores on the outer part of the cotton. The inoculated tubes are then numbered and incubated. In due time the cultural characteristics are noted and the observations entered in a suitable note-book.

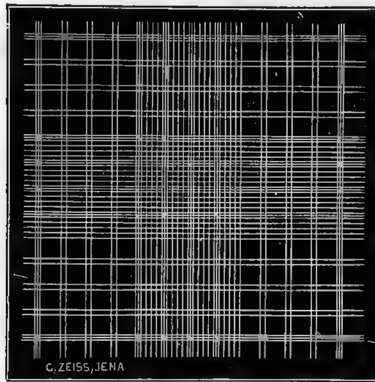


Fig. 41.—Turck ruling of the Thoma-Zeiss hemacytometer. This is especially useful if it is desired to combine the bacterial count with the spore and yeast count. The smaller areas (1–400 sq. mm.) may be used for making the bacterial counts, while the larger areas (1–25 sq. mm.) may be used for making the spore and yeast counts.—(*Carl Zeiss.*)

Subcultures may also be made in Petri dishes, on potatoes, in tubes containing bouillon broth, blood serum, milk and other media with or without indicators.

C. *Studying Anaërobic Microbes.*—Some microbes have anaërobic tendencies (facultative aërobes) and some are absolutely anaërobic (obligative anaërobes). The deep stab culture will show anaërobic tendencies. If such tendency exists, development will be more active near the bottom of tube (in the line of the stab). The culturing of obligative anaërobes requires special apparatus though the methods are not in any way difficult. The following methods are used:

a. Deep stab culture. This has already been sufficiently explained. It merely indicates possible anaërobic *tendencies*.

b. High-culture methods. Fill the tube of a deep stab culture, shallow stab or streak, with liquid agar or gelatin and incubate in the usual way. The medium to be poured must not be warmer than is absolutely necessary

to render it liquid. This brings out possible anaërobic tendencies to a more marked degree than does the simple deep stab culture.

c. Make an Esmarch roll tube culture as follows: Roll a dilution gelatin or agar tube culture (1 : 10, 1 : 100, 1 : 1000, etc.) so that all of the medium (5 cc. to 10 cc.) is spread over the inner surface of the tube to within a short distance of the cotton plug. Keep on rolling slowly until the medium has set. Roll on ice, under the tap water, in ice water, holding the tube at the proper slant. When the medium has set, fill in the entire tube with liquefied gelatin or agar; cool, and incubate. Like the other methods described, this will show possible anaërobic tendencies.

FIG. 42.—Hanging-drop culture, sectional profile view. These slides can be procured from dealers in microscopical supplies.

d. Various methods are used to either remove the air (vacuum), displace the air, or remove the oxygen from the air. In the so-called Buchner method, potassium hydroxide and pyrogallic acid are used to take up the oxygen of the air. The air in a suitable container may be replaced by hydrogen by means of a Kipp generator. As it is not likely that the pharmacist will have any occasion to employ these methods we shall pass them by with this mere mention. The full description of the methods will be found in any of the larger works on medical bacteriology or in the larger text-books on bacteriological technic.

D. *Microscopical Examination of Microbes.*—The compound microscope is used in examining hanging-drop cultures, water mounts and cover-glass preparations. To make a hanging-drop culture, hollow ground slides (concave center) are required. Touch a small drop of the culture to be examined on the center of a clean and heat-sterilized cover-glass, by means of a heat-sterilized platinum wire loop. Smear a little plain petrolatum around the rim of the concavity of the slide and invert the cover-glass preparation upon the slide, pressing it gently in place on the petrolatum. Examine for a period of several hours or longer as may be desired. Cell division, spore formation, etc., can be studied very conveniently. Observations on the effects of temperature and rate of septation may be made. The hanging-block preparation is made by touching the surface of a cube of nutrient agar with the bacteria and then applying this bacterial side against the cover-glass and mounting like the hanging drop. The bacteria will be found close to the cover-glass.

Bacteria can be examined mounted in water on a slide covered with cover-glass, in order to make observations regarding motility. Of course it is not desirable to examine pathogenic microbes in this manner because

of the possibility of infection. In any case, great care should be observed in making the mounts. The slides, covers and needle used must be sterilized, every antiseptic precaution must be observed; and avoid placing an excess of the material on the slide. As soon as the observation is completed (few minutes to half an hour) the mount (slide cover and all) should be placed in a 5 per cent. solution of carbolic acid preparatory to cleaning.

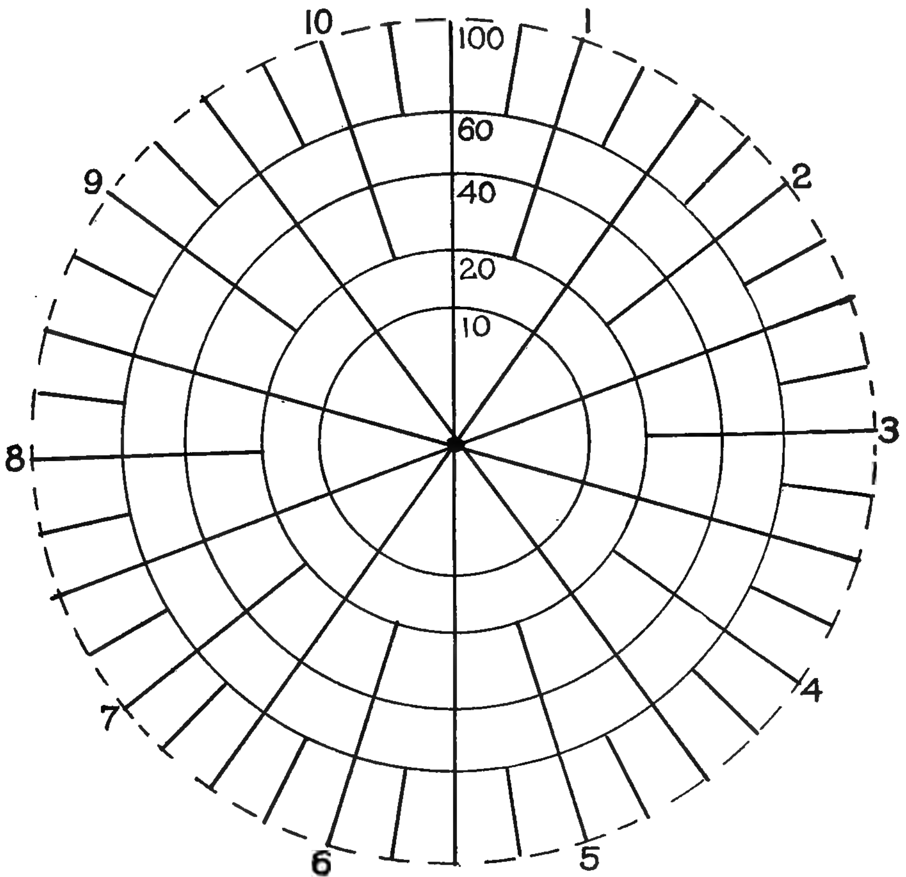


FIG. 43.—Jeffer's circular counting plate for Petri dish cultures. The entire area (100 sq. cm.) is marked off into ten equal sectors of ten sq. cm. each.

Cover-glass preparations, temporary and permanent, are made as follows:

a. Clean a cover-glass thoroughly, dry it well and heat it. The heating will cause the smear to spread better and to adhere better. The slides to be used must also be clean and dry.

b. By means of the platinum needle, spread a bit of the bacterial growth or culture over the greater portion of the surface of the cover-glass. Add a droplet of water, if desired, to separate the bacteria more. Spread evenly. Do not use too much material, as it will make an unsightly mount.

c. Air-dry the smear preparation. This requires but little time, perhaps a minute or two.

d. Pass the cover-glass preparation through the flame of a Bunsen burner four times. This must not be done too slowly as that will char or burn the microbes, nor yet too quickly, as that would not coagulate the albuminous matter and thus fail to fix the microbes upon the cover-glass. A little experience will soon teach the proper speed. Four seconds, or a little less, is the average time in which to make the four passages through the flame.

e. Place a drop or two of the stain on the fixed smear and allow it to act long enough to stain sufficiently, holding the cover-glass over a flame to warm the preparation. Do not heat it more than 60° to 70° C. On an average the stain will be sufficiently deep in five minutes. Fuchsin requires longer time than does methyl-blue or gentian-violet.

f. Wash off the excess of the stain under a small hydrant stream or by means of a wash bottle, or by moving it about in a dish of water.

g. After washing, the preparation may be examined as a temporary water mount. If it is satisfactory it may be made a permanent mount by turning the cover-glass up again and allowing the water to evaporate and then mounting in Canada balsam with xylene, oil of cloves or some other diluent for Canada balsam. Oil of cloves acts on the stain for which reason xylene, benzene or some other balsam diluent of the coal-tar series is preferable. Special staining methods have already been explained. The above is a general method which will serve most purposes. It should be kept in mind that the staining process shrinks the microbes somewhat. The ordinary staining methods do not bring out the cilia. The fact that the microbe is motile is evidence that cilia are present, though it cannot be known whether they are unipolar, bipolar or general, single or multiple.

CHAPTER VII

SYMBIOLOGY—THE BIOLOGICAL RELATIONSHIPS OF ORGANISMS

1. GENERAL INTRODUCTION

Symbiology is the science which treats of the biological relationship of living organisms, animal and vegetable. A general discussion of the subject is essential to a clearer comprehension of the science of bacteriology or microbiology. It will be found that in the treatment of bacteria in disease we are dealing solely with symbiotic relationships. The term symbiosis has been variously interpreted and misapplied. The most common mistake is to define the word as a mutually advantageous biological or physiological association of two or more organisms. Some confuse symbiosis with commensalism. By commensalism is meant the living together of two or more parasitic organisms upon a common host, and is therefore a form of compound symbiosis. In this case the common (commensal) parasites apparently bear no harmful relationship to each other, and may and often do bear a mutualistic relationship toward each other.

Symbiology in the broader sense, therefore, means the science which treats of parasitology, of symbioses in the narrower sense, of commensalism as above defined, of paracytosis, of patrocytosis, of leucocytosis, and of all other forms of intimate physiological and pathological associations of living things. The subject, in the broader and more comprehensive sense, has not received the consideration which it deserves, although some of the special phases have been very fully treated, as for example general parasitology and leucocytosis. Nothing more can be done than to outline the subject and to suggest that any special discussion or fuller treatment be looked up in some of the excellent recent monographs (example—Herms, William B. Medical and Veterinary Entomology. The MacMillan Company, 1915).

A statement of cytology and of embryology is essential to the better understanding of symbiology and the related sciences adenology and bacteriology. The following is a brief summary of the subject.

Van Leeuwenhoek, Hooke and Malphigi (about 1660) are usually credited with having made the first observations regarding a cellular structure in plants. The first published illustrations of plant cells per-

tained to cork tissue. Nothing further of moment was done until about 1833 when Robert Brown again took up the study of the cellular elements of plants. Thus it will be seen that a period of nearly two hundred years had elapsed since the first observations on plant cells during which no notable progress was made in the study of cytology. This long period of inactivity was no doubt occasioned by the inadequacy of the simple microscope and the fact that the compound microscope was not perfected sufficiently to be of any great advantage over the simple microscope, until about 1825. By 1838 and 1839 Schleiden and Schwann were ready to formulate a cell theory of living things, based largely upon the study of plant tissues and organs. At this time it was generally believed that the cell-wall represented the basic or essential part of the cell. In 1846 von Mohl called attention to a slimy or mucilaginous substance enclosed by the cell-wall which he called *protoplasm*, believing it to be the primal living substance. Dujardin, Cohn and others, now began to give some attention to the animal structure, declaring that this also consisted of cellular elements in which were often noticeable certain slimy or gelatinous substances to which Dujardin gave the name *sarcode*. Kölliker noted the fact that the animal cell was frequently devoid of a cell-wall. Max Schultze declared that the protoplasm of the plant cell and the sarcode of the animal cell were in all essentials alike. Since 1870 a multitude of keen observers and able investigators have given their attention to the plant cell and the animal cell and some of the theories based upon the discoveries made have become classics in scientific annals. To review these is impracticable and unnecessary for the present purpose. Attention may however be called to the more important investigations and theories.

It may be recalled that Harvey in the 16th century declared that all life came from an egg (*Omne vivum ex ovo*) which, after the formulation of the cell theory, was changed to the declaration that every cell came from a preëxisting cell (*Omnis cellula ex cellula*), and, no life excepting from a previously existing living organism (*Omne vivum e vivo*). The cell-wall lost interest and the entire attention was now centered upon the cell-contents. For a time an attempt was made to differentiate between living and non-living or dead cell contents, but soon the conclusion was reached that all cell parts and cell constituents were the product of plasmic activity at some time since their coming into existence. Nor did it take long to reach the conclusion that even the cell was not the ultimate unit of living structure, that it was rather a living complex of which we know very little and of which we cannot know very much until the chief mechanical aid to biologic investigation, namely the compound microscope, is more highly perfected and until the science of physical and biologic chemis-

try is more advanced. Certain text-books still persist in naming and tabulating the physical properties of the cytoplasm; for example stating that it is viscid, stringy, slimy, tenaceous, semi-liquid, semi-solid, colorless, etc., etc. It may be recalled that so eminent an investigator as Bütschli described plasm a "Wabenartig;" others that it was fibrillated, or granular, etc.

It may be recalled that Caspar Wolff advanced the theory of epigenesis or the development of individual characteristics through environment, after the union of the gametes or reproductive cells. Wolff may therefore be considered as the biological champion of the believers in the formation of character through environmental influence. In 1892 Weismann formulated his epochmaking theory regarding the continuity of the germ plasm, of the pre-formation and the pre-determination of the physical, mental and moral traits of the individual which were supposed to be held or bound within the reproductive cells. An attempt was made to draw a sharp line between the germ cells or reproductive cells and the other body cells or the so-called somatic cells. It is notable that the major theoretical deductions of Weismann have in the main been proven correct in the light of subsequent cytologic and embryologic investigations.

Various theories were advanced with a view to explaining the mechanism of the transmission, from cell to cell and from individual to individual of the inherent or hereditary properties and characteristics of the cell and of the individual. Darwin very ingeniously assumed the existence of biologic molecules which he called *gemmules*. It was supposed that each and every cell possessed the biological properties of every other cell of the body, represented by the gemmules. Thus it was assumed that a kinetic secreting cell of a gland for example, was also a potential nerve cell; or, that any somatic cell might also be a potential germ cell. De Vries followed out a similar line of reasoning in his theory of *pangenesis* in which he suggested that the physical carriers of the hereditary qualities of the cell were the theoretically assumed *pangenes* which are to be compared to the gemmules of Darwin. Naegeli advanced the micellar theory in which it was assumed that certain biologic molecules and aggregates of such molecules (the micellæ and the pleons) directed or controlled the growth of the cell and all other cell activities. Weismann suggested that the hereditary properties of the germ plasm resided in the *idants* (composed of *ids*) which were also theoretically assumed to be biologic molecular bodies, comparable to the gemmules of Darwin and the pangenes of de Vries. The structure of the cell and its many constituents received attention, more especially the nucleus which was and still is believed to be the essential part of both the germ cells and the somatic cells, in the animal as well as in the vegetable organism. The chromatin bodies of the nucleus

(chromosomes) were considered to be the carriers of and the transmitters of the hereditary characters of the cell and we have come to believe that there can be no new cell except from a mother nucleus, at least as far as pertains to cells which have nuclei. While it is generally believed that the germatic and the somatic cells have distinctive inherent properties which are not capable of being transmitted from the one kind of cell to the other, there are those who maintain that the nucleus, if not the cell plasm, has a dual nature, that it possesses both somatic and germatic properties, and that the somatic cells and the germatic cells are therefore potentially interchangeable. In fact some investigators have suggested the possibility of the functional interchangeability of somatic and germatic cells and also of the male and female germ cells, a contention which has been proven to be correct at least as far as it applies to the lower forms of plant and animal life. Of great interest have been the recent experiments in ovarian and testicular transplantation made by Castle, Stanley and others, the results of which tend to prove the correctness of the Weissmannian theory of the transmission of hereditary qualities. For example, the Guinea pig offspring derived from a transplanted ovary possessed the qualities of the mother from which the ovary was taken and not those of the pig into which the ovary had been transplanted.[1] We must not forget the epochmaking experiments of the Austrian monk Mendel (1822–1884) and the Mendelian law of the transmission of hereditary qualities. Mendel demonstrated that the gametic fusion of different ancestral characteristics did not give rise to a blend of such characteristics, but rather that there was a continuation of the hereditary qualities of both gametes in dominant and recessive proportions. It is surprising that this fundamental principle or rule in gametic or sexual reproduction was not noted earlier. It must have been apparent to all observers that the combining of male and female hereditary sex characteristics, as must be the case in every gametic fusion, did not as a rule result in a hermaphrodite or sexually neuter being. The child does not inherit a blend of paternal and maternal characters. A son may inherit the physical characters from the maternal side of the house and the mental and moral characters from the paternal side. The offspring of an ill tempered and an indifferent parent does not grow into an even or happily tempered person. Both characteristics may be present, one dominant and the other recessive but not as a blend. In this connection might be mentioned the theory of male dominance as promulgated by Galton and others. That is, the offspring manifests to a dominant degree the paternal hereditary qualities. There are however numerous exceptions to this rule.

[1] Experimental tissue transplantations, tumor transplantations and grafting, show similar results.

Prior to the discovery of the cellular structure of plants and animals, there was some discussion and theorization as to an ultimate unit of living structure; that is, what might be the smallest part of a higher plant, for example, which would continue to exist and mature. Many held that the *phyton* or *phytomer* (node, *i.e.*, node and internode) was the smallest plant part which would continue to exist vegetatively and develop into a new mature individual. It is however known that even smaller cell aggregates of a plant can be induced to form a new individual, as leaf and part of leaf (Begonias), bits of rhizome, of tubers and of stems. In the case of the lower plants and animals the reduction can even be carried to the individual cell. The Saccharomycetes are probably multicellular plants in the process of formation and in this group any single cell is capable of forming new cell aggregates by the budding process. In the class Spongilla a group of a few cells will form a new sponge mass. In the lichens, lower algæ, fungi, and liverworts, small bits representing a few cells will mature into new individuals.

With the advent of the cell theory the immediate conclusion was reached that the cell represented the ultimate unit of living structure but as already stated we now know that the cell itself is an aggregate of different kinds of more or less highly differentiated living units. In the lower forms, as amebæ, paramecia, bell animalculæ, etc., the cell may be divided mechanically and the several fragments will each develop into a new mature cell. Certain plastids, nuclear chromosomes and plasmic granules (plasomes, chondriosomes, etc.) will live for a time outside of the cell, in water, in isotonic solutions and in the presence of certain active plant constituents (caffein, asparagin). Tissues and organs have been transplanted from one animal to another (skin grafting, bone grafting, ovarian grafting, cancer grafting, etc.) and certain tissues have been induced to grow in artificial culture media (epithelial cells, muscular tissue, etc., in blood plasm) but no one has as yet succeeded in developing a higher plant or animal from a single detached somatic cell, nor has any one succeeded in perpetuating, in artificial media, any of the living cell inclusions. There appears to be no difficulty in keeping groups of living tissue cells, which are kept in an undisturbed trophic relationship, alive for considerable periods of time, but to induce such cell aggregates to grow and to multiply by septation is apparently more difficult. Fruits, seeds, eggs, ova and some larval forms, will remain viable for long periods under natural and also under certain artificially maintained conditions. The cells and certain plasmic cell inclusions of the apple, the grape, the melon, the pumpkin, the tomato, etc., will remain alive until decomposition sets in or until the loss of moisture becomes excessive. Sections of fleshy roots, tubers, fruits and rhizomes mounted in water or in isotonic solutions

will show living cell elements which may be studied for many hours and even days, and some of the plastids, chromophores and nuclei, will not only remain alive for weeks when suspended in the hanging drop but will also increase in size and in rarer instances will multiply by septation.

We have from the first recognized and admitted the transmissibility of the parental characteristics *via* the male and female germ cells, and such hereditary transmission is undeniable. In order to explain the phylogenetic and ontogenetic relationship of the somatic cells and the germatic cells, we must survey our present conception of the origin of the gametes or the sexual reproductive cells.

We may assume that the original living structures or organisms consisted of individualized bits of more or less complexly differentiated plasmic substances and we may also assume that these plasmic units were biologically somatic or trophic in character rather than germatic or reproductive. We can readily comprehend why in the very nature of things these living bits of plasm were self limited as to size and also as to duration of existence, in all probability largely due to the limitations of available food materials in the immediate vicinity of the originally motionless plasmic units.

The essential of reproduction is the detaching of a single cell or a group of cells from the parent cell or parent body, capable of continued existence, and growth. Three types of reproduction are generally recognized, vegetative reproduction, spore reproduction and sexual or gametic reproduction. In the lower plants and animals all three methods may be observed. In the alga Ulothrix, for example, a portion of the vegetative filament may become detached and such detached cell or group of cells will continue growth and septation and finally develop into a new mature filament. As the conditions for the purely vegetative method of reproduction became more and more unfavorable, the contents of certain specialized cells of the filament were formed into spores which possess the unusual quality of being able to tide over an unfavorable period. In time even the spore was no longer sufficient to enable the organism to survive the changing environmental conditions and the gametic method was developed. There certainly can be no doubt as to the priority of the vegetative method of reproduction as represented by the simpler forms of cell septation and by budding and the renewed growth of detached cell groups. In sexual reproduction we have the union or fusion of the chromosomes and perhaps other essential elements of two different cells (male and female gametes) of the same species. Since the somatic or vegetative cell and the vegetative methods of reproduction preceded the gametes and the sexual method of reproduction, we must assume that gametes are somatic in origin, both phylogenetically and ontogenetically.

It is generally admitted that gametic or sexual reproduction secures or attains a union or fusion of extreme variations of living cells which resulted from the variations in environmental influence, which union or fusion produced an optimum adaptability to the causative variations in the environment. Let us suppose that two groups (I and II) of unicellular organisms, perhaps of the amebic type, should by chance be placed in different environments, group I in a medium with an ample food supply, and group II in a medium with insufficient food. The individual cells of group I would grow comparatively larger and become more sluggish and inactive. The individuals of group II, because of insufficient food would become smaller and develop increased motility for the purpose of securing more of the scant food. As a result of this environmental difference there would in time result two sets of living cells derived from one and the same species which would differ morphologically and physiologically. Should these two groups be compelled to continue in these environments, death or extinction would probably follow, on the one hand because of hypernutrition and resulting inertia, and on the other hand because of insufficient food. We can imagine the condition of one or more of the smaller and starving but more active cells finding one or more of the hypernourished and inactive larger cells and seizing upon these primarily for the purpose of securing a food supply. This attack on the part of the smaller cell would probably result in more or less reaction in the larger cell. Perhaps the withdrawal of some of the proteid excess restored or awakened or aroused some of the lost energy. In brief, the biological association of these different cells proved mutually beneficial. It is reasonable to suppose that such primal gametoid association should be temporary, rather than a permanent fusion, as in the gametic cell fusion in higher plants and animals. Such temporary gametoid cell associations occur in the group Paramecium, and represents the lowest or least specialized form of sexual reproduction. In the illustration cited, the larger cell might be considered the female gamete and the smaller one the male gamete. There are gametes which appear to be identical as to size, form and color, but it is irrational to suppose that such germatic cells are identical physiologically and chemically. We cannot imagine what might be the gain in the gametic union, either temporary or permanent, of two identical cells. In other words it is extremely doubtful if there are genuinely isogamous plants or animals.

We can illustrate the advantages resulting from the union of the properties of two gametes by the classical chart by Wilson. We may represent the properties of the originally somatic cells by the first four letters of the alphabet. In one group of cells the properties developed to the maximum (A, B, C, D), in the second group the same properties were

reduced to a minimum (a, b, c, d). In the gametic union these properties would be combined in the zygote, at least we can assume that such might be the case. In the cell septations which would result from this zygote the properties (inherited) of the two gametes might appear in the daughter cells in sixteen possible combinations and it is readily comprehensible how and why some of the combinations would be more suitably adapted to the environment than others and these would secure the survival of the race or species.

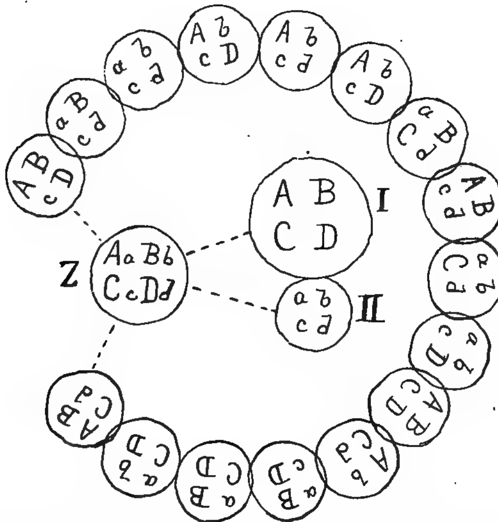


FIG. 44.—Illustrating the redistribution of hereditary properties after the fusion of the female gamete (I) and the male gamete (II), forming the zygote (Z), which upon starting a new septating cycle gives rise to cells in which the original properties of the two gametes (A, B, C, D, and a, b, c, d) may be rearranged in sixteen different combinations. (Adapted from the chart by Wilson.)

The investigations of Bütschli, Calkins, Hertwig, Jennings, Maupas and others, led to the conclusion that the union of gametes in sexual reproduction had the effect of adapting a few out of many to the environment, through restoration or augmentation or restimulation of weakened activities. The somatic existence of single-celled animals and of the many-celled animals, has occasioned much discussion. It has been customary to speak of single-celled animals such as the paramecia, the amebas, etc., as endowed with eternal life, barring accidents, and that the complex organisms as man, for example, is doomed to suffer an unavoidable death of the somatic body. No such difference exists if we draw the correct biological parallellism. If we compare the life cycle of the single-celled organism in a given medium, with the life cycle of the cells of the body of a higher organism, we note a very close analogy. If we inoculate a given

medium with a given species of paramecium and keep the food supply constant and uniform, we find that sooner or later the organisms gradually divide or septate less actively until finally all septation ceases and the medium becomes freed of living paramecia. In other words, the organism has completed its cycle of somatic existence, which may have lasted hundreds and thousands of generations. By means of certain stimuli, as strychnine, alcohol, etc., the cycle may indeed be prolonged by many additional generations but even these stimuli will not make it possible to maintain the cycle indefinitely. In the case of bacteria, biological cycles have been maintained for many years, apparently without appreciable diminution of the septating powers of the cells, but bacteriologists declare that sooner or later a strain of bacteria will deteriorate and take on irreparable changes. In a similar manner the somatic cells of a many-celled animal complete the biological cycle and somatic death follows. In the one case the individual cells are apparently all alike and live apart, and in the second case the individual cells differ morphologically as well as physiologically and are united by growth and have become specialized into tissues and organs. Just as the continued existence of the single-celled race is made possible through the gametic relationships of certain cells just so the life of the somatic cells of higher plants and animals is made continuous through the reproductive cells.

Within recent years many researches have been made in cytology and embryology. Of these the most interesting were the discovery of the sex chromosomes and the relationship of these bodies in the male and female gametes, by Boveri, Henking, McClung, Wilson and others. The chemism of the egg cell received the attention of Baltzer, Herbst, Loeb and many others. It was found that the egg cell of the sea urchin, of the frog and of other animals, could be induced to segment without the presence of the male cell (Herbst, Loeb). The most spectacular discovery was that of the *sex determinant* in the chromosomes of the male or sperm cell. It was found that the germ cells of higher plants and animals differ from the somatic cells in the reduction of the chromosome bands (24 in the somatic cells as against 12 in the germatic cells, as a rule). The 12 chromosomes of the female germ cell fuse making 6 larger chromosomes. In the male germ cell a most remarkable irregularity in the union of the chromosomes was observed, upon which irregularity the determination of sex depends. Some of the sperm cells contain 12 chromosomes and an extra larger chromosome, which is the sex determining chromosome. There are therefore two kinds of male reproductive cells, one with six fused chromosomes and the other with seven chromosomes (six fused and one single). The latter are the male producing sperm cells. The male and female producing sperm cells are supposed to be present in about equal numbers. Which

kind of sperm cell will fuse with the egg cell is manifestly a matter of chance. In some animals the male sex determinant is a double or fused but larger chromosome and again the male sex chromosome may be double but remain unfused or uncombined. It is of interest to note that while the male cell is as a rule much smaller than the female and suffers practically a complete loss of identity at the time of fertilization of the egg cell, it is nevertheless the dominant factor in the transmission of hereditary qualities.

We may sum up the essentials of our present knowledge of cytology as follows:

1. The somatic cell preceded the germ cell, and all living cells and cell units must have been derived from a plasm of somatic or trophic character.

2. Gametes (both male and female) are merely differentiated somatic cells of the same kind and are the product of extreme variations in the environment. Germatic cells differ from somatic cells in the reduced chromosomes.

3. Germatic chromosomes as well as somatic chromosomes are living plasmic complexes derived from the cell plasm. The plasm in all probability consists of and contains the ultimate units of living structure which ultimate units are the creators and generators of the living formed cell constituents of all kinds.

4. Gametes have the property of combining or fusing certain substances which are believed to be more or less essential to the continued septation of the egg-cell. Loeb and others have proven experimentally that the male germ cell is not absolutely essential to embryonal development.

5. The mitotic (karyokinetic) changes in the somatic cells manifest some of the characteristics of the fusion of the gametic chromosomes. The biological and physiological activities of the cell (microcosm) are very closely analogous to the activities of the cells forming the body of the individual (macrocosm).

6. The biological association (symbiosis) of cells of the same somatic origin (resulting from septation) in the multicellular organism, is an evolutional incident, probably occasioned by the variations in the environment. Associations of cells of this kind are in process of formation at the present time among the Saccharomycetes, the group bacteria, the lower algæ and among the lower groups of the animal kingdom.

7. We can readily comprehend how the originally homogeneous cells resulting from septation, composing the complex organism, gradually differentiated into tissues and organs each endowed with specialized activities and functions.

8. There are indications however, that the cells of certain organisms are of heterogeneous origin. In the water net (Hydrodictyon) for exam-

ple, the cells are unquestionably of homogeneous origin; whereas in the lichen group we know for a certainty that the cells are of heterogeneous origin (alga and fungus). It is highly probable that some of the living elements of the cells of higher plants are of heterogeneous origin, likewise those of such animals as the chlorophyll bearing *Hydra viridis* and the chlorophyll bearing amebæ.

9. Whether or not the cells resulting from septation remain free or uncombined as in the protozoa, bacteria, single-celled algæ, etc., or united as in many-celled plants and animals, is incidental in the order of evolution. In both cases septation is cyclical, that is, it continues until the septating power is exhausted and death of the entire somatic cell association follows. In both cases extinction of the cyclical and individualistic and autonomous cell groupings is prevented by spore formation and by the gametic fusion of certain specialized cells.

10. All evidence points to the biological fact that as the association of cells of the same kind became closer and closer physically, there was developed a corresponding increase in the biological and physiological interdependence of the cells, with a gradual reduction or lessening of the individualism or independence of the cells. In the lowest many-celled plants and animals, a single cell still retains the power to develop into a new individual, as in Saccharomyces, diatoms, desmids, water net, lower filamentous algæ, streptococci, etc. As the cell grouping became more and more intimate and interdependent biologically, the individual cell could no longer dissociate itself and develop into a new group. The individual cell can only septate while in contact or biological association with its fellows. Fragments representing a variable number of cells still in biological association, of certain lower plants and animals, as among the algæ, the fungi, the lichens, hydras, sponges, liverworts, etc., etc., could still develop into new organisms. Finally the somatic cells of the organism lost wholly the power of developing into a new individual, no matter how large the dissociated fragment might be, as is the case in all higher animals and in many of the higher plants.

11. The living inclusions of the cell are even more intimately interdependent biologically (somatically) than are the cells composing the individual, and it is therefore not surprising to find it very difficult, if not absolutely impossible, to induce such plasmic cell units to multiply when dissociated form the mother cell. Amyloplastids, chloroplastids and leucoplastids have been seen to increase numerically outside of the cell in the hanging drop but so far no one has succeeded in inducing such numerical increase to proceed to any very considerable cell mass formation. The indications are that any such numerical increase is more apparent than real. That is, the plastids which were already present in the embryonic

stage at the time the hanging drop was prepared simply grow to maturity, without actual numerical increase. There are however indications that there is also actual increase by septation or by extension from the cell plasm.

That the fusion of gametes is intimately bound up with the activities and evolutional development of the somatic cells, is indicated by the fact that only those gametes which are derived from very closely related organisms, will combine sexually. While it is quite evident that gametic reproduction is the product of environmental influences which have been at work for ages, the why and wherefore of such reproduction is not clear. To state that certain proteid stimulins or perhaps enzymatic bodies, such as fertilizin, spermin, etc., are concerned in sex fusion and in sex differentiation, does not materially clear the situation, unless we can explain how and why these substances produce the effects ascribed to them. Sex evolution still is and for some time to come will continue much of a mystery.

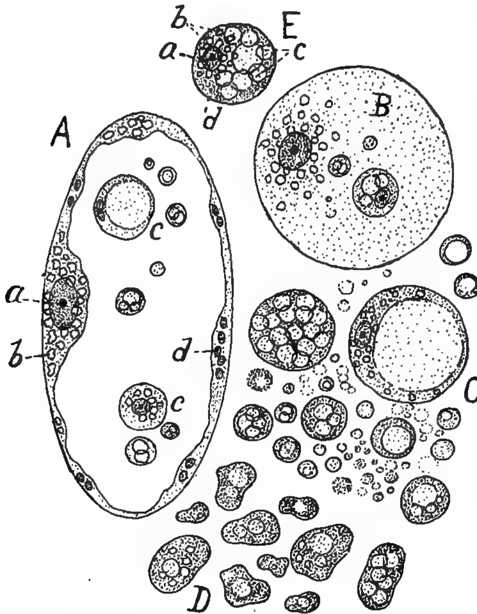
We may assume that it is an established fact that the somatic cell preceded the germatic cell and that formed and living cell inclusions, such as the chromoplasteds, chloroplastids, amyloplastids, leucoplastids, chromosomes, directive spheres, chondriosomes, mitochondria, etc., have their origin (phylogenetically as well as ontogenetically) in the cell plasm, probably derived from fusing and septating plasmic granula and from other as yet unrecognized ultimate plasmic elements. We are therefore also justified in assuming that the essentials of cell growth and of reproduction reside in the plasm of the somatic cell and that mitosis which for several decades has been looked upon as the dominant and all-important cell activity, is of secondary significance.

We have indicated the genetic relationship of somatic and germatic cells, of single-celled and many-celled organisms, of cells and of cell contents, of cell plasm and of chromosomes. It is believed that the observations of sphærocytes is further conclusive evidence that the cell plasm is the source of all living inclusions and that the plasm of the somatic cell of higher organisms may under certain conditions, produce not only a single cell but also an indefinite number of secondary cells. This is evident from the study of plant sphærocytes.

The sphærocytes of plants are living structures derived from the cell plasm, possessing the characteristics of the cells from which they are derived. They occur largely extracellular, that is, outside of the mother cell from which they originated, although in some fruits, as the grape and the tomato, many are found within the cell. In the squash they appear to be very largely if not wholly intra-cellular. In size they are extremely variable, ranging from the limits of microscopic vision to over 100 microns

in diameter. The normal form of the sphærocytes, as the name would indicate, is that of a perfect sphere, although some are more or less irregular in outline and some show marked ameboid movement. Actively motile sphærocytes are comparatively numerous in the green tomato and also in the green grape. The smaller and probably the younger sphærocytes of the fruits thus far examined have the following characteristics in common:

1. They are spherical in form excepting the motile forms referred to.



FIGS. 45–48b × 450.

FIG. 45.—Different forms of sphærocytes from the mucilaginous tissue of tomato seeds. A, a fully matured sphærocyte cell (Gliding cell); a, nucleus with nucleolus; b, reddish brown coloring granules; c, intra-cellular sphærocytes; d, chlorophyll granules. B, sphærocyte nearly mature, showing several endo-sphærocytes. C, sphærocytes in various stages of development. D, amebo-sphærocytes of the ripe tomato. E, a mature nucleo-sphærocyte; a, nucleus with nucleolus; b, reddish brown coloring particles; c, vacuoles; d, granular cell-plasm.

2. The plasmic contents are rather delicately granular but there is no evidence of streaming plasmic motion. The plasmic granula often show slight active and also Brownian motion.

3. There is no outer limiting membrane or cell wall, at least none is demonstrable by the usual staining methods.

4. Vacuoles are generally present and are very variable in size. In some sphærocytes a single vacuole may occupy the greater portion of the cell, leaving a mere meniscoid outer rim of granular plasm. More gener-

ally there are two or more smaller vacuoles, sometimes as many as twenty or thirty, again there may be one comparatively large vacuole and two or more smaller vacuoles.

5. There is a striking similarity between the younger sphærocytes as above described and the resting or encysted forms of amebæ. In fact so striking is this resemblance that it was at first supposed that they might be stages in the life history of certain amebæ, but further observation proved this supposition erroneous. Sphærocytes disappear with the advent of decomposition whereas amebæ thrive in the presence of decaying substances.

6. The colorless younger sphærocytes are generally without nuclei; at least none could be detected by the usual staining methods.

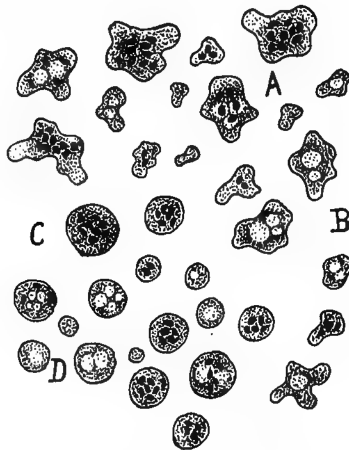


Fig. 46.—Amebo-sphærocytes of the green tomato. A, actively motile forms with chlorophyll granules. B, actively motile forms without chlorophyll granules. C encysted amebo-sphærocytes with chlorophyll granules. D, encysted forms without chlorophyll granules.

The sphærocytes are very abundant in the mucilaginous layer enclosing the seeds of the tomato (Schleimhülle), where they occur in all stages of development, ranging in size from the very limits of microscopic identification (about one micron in diameter) to mature mucilaginous tissue cells (275 microns in diameter). They originate in the cell plasm and are soon extruded from the cell plasm whereupon they continue an independent existence within or without the mother cell. The extra-cellular forms no doubt make their escape from the cell by way of the pores of the cell-wall. Some may be derived from intercellular plasmic threads.

Occasionally groups or clusters of sphærocytic pulp cells occur in certain areas of the tomato pulp, more especially near the epidermal layers. It would appear that under ordinary or usual conditions the sphærocytes

are formed sparingly, or at least they do not develop to larger size in considerable numbers. Due to the action of certain stimuli they may develop very rapidly in large numbers, forming a new or an additional tissue identical with or very closely similar to the tissue from which they had their origin. In other words sphærocytes may give rise to a neoplasmic growth, of which the mucilaginous layer enclosing the seed of the tomato is a striking example.

The following different kinds of sphærocytes may be recognized in the tomato. These are, in all probability, different stages in the development of a sphærocyte.

a. *Leuco-sphærocytes.*—These are the youngest and earliest stages in the development of sphærocytes, as has already been explained. They usually range from one micron to about six microns in diameter.

b. *Amebo-sphærocytes.*—These resemble the leuco-sphærocytes in that they are colorless and contain vacuoles. They differ in that they are always irregular in form and show a very slow to a rather marked ameboid movement, resembling the motion of the leucocytes of the blood and of true amebæ. The plasmic contents are more distinctly granular than that of the leuco-sphærocytes and the vacuolesar e generally fewer, usually from one to three. They resemble amebæ excepting that there is no distinct hyaloplasm (ectoplasm). In the ripe tomato these bodies are few in number but in the green tomato they are quite numerous and show very marked ameboid movement. They vary from 15 to 30 microns in diameter. Most of them contain chlorophyll, from three to twenty or even more unchanged chlorophyll granules of the same type and kind as occur in the normal peripheral pulp cells of the green tomato. They encyst quite readily and in this form they cannot be distinguished from the larger leuco-sphærocytes, especially those which are free from chlorophyll granules. Since these actively motile cells occur in the intact fruit tissues, especially abundant in the mucilaginous layer of the tomato seed, it is reasonable to assume that they are derived from the plasmic elements of the tomato itself and are not true amebæ which might have entered from the outside. Perhaps 10 per cent. of the total number of the sphærocytes are of the ameboid type and about 70 per cent. of these contain chlorophyll. There are indications that all of the sphærocytes pass through the ameboid stage.

What analogy there may exist between the amebo-sphærocytes of the tomato and the amebocytes of the Spongilla group is not determined. The sponge amebocytes which are said to arise from the archeocytes are capable of ameboid movement and will form into new sponge cell aggregates. That the amebo-sphærocytes of the tomato are capable of multiplying by septation is probable and that they may form new tissue cell aggregates

is a fact if the postulate herein submitted, to the effect that they are immature pulp cells, is correct.

c. *Nucleo-sphærocytes.*—These resemble the leuco-sphærocytes as to form and as to presence of vacuóles, but differ in that they contain nuclei and distinct nucleoli and usually also brown coloring matter. The nuclei are comparatively large and resemble those of the mature pulp cells of the tomato. Dull brown to reddish brown chromophores are usually aggregated about the nucleus. There is no evidence of the presence of an outer membrane or cell-wall. In the green tomato the nucleo-sphærocytes may also show chlorophyll granules aggregated about the nucleus.

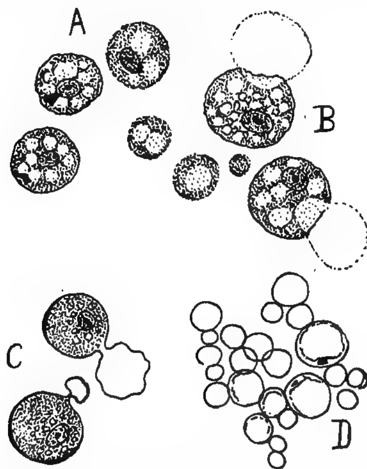


Fig. 47.—Nucleo-sphærocytes from hanging drop, twelve days old. A, living nucleated sphærocytes. B, dying sphærocytes showing extruding vacuoles. C, septation (?) as observed in hanging drop. D, a group of dead sphærocytes in hanging drop.

The most remarkable characteristic of the nucleo-sphærocytes is their great vitality. Crushed tomato pulp in the hanging drop incubated at 25° to 30° C. and also kept at normal room temperature, showed that the nucleo-sphærocytes will remain alive for many months whereas the normal tissue cells and inclusive of most other forms of sphærocytes, die almost at once or at the longest within a period of twenty-four to forty-eight hours.

d. *Chromo-sphærocytes.*—These are generally larger than the nucleo-sphærocytes and contain a variable number of reddish brown coloring bodies (chromophores), irregular in outline, apparently identical with the coloring bodies of the tomato parenchyma. Abundant vacuoles are found. In the hanging drop, the chromophores become diffused through the plasmic substance of the sphærocyte, losing their morphological identity entirely.

e. *Chloro-sphærocytes.*—These are comparatively few in the ripe tomato. In the unripe tomato they are very abundant. They may also contain brown chromophores and they generally have distinct nuclei. The chlorophyll granules are elliptical in form and are identical with the chlorophyll granules of the peripheral pulp cells of the tomato.

Chlorophyll granules are very short lived outside of the mother cell. In the hanging drop they disintegrate very readily, much more readily than do the brown coloring bodies.

f. *Amylo-sphærocytes.*—These are always nucleated and are simply nearly mature sphærocytes which contain a few starch granules. They may also contain brown chromophores and chlorophyll granules.

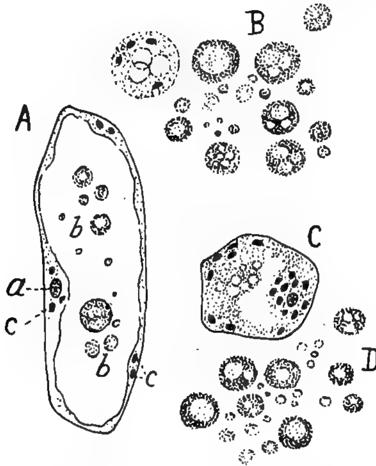


Fig. 48.—Sphærocytes of the grape. A, C, Mature pulp cells. B, D, sphærocytes in various stages of devolpment.

Observations in the hanging drop showed that the chloro-sphærocyte and the amylo-sphærocyte died as quickly as did the mature pulp cells. The amyloplastids are however quite resistent and under certain conditions will continue growth for some time in the hanging drop. Asparagin (1–1000) appears to stimulate the amyloplastids.

Hanging drops of the tomato pulp rich in sphærocytes, incubated at 30° to 35° C. showed that many of the nucleo-sphærocytes kept alive for fifteen months and longer, whereas most other forms, including the mature tissue cells, usually died within a period of twenty-four hours. The most marked change in the living nucleo-sphærocytes in the hanging drop was a slight increase in size of the cell and of the nucleus and a very pronounced darkening of the plasmic substance. At first the vacuoles increased in number and also in size, some of the more peripheral ones

becoming extruded. In the course of four or five weeks the vacuoles gradually became smaller and smaller and finally disappeared altogether. In time many of the sphærocytes developed plasmic threads which extended from side to side across the cell. In many cases the nucleus divided into from several to perhaps as many as thirty and more, secondary nuclei; these secondary nuclei being invariably smaller than the mother nucleus.

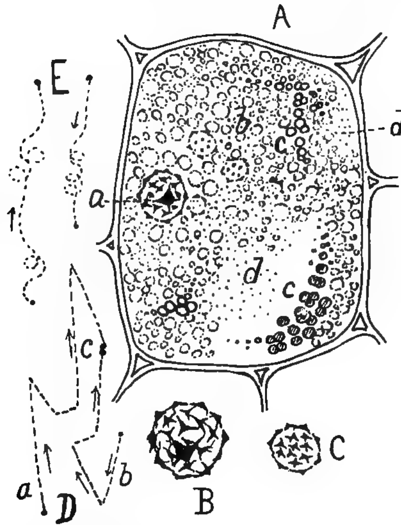


Fig. 49.—Sphærocytes of the pulp cells of the immature squash. A, pulp cell; a, nucleus with irregular branching nucleolus; b, sphærocytes in various stages of development; c, starch granules; d, plasmic granules, some of which are capable of very active movement. B, nucleus enlarged, showing irregular and branching nucleolus and outer irregular branching particles. C, a sphærocyte more highly magnified, showing outer irregular branching particles resembling those of the nucleus. D, illustrating motion of two plasmic granules (a, b), which meet at (c), where they come to rest. E, illustrating a more complex form of motion of plasmic granules.

Fig. 49, × 1000; D and E represent the directions and distances traveled by four different plasmic granules within a period of about 5 seconds, going at a speed of from 3 to 8 microns per second. At that rate the plasmic granule travels from 0.18 to 0.48 millimeters in one minute.

One of the functions of the sphærocytes is to continue the life of the plant part, as fruit or seed, after the plant part has become separated from the mother plant. That is, the plasmic activities are centered in the sphærocytes, rather than in the cell plasm or in the nucleus of the mother cell. A second and perhaps equally important function is the warding off of infections. The nucleo-sphærocytes in particular show a high resistance to the successful invasion by the various organisms of infection, as rotting bacteria, yeasts and molds. Hanging drop cultures rich in sphærocytes remained free from infection even with rather careless manipula-

tion and occasional exposure to air. The greater keeping power of ripe fruit, as compared with green fruit of the same kind, is no doubt due to the fact that the ripe fruit contains comparatively more sphærocytes than does the green fruit. The further study of these highly interesting structures in the plant kingdom, and their analogues in the animal kingdom, will no doubt reveal new chapters in the study of cytology and also in immunology.

The entire discussion of cytology, of the relationship of the cells (somatic as well as germatic) of living organisms, unicellular as well multicellular, etc., is intimately associated with the problems of symbiosis in the broader sense, as has been indicated in the preceding. We shall now take up the more specific forms of symbioses, generally recognized as such.

2. The Phenomena of Symbiosis

Introduction.—All living organisms manifest a more or less intimate biological interdependence and relationship. In fact, their very existence depends upon this condition; therefore no organism, no matter how simple or how complex its structure may be, is the result of a wholly independent phylogenetic development. Upon careful study and investigation it is found that, although this interrelation and interdependence varies greatly as to quality and quantity, there may be found innumerable intermediary phenomena which make it difficult to draw the dividing lines. Such a difficulty is, for instance, encountered in attempting to distinguish between mere "associations" or societies (according to Warming and others) and true symbiosis. Both are evident phenomena of biological interdependence with the general difference that in the former the interdependence is remote, in the latter more close.

Great difficulty is encountered in limiting and defining the biological relationships in the animal kingdom. Highly automobile organisms do not permit the ready establishment of symbiotic relationships as we have come to understand them. Symbiosis presupposes a certain relative fixedness of the organisms. We may find clearly defined symbioses between highly automobile organisms and those which are comparatively non-motile. Here it is very essential to keep distinct the difference between auto-mobility and passive motility (immobility). The former tends to counteract or reduce the occurrence of symbiosis; the latter favors its occurrence as well as its adaptive modification, as will be explained later in the discussion. The most clearly defined and most highly specialized forms of symbiosis occur between non-motile organisms.

Motility or non-motility of organisms has little or no direct influence upon the more remote biological relationships. From the fact that these latter phenomena are most conveniently limited, geographically,

it becomes evident that they are largely dependent upon the influence of the soil, the climate, moisture, etc. (meteorological influences).

The largest and, at the same time, the most remote association of organisms is the hemispherical. The faunal and floral differences between the eastern and western hemispheres are considerable, as every naturalist can testify. In each hemisphere we again recognize subdivisions of associations, which may be designated as zonal. Here the interdependence is more marked, and is primarily dependent upon the influence of temperature and light. The fauna and flora of the tropics is essentially different from that of the temperate zone, and this again is different from the arctic. Each of the zonal areas is again subdivided into numerous larger or smaller geographically delimited societies, dependent upon local influences, as soil, elevation, moisture, sunlight, etc. For example, life in the Mississippi valley is essentially different from that in the Rocky mountain region. In each of these divisions we again find numerous smaller societies. The process of subdividing could be carried on indefinitely. These smaller subdivisions may be natural or artificial, as pond, brooklet, meadow, field, roadside, town, city, etc., each of which has its peculiar fauna and flora.

Within each of these numerous associations, great and small, we find the organisms acting and reacting upon each other. Here there seems to be a mutualistic association of two or more organisms, while the next door neighbors may be engaged in a fierce struggle for existence. A single example will suffice to illustrate this. The wood-peckers and trees evidently form a mutualistic association, while insects and larvæ are diligently hunted by the wood-pecker. Weasel and wood-pecker again are antagonistically related.

Definition of Symbiosis.—Etymologically the word symbiosis signifies "a living together." It is therefore peculiarly fitted for use in the broader sense, as including all phenomena of "living together." Owing to the mutability and imperfections of a language the etymology of a word is not sufficient to limit its application. A careful definition or explanation is always necessary. Symbiosis may be defined as *a contiguous association of two or more morphologically distinct organisms, not of the same kind, resulting in a loss or acquisition of assimilated food-substances.* This definition is by no means perfect. It will, however, be left to further discussions to point out and explain its deficiency.

The Origin of Symbiosis.—It is self-evident that before a symbiotic relationship between morphologically distinct organisms could be established it was absolutely necessary that they be brought in close proximity, or in actual contact. It is also clear, from *a priori* reasoning, that there could be no inherent tendency within these organisms to attract or repel

each other; nor could the first contact have been co-incident with morphological and physiological adaptations. The very conception of symbiosis implies something secondary, and in a certain sense something abnormal. The establishment of marked symbioses required long periods of time; just when they began is impossible to determine. It is, no doubt, justifiable to assume that a number of lowly organized organisms existed in a natural state, manifesting no symbiotic phenomena, because competition (for space) had not yet resulted from over-production. It may also be assumed that symbiotic phenomena began to manifest themselves during the earliest geologic ages. All the multitudinous phenomena of antagonistic symbiosis, and of mutualistic symbiosis, represent highly specialized biological conditions which were initiated by the first contact of morphologically distinct organisms. This contact set up a new phase in the environment. An unforseen struggle was the result, since it is reasonable to assume that the primal contact relationships of contiguous organisms was antagonistic rather than mutualistic. As already indicated, organisms were not primarily adapted to form or enter into symbiotic relationships, yet there is every reason to suppose that natural tendency must have been to respond toward the contact organism as toward the environing substrata. The living organism takes its nourishment and other essentials for existence, from the environment. The organism induces destructive or katabolic changes in its environment, and the foreign organism with which it was accidentally brought in contact, was simply a new bit in the environment. This primal or incipient antagonism engendered by this accidental contact of living organisms, being essentially mutualistically antagonistic, as indicated, must have tended to drive the contiguous organisms away from each other, which they were free to do, for as yet there were no morphological adaptations by which one organism might attach itself to another. Subsequently, the antagonism may have been increased or even entirely modified, changing into mutualism or other highly complex symbiotic associations. These changes are intimately bound up with the questions of "struggle for existence," "survival of the fittest," "evolution," "adaptive changes," etc., etc. We may cite the example of parasitic fungi for the purpose of explaining the probable origin of antagonistic symbiosis. Most fungi are, no doubt, derived from algæ, as certain morphological similarities would lead us to believe. Owing to lack of space, or to over-productiveness, certain algæ frequently came in contact with more highly organized plants and animals, from which they absorbed (by osmotic action) various organic food-substances, thereby reducing the necessary activity of chlorophyllian assimilation. Co-incident with the first contact and resultant change in function, there was a corresponding change in structure. As the opportunities for the symbiotic association

continued (perhaps more or less interruptedly), the morpho-physiological changes progressed in the direction of parasitism and away from independence. Finally the originally independent chlorophyll-bearing and carbon assimilating organism became wholly dependent upon an organic food supply and sustained a total loss of the chlorophyllian function. There is no doubt that the host plant or host plants are also more or less affected by the symbiosis. The relative morpho-physiological changes are approximately in proportion to the size (volume) and biological activity of the associating organisms.

It is necessary to keep distinct the difference between mere associations and societies of organisms, and symbioses proper. There is scarcely a problem of economic significance which is not directly associated with some form of symbiotic relationship of organisms. One needs but call to mind the recent discoveries in the treatment of disease, modern surgery, agriculture, dairy industries, etc. A mere mention of all the experimentations and discoveries in connection with symbiosis would fill volumes. Much careful research is yet necessary in order to clear up the uncertainties in regard to the biological significance of many of the symbioses. In order to impress this uncertainty more fully we shall mention a few symbiotic phenomena which are either not recognized as such, or improperly classified, usually as parasitism.

Unclassified Symbiotic Phenomena.—Under this heading will be briefly mentioned numerous and varied phenomena which are of undoubted symbiotic nature, but are not as yet clearly understood. Some of these phenomena are of a very complicated nature and indicate a long phylogenetic development. In many instances the morphological adaptation and relationship of the organisms is so remote as to awaken serious doubt as to its symbiotic nature. Under this category belong the mutual adaptation of plants (entomophilous and other flowers) and insects; also the various forms of mimicry; the association of various species of aphidæ and ants upon certain plants; besides many other phenomena. The association of trees, such as the myrmocophilous *Cecropias* and representatives of other genera, with ants, is by many designated as true mutualistic symbiosis.

The relation of the male and female reproductive cells is of a truly symbiotic nature. It represents a highly specialized form of individualism. The relationship existing between the immature embryo and the food-supplying parent-stock is evidently a form of symbiosis. There are numerous instances in both the animal and vegetable kingdom in which the more or less imperfect but complete second generation lives in a symbiotic relationship with the first generation. The relationship existing between sporophytic and gametophytic generations may be considered symbiotic in nature even though the two generations are parts of the

same ontogeny. The two generations form a highly specialized symbiosis (individualism). There are many other phenomena of a complicated nature which are designated as true parasitism by some authors while others do not ascribe to them any symbiotic relationship.

Several species of crab belonging to the genus *Stenorhynchus* are usually covered by a growth of algæ, sponges and other lower plants and animals. This is perhaps a case of accidental symbiosis. The habitat of the crab combined with its slow movement makes the chitinous skeleton a suitable substratum for the attachment of various aquatic organisms. The covering may serve some protection but this is evidently of no significant importance. Species of the closely related genus *Inachus* are also covered by a similar growth but here the plants and animals serve as food for the crab. Brehm states that the crab even transplants hydroids, algæ and other organisms upon its back, thus converting itself into a traveling economic zoologic and botanic garden. Another crab is totally hidden by sponges growing upon it which enables it to approach its prey unperceived as well as to hide from its enemies. Although some of these phenomena seem very complicated, there is no evidence of marked symbiotism. If more than mere accidental symbiotism does exist, no experiments have been made to demonstrate whether it is antagonistic or mutualistic.

The hermit crab is morphologically adapted to live in the empty shells of certain snails. The last pair of legs are much shortened and serve the special function of holding the shell. The coleopter *Necrophilus subterraneous* attacks live snails, eats the animal and then moves into the empty shell. The crayfish *Phronima sedentaria* eats species of Doliolum and Pyrosoma and utilizes the empty skeleton as a dwelling place, paddling it about by means of its claws. Although these phenomena are in part of symbiotic nature, yet one must hesitate to place them in this category since the hunting, killing and eating process is not true parasitism) antagonistic symbiosis). According to definition, symbiosis necessitates a prolonged contiguous relationship. This is not the case with the carnivorous animals and their prey. The apparently wonderful adaptations of the crab and other related animals, to the snail-shell and to the outer skeletons of crustaceans, is perhaps purely accidental unless it can be proven that the structural conformations are the result of phylogenetic development.

Climbing plants are interesting as they mark the beginnings of a highly complicated form of symbiosis. The plants form a close association with their supports, which in most cases are living plants; especially is this the case in the dense jungles of the tropics. Whether these plants cling to their support by means of twining stems, tendrils, suctorial organis or aërial roots, there is more or less absorption of soluble food-substances

from the living support and in so far it constitutes a symbiotic relationship. The morphological adaptations favoring climbing are however primarily for the purpose of bringing the assimilative tissues nearer the sunlight, and away from excessive moisture. The support is necessary in order to enable them to enter into successful competition with other plants. In many instances the supporting plant plays the part of a host as in true parasitism (Cuscuta). There is little doubt that the members of the Dodder family were originally climbing plants which took almost their entire nourishment from the soil and air. The contact with the supporting plants gradually developed a wholly parasitic habit. In many of the climbing plants the supporting function predominates while the symbiotic relationship remains practically zero. This is especially true of the large thick-stemmed climbers of the tropics.

Highly interesting though little understood, are the frequently occurring neoformations in animals, such as tumors (epithelioma, limpoma, osteoma, sarcoma, carcinoma, etc.) and cysts of various kinds. It is supposed that these growths are neoformations arising from the development of dormant embryonic cells. These pathologic growths are special body cell proliferations, as has already been stated. Why certain tissues should suddenly take on this highly antagonistic attitude toward the rest of the body cells (somatic cells) is not known. It is a fact that these circumscribed degenerative cell proliferations present all of the characteristics of a foreign attacking parasite, sapping the vitality and even destroying the life of the host. Much attention has been given to cancer research within recent years but no conclusions have as yet been reached, neither as to cause or as to cure.

In conclusion we shall cite a few symbioid phenomena from the insect world and show how they are gradually converted into undoubted symbioses. Different species of wasps narcotize or paralyze spiders, crickets and caterpillars, by stinging, thus rendering them motionless. How the wasp learned to perform this remarkably delicate operation, through which the animal operated upon is paralyzed and rendered entirely helpless without destroying life, is not known. Not even the most skilled surgeon now living can perform an operation of this kind with the precision and the nicety with which the apparently awkward and plundering wasp performs it. In this condition the narcotized insects are sealed into the wasp's nest containing the eggs, in order to serve as food for the young wasp. This condition becomes more complicated by the intrusion of another wasp which unobserved lays its egg in the nest already supplied with the necessary food. The foreign egg develops first and the young wasp not only eats the food supplied by its foster mother, but also the eggs. From these conditions to true parasitism is only a step. Some

wasps lay their eggs directly into the tissues of the caterpillar. The egg develops and the young larva feeds upon the less vital tissues of the host so as to prolong life as much as possible. Finally only the outer tegument of the host remains which is utilized as a protective covering during the resting stage.

We must also mention the phenomena of grafting (plant), tissue transplantation, tumor transplantation, gland transplantation, organ transplantation, etc. These are usually not designated as symbioses. In successful tree grafting, for example, there is established an apparently perfect symbiosis of a mutualistic character. In successful cancer transplantation there is established a form of symbiosis which is truly parasitic. Skin grafting, as practised in cases of severe burns constitutes a symbioid cell association of the mutualistic type.

Phenomena of True Symbiosis.—The phenomena of symbiosis here defined have been more or less discussed by scientists and have received recognition. Authors are, however, at variance as to their exact limitations which makes the definitions subjectively variable. The phenomena of symbiosis may be classified as follows:

I. Incipient Symbiosis (Indifferent Symbiosis).
  1. Accidental Symbiosis.
  2. Contingent Symbiosis (Raumparasitismus).

II. Antagonistic Symbiosis.
  1. Mutual Antagonistic Symbiosis (Mutual Parasitism).
  2. Antagonistic Symbiosis (Parasitism).
    a. Obligative Antagonistic Symbiosis.
    b. Facultative Antagonistic Symbiosis.
  3. Saprophytism.
    a. Facultative Saprophytism.
    b. Obligative Saprophytism.

III. Mutualistic Symbiosis.
  1. Nutricism (Semi-mutualistic Symbiosis).
  2. Mutualism.
  3. Individualism.
    a. Semi-individualism.
    b. Complete Individualism.

IV. Compound Symbiosis.

V. Cytosis.
  1. Autocytosis.
    a. Patrocytosis—Phagocytosis, tissue regeneration, etc.
    b. Paracytosis—Epithelioma, cysts, etc.
  2. Heterocytosis. Consortism. Commensalism.

These phenomena are represented by the association of widely, different organisms. Organisms similar to those which enter into an antagonistic symbiosis may also occur in mutualistic symbiosis. This seems to indicate that the development of these associations depends largely upon opportunity (environment). To some extent, however, the organisms control or modify the symbiotic relationship. A classification of the phenomena indicating their phylogenetic relationship can therefore not be based upon the phylogenesis of the organisms which enter into heir formation. One can only indicate the physiological relationship of the phenomena and their approximate relative evolution.

I Incipient Symbiosis (Indifferent Symbiosis)

Under incipient symbiosis are included the multitudinous phenomena of symbiotic relationships, which have not yet acquired evident antagonistic or mutualistic characters. In many instances there are marked morphological adaptations, but without any *apparent* corresponding functional modification. In far the greater number of cases there is simple contact, resulting from over production. In view of this fact one may be criticised for recognizing such relationships as symbioses. From *a priori* reasoning one is, however, forced to conclude that the first symbiotic activities began with the first contact of organisms. Incipient symbiosis, therefore, forms the basis or common source of all symbiotic phenomena. From it gradually emerged highly complicated morphological and physiological adaptations of originally independent (self-sustaining) organisms. There is also little doubt that as our methods of investigation become more highly perfected, many of the symbiotic phenomena now considered as indifferent will be relegated to the realms of antagonistic or mutualistic symbiosis.

1. *Accidental Symbiosis.*—This represents the least specialized form of symbiosis, but is of wider occurrence than all the others combined. Accidental symbiosis is represented by the mere coming in contact of two or more morphologically distinct organisms; such contact being, however, sufficiently prolonged to give it the semblance of a symbiosis. Mere momentary contact is not symbiosis as here understood.

Accidental symbioses are particularly numerous where there is luxuriant growth, hence where competition is great, as in the tropics and in highly productive soils generally. The lower parts of plants in green-houses are covered with bacteria, hyphal fungi, algæ and more rarely some of the lower protozoa. The epidermal cells of many plants contain more or less bacteria. Submerged plants are covered with mollusks, hydras, tubulariians, amebas, vorticellas, etc. The larger land and water organisms furnish hiding places and protection for hosts of smaller organisms.

In fact, no organism is free from the accidental association with other organisms.

In all of the instances mentioned there is no perceptible evidence of either antagonism or mutualism. Injurious results may result, due to mechanical causes. Slight morphological changes usually result, but such changes seem to have no effect upon the life-history and development of the symbionts.

To the category of accidental symbiosis also belong the association of climbing plants and their living supports. The symbiotic relationship was at first merely accidental. It is a striking example illustrating how marked and highly specialized morphological adaptations favoring one function may initiate widely different morpho-physiological changes. In the case of climbing plants, it is impossible to know when the symbiotic relationship begins to overbalance the function of mechanical support. It is just as difficult to determine when marked symbiotic phenomena began to manifest themselves. It is safe to conclude, however, that the morphological changes favoring climbing and support progressed considerably before any marked symbiotic relationships occurred.

It is also evident that accidental symbiosis is a condition readily subject to change, since the permanency of symbioses is in direct proportion to the degree of mutualistic specialization. Each plant and animal may enter into accidental symbiotism with other plants and animals. In a given animal this association changes with a change of locality, of temperature, or of moisture; in fact, with every change in the environment. The absence of all permanency in morphological and functional relationship, characterizes accidental symbiosis. It resembles a form of haphazard experimentation on the part of nature to determine whether or not a definite symbiotic relationship can be established.

2. *Contingent Symbiosis.*—In this form of symbiosis the relationship of the organisms is already sufficiently marked to give the semblance of an elective affinity, although the functional interdependence is as yet not manifest. It is of wide occurrence among widely different organisms. Many phenomena heretofore recognized or variously classified as parasitism, perhaps belong to this category. Most of the phenomena recognized by the German scientists as *Raumparasitismus* also belong here. The citation of a few examples will suffice to explain the nature of contingent symbiosis, and to distinguish it from mere accidental symbiosis as well as from the more highly specialized forms of symbiosis.

There is a difference between the bacterial flora of the digestive tract of man and that of the chicken or dog. Certain bacteria, which have not yet become markedly antagonistic or mutualistic in their symbiotic associations, show a preference for one digestive tract which indicates that

there must be some elective affinity. That the elective affinity is only slight is evident from the fact that the bacteria referred to will very readily grow and multiply upon artificial culture media, and may be induced to change hosts. Some algæ show an elective affinity for certain living substrata. *Sirosiphon pulvinatus* occurs quite constantly upon species of *Umbilicaria* and *Gyrophora*. *Pleurococcus punctiformis* occurs upon the young thallus of *Cladonia* and *Baeomyces*. *Pleurococcus vulgaris*, on the other hand, occurs upon the most varied substrata living and dead; hence this association is evidently only accidental, as the alga shows no preference for any particular host. It has, perhaps, a slight biologic preference for some of the *Polyporei*.

Some of the higher crustaceans select certain corals, among which they live, without forming any marked symbiotic relationship. In one locality (geographical area) *Hydra viridis* seems to prefer one vegetable substratum (Nuphar), while in another locailty it prefers to live upon another plant, *Lemna polyrhiza*. Some Rotifera show a preference for certain plants to which they attach themselves. Certain algæ, as species of *Dactylococcus* and *Euglena*, show a decided tendency to locate upon such animals as cyclops, snails, and clams. Some mammals (sloth, ant-eater, and others), have algæ living upon them. The symbiosis of snails with corals is perhaps contingent. Some sponges and hydroids show a preference for animals, others for plants. Marine life in particular, presents many forms of contingent symbioses. The instances cited are sufficient to indicate the nature of contingent symbiosis. Many require further careful study before anything definite can be stated as to their biological activity and as to their relationship to other symbioses.

II. Antagonistic Symbiosis

The phenomena included under this head are of wide occurrence and were the first to receive the attention of scientists. The term as here used includes mutual antagonistic symbiosis and antagonistic symbiosis proper. The former is not generally recognized by authors. The latter is more commonly known as parasitism. There are no objections to the use of the term parasitism, since it has become clearly defined and definitely restricted in its application. It is, however, recommended that the term antagonistic symbiosis be substituted for the sake of uniformity in terminology.

From the nature of things the morpho-physiological specializations and adaptations of antagonistic symbiosis are limited. Although one of the symbionts may be highly benefitted the other is always injuriously affected. This injurious effect may finally reach the stage where it will react upon the parasite, thus indirectly resulting in the mutual destruction of the symbionts. In far the greater number of instances the host is not de-

stroyed, nor even seriously injured, although its morphological changes tend in that direction; a condition which will of necessity react upon the parasite. From this it also becomes evident that it is desirable for the parasite to locate upon a host whose vitality and biological activities are many times greater than its own. This we find to be the case, the host is quite generally a large plant, while its parasites are comparatively small.

Strictly speaking, antagonistic symbiosis is therefore a destructive association. The morphological and physiological changes tend toward dissolution rather than evolution. It is a change from the higher to the lower, hence a katabolic change. There is, however, no doubt that symbioses which were originally antagonistic may subsequently be converted into mutualistic symbiosis. Reinke expresses the opinion that the lichen prototype was the result of the parasitic association of a fungus and an alga (Nostoc). This transition from antagonism to mutualism, however, takes place early in the phylogeny of the symbiosis.

As has already been indicated, the majority of symbioses were perhaps originally more or less antagonistic, although actual experiments are wanting to prove this. Incipient antagonistic symbioses are, however, in existence, represented by some Chlorophyceae and Cyanophyceae, in and upon higher plants. In time these algæ will no doubt lose their chlorophyllian function and depend entirely upon the organic food supply of the host.

1. **Mutual Antagonistic Symbiosis (Mutual Parasitism).**—Mutual parasitism as such has heretofore received little or no recognition. It is a phenomenon characterized by the mutual antagonism of the symbionts and is therefore essentially different from antagonistic symbiosis proper, or parasitism. It is a relationship which can not readily occur. If, for example, two or more symbionts nearly equal in size and in vitality, enter into a relationship of mutual antagonism two things may occur. Owing to the antagonism a prolonged symbiosis is impossible, and the symbionts will return to the original substrata, or they will mutually destroy each other. It is, however, highly probable that an association of organisms, which was at first more or less mutually antagonistic, later developed into antagonistic symbiosis proper or even into mutualistic symbiosis. As an illustration of mutual antagonistic symbiosis, we may mention the cells of any pathologic growth, as carcinoma, epithelioma, etc. The cells composing the growth are antagonistic toward each other, as well as toward the normal cells of the organism upon which they occur. This antagonistic relationship of the tumor forming cells is indicated by the fact that they are much weakened in comparison with the normal body cells. In

the numerous cases of socalled tissue degeneration the cells of the particular tissue assume a dystrophic relationship toward each other, resulting in their mutual destruction, carrying with them the host which gave them origin and of which they formed a part.

Complete and simultaneous mutual antagonism of symbionts of equal potentiality or virulency is certainly of rare occurrence. Further careful study may reveal phenomena of this nature. Various forms of mutual antagonism do, however, occur. It exists, for example, between normal cells of plants and animals and certain disease-producing germs (bacteria, etc.) The ability of the cells to resist the attacks of certain germs is spoken of as "physiological resistance" or "natural resistance." In fact, the recent investigations and discoveries in regard to immunity are based upon this mutual antagonism between host and parasite. This antagonism varies greatly between different organisms. Phagocytosis is another example of mutual antagonism. Under ordinary circumstances the phagocytes destroy all of the germs with which they come in contact, thus preventing the occurrence of diseases or other intoxication. Under certain conditions the germs, however, gain the upper hand and destroy the phagocytes.

2. **Antagonistic Symbiosis (Parasitism).**—Antagonistic symbiosis in some of its forms is familiar to all. We will briefly mention some of the more important relationships of host and parasite.

In many instances the host is destroyed without any preliminary morphological changes. The parasite simply enters the cells and destroys them by assimilating the plasmic contents. This form of symbiosis Tubeuf designates as *Perniciasm*. In other instances, also belonging to perniciasm, there are slight secondary changes morphologically before death takes place, as galls or swellings.

In other instances death is the result of enzymatic action, or due to ptomaines or toxins produced by the parasite, as in various diseases of animals as well as of plants. Some parasites dissolve the cell-wells of the host, while others simply lie in contact with the cells and absorb the contents by osmotic action. In a great number of instances hypertrophies and abnormalities in growth are induced (galls, hypertrophied fruits and leaves; enlargements in animal tissues). Again, atrophy, or a total check in development, may occur.

In some forms of parasitism the host adaptation has become highly specialized. In the phenomena known as heteroecism the successive generations of the parasite develop upon different host-plants. For example, *Puccinia graminis* developes its aecidiospores upon *Berberis vulgaris*, while its teleutospores are developed upon some of the grasses, as wheat or oats. Most parasites do, however, not have successive auto-

genetic generations. Many are limited to one host-species, or even to definite tissues or organs.

One organism may enter into different forms of symbiosis. For example, the bacillus of typhoid fever may enter into an accidental (perhaps contingent) symbiosis with the oyster, while with man it forms an antagonistic symbiosis. The bacillus of Asiatic cholera, likewise, may live in and upon various animals without any injurious effects, but as soon as it finds its way to the intestinal canal of man it acts as a true parasite. The differentiation into facultative and obligative parasitism depends upon the ability that some organisms have of living as parasites and saprophytes, while others are absolutely dependent for their existence upon association with the living host.

The most common parasites are the fungi. The Schizomycetes form antagonistic symbioses, preferably with animals. The higher parasitic fungi predominate upon vegetable tissue. Many diseases of animals are also due to the higher fungi. Algæ occur parasitically in and upon plants and animals. Many of the Chlorophyceæ and Cyanophyceæ occur as parasites upon higher plants. Many of the marine algæ are parasitic upon each other as well as upon marine animals. Higher plants are often parasitic (Mistletoe, Dodder, Indian Pipe, etc.). Protozoa occur parasitically upon animals. A sporozoan causes malaria. Still higher animals occur parasitically in and upon animals and plants, producing manifold injurious effects.

Most interesting is the phenomenon of sex-parasitism in which one sex, usually the male, lives parasitically upon the other. In one of the parasitic crustaceans the male is entirely dependent upon the female for its sustenance. Among the Bouellias the male is represented by a mere fertilizing structure, parasitic within the reproductive organs of the female.

We may also mention the parasitic relationship of embryos and the mother-organisms. This has already been referred to as a questionable form of symbiosis. Klebs is, however, of the opinion that it is true parasitism. The embryo of a plant derives all its nourishment from its parent, and in addition takes from it certain materials which it stores for future use (cotyledons, endosperm). Even after birth the young of many animals remain in parasitic association with the parent. Of the numerous eggs of the black salamander, only one develops a young animal, which eats the remaining eggs.

3. **Saprophytism.**—Saprophytism is not true symbiosis. This is a condition which in many instances was no doubt phylogenetically derived from parasitism as we have all gradations between obligative parasites and obligative saprophytes. It is quite reasonable to assume that in many cases of parasitism in which the death of the host was the final

outcome, the parasite adapted itself to the dead substance and used it as food. In some instances saprophytism no doubt originated as such. Dead organic matter occurs plentifully everywhere and forms a suitable substratum for a number of animal as well as vegetable organisms, having special morpho-physiological adaptations for utilizing such as food supply. This preference was no doubt gradually acquired.

III. Mutualistic Symbiosis

This form of symbiosis differs from the preceding in that the relationship of the organisms is mutually beneficial. Each symbiont possesses or has developed a specific character which is useful for the other symbionts. As in the preceding forms of symbioses, widely different organisms may enter into its formation. The morphological changes accompanying the functional adaptations may be very marked or scarcely perceptible, nor is the adaptation quantitatively and qualitatively equal for all the symbionts. The adaptation is complementary, one organism supplies a deficiency (morphological or physiological) of the others. Theoretically there is no limit to the degree of specialization and perfection which this form of symbiosis may attain. In fact, mutualistic symbiosis implies that there is an increased specialization and fitness to enter into the struggle for existence. This is most beautifully illustrated in the case of lichens. These plants are of wider distribution and possess greater vitality and physiological activity than either of the symbionts. They occur in the tropics as well as in the extreme north; in the lowest valleys as well as on the highest mountain peaks. Bonnier has shown that their vitality is greater than that of any other morphologically similar plants. Likewise the mutualistic symbiosis occurring in the Leguminosæ, adapts these almost equally well to rich and poor soil, thus giving them a great advantage over other plants. Our knowledge of the higher forms of mutualistic symbiosis is as yet too incomplete to permit us to make statements as to the full benefits resulting therefrom.

1. **Nutricism.**—Nutricism establishes a connecting link between the lesser marked symbioses and mutualism. It may be defined as a form of symbiosis in which one symbiont nourishes the second symbiont without receiving any benefit in return. It might therefore be designated as one sided or incomplete mutualism. Absolute nutricism, as above defined, does perhaps not occur, for, as already indicated, it is not reasonable to assume that any symbiotic relationship exists in which all of the symbionts are not more or less mutually affected. There are, however, a few instances in which one symbiont is very materially benefited, while the other is not materially benefited. The most marked example is met with in the *mycorhiza* of the Cupuliferæ. A mycorhiza is the association

of a hyphal fungus with the younger rootlets. The function of the fungus, which forms a network about the tips of the terminal rootlet, is to supply the tree with certain food-substances and moisture taken from the soil. It also supplants the function of the hair-cells which are wanting in the mycorhiza. It has been proved, experimentally, that the tree is greatly benefited, while no evidence could be found to indicate that the fungus is benefited. The hyphæ always remain on the outside of the root, and therefore form an ectotrophic association. The endotrophic mycorrhiza of orchids have not yet been sufficiently studied to determine the kind of symbiosis which they represent. Tubeuf designates it as nutricism.

In *Cycas revoluta* we find a form of symbiosis which is evidently nutricism. It is found that in the majority of cultivated cycads there are numerous tubercular outgrowths from the roots, which usually contain a species of *Nostoc* between the cells of a specialized parenchyma. This is evidently not a form of parasitism as is indicated by the fact that the cycads bearing the greater number of tubercles are in no wise injuriously affected; neither has it been proven that the host is benefited. There is, however, no doubt that the *Nostoc* is dependent upon the host for its food supply. It may therefore be looked upon as a case of nutricism, in which the host acts as the transfer agent.

Klebs cites an interesting example which is, no doubt, nutricism. The crayfish *Pagurus Prideauxii* is quite uniformly infested by one of the actinias (*Adamsia palliata*). The latter is said to be absolutely dependent upon the former for its food-supply. The crayfish receives only a slight benefit if any.

2. **Mutualism.**—This form of symbiosis was first described by Reinke and de Bary among botanists and van Beneden and Klebs among zoologists. By mutualism is meant a form of symbiosis in which the symbionts mutually benefit each other, but are still capable of leading an independent existence. It is an association of wide occurrence and in many instances reaches a high degree of morphological and physiological specialization.

The most striking example occurs in the root-tubercles of the Leguminosæ. The tubercles are neoformations induced by the *Rhizobia* which grow and multiply in the parenchyma cells. The *Rhizobia* take their food supply direct from the plasmic and other cell contents of the host; in return the latter receives certain nitrogenous compounds formed by the bacteria in the process of binding the free nitrogen of the air. It has been proven experimentally that the symbionts may exist independently, but thrive much better when in association, especially in poor soil.

To this category also belong the association of ants and trees in the

tropics, which has already been referred to. A given species of ant lives upon and obtains its food supply from the branches of a tree (*Cecropia*); in return the ants protect the tree against the attacks of another species of ants. The ants live within the transversely divided hollow stem to which they gain access by eating away the thin lateral (outer) area. The thin outer membrane of which there is one to each hollow chamber and the chambers themselves are, however, perhaps not the result of the symbiotic association. The preëxisting morphological characters simply happen to make possible the establishment of this particular form of symbiosis.

In the insectivorous plants (Drosera, Dionæa, Nepenthes) we doubtless have another example of mutualism. Formerly it was generally believed that the plant itself digested the insects which it caught, by the aid of irritable glandular hairs and other special organs. It is highly probable that the insect digesting ferment is secreted by bacteria which live upon the plant.

A most remarkable instance of mutualism occurs in the animal kingdom. The very inactive polyp *Actinia prehensa* lives firmly attached to the inner sides of the claws of the crustacean *Melia tessellata*. The *Actinia* aids in killing the prey of the crayfish while the latter carries its guest from place to place thus giving it better opportunities for securing a sufficient food-supply. Möbius states that this association occurs with all the representatives of *Melia tessellata*, both male and female, and that it is almost impossible to remove the symbionts without injuring them.

Many of the symbiotic associations of algæ with animals are perhaps mutualistic. Many *Actinias* contain single-celled algæ which elaborate food-substances for the use of the polyp. Brandt states that as long as this animal contains no algæ, it feeds upon the organic substances in the immediate vicinity, but as soon as it becomes associated with the algæ it depends upon these for the supply of organic food. Further research is necessary to determine whether or not this is true mutualism.

3. **Individualism.**—This form of symbiosis differs from mutualism in that *one* or *more* of the symbionts is absolutely dependent upon the other for its existence. It therefore represents a more highly specialized form of mutualism, from which it is no doubt phylogenetically derived. Individualism may be divided into semi-individualism and complete individualism. In the former at least one of the symbionts is incapable of existing independently, however, the organism of which it was a part cannot survive. In complete individualism none of the several symbionts can continue to exist independently. A new individual, a new autonomy, is the result. It cannot be denied that the association of the symbionts

is less close and even less interdependent than it is among the several living inclusions of the cell of higher plants and animals, and even less closely interdependent than the associations of the somatic cells of the higher multicellular organisms, but is an independent autonomous structure nevertheless.

(*a*) *Semi-individualism.*—This is perhaps of wide occurrence. It is represented by the lower lichens in which the algal symbiont is capable of leading an independent existence, while the fungus can not. In the lowest crustaceous lichens there is perhaps mere mutualism, since several investigators state that the symbionts may live independently as fungus and alga. Some algæ seem to form semi-individualism with animals. According to Kühn, *Pleurococcus brachypodis* and *Pleurococcus chlopodis* occur only upon the body (among the hair) of the two and three-toes sloths. Simple-celled, chlorophyll-bearing algæ or chlorophyll-bodies have been found in representatives of the following genera of the animal kingdom; Ameba, Dactylospora, Difflugia, Hyalosphænia, Helepera, Arcella, Cochliopodium, Actinosphærium, Rhaphidiophrys, Acanthocystis, Heterophrys, Chondropus, Spærastrum, Ciliophrys, Vorticella, Epistylis, Ophrydium, Vaginicola, Euplotes, Urostyla, Uroleptus, Stichotricha, Spirostomum, Blepharisma, Climacostomum, Stentor, Cyrtostomum, Microthorax, Paramecium, Loxodes, Coleps, Lionotus, Amphileptus, Lacrymaria, Phyalina, Holophrya, Euchelyodon, Euchelys, Spongilla, Hydra, Vortex, Mesostomum, Hypostomum, Derostomum, Couroluta, Anthea, Bouellia, Idotea. In many instances the green particles occurring within the animals are simply remnants of chlorophyll derived from the algæ upon which the animal feeds. In other instances there is an undoubted symbiotic association of an alga and the animal.

(*b*) *Complete Individualism.*—The best known and perhaps the most typical form of complete individualism is represented by the higher lichens. Most authors are agreed that the fungal symbiont has entirely lost the power of independent existence, while the alga may exist independently. Some recent experiments would, however, indicate that the algæ likewise have lost the power of continued independent existence. Lichens would therefore form a complete individualism. The association of algæ with *Hydra viridis* and with forms of soil amebae and ciliata, perhaps belongs to this category.

The true significance of the lichen symbiosis does not receive general recognition. Botanists still persist in classifying them among the fungi. Some place them in a separate and independent group, recognizing the fact that they "are not as other plants" but steadfastly refuse to recognize the true reason why they should be given an independent group position. Most of the botanists see in the relationship between alga (the gonidia of the

older lichenologists) and the fungus which make up the lichen individual, nothing more or less than parasitism. Some consider the fungus as the parasite, others the alga. Fünfstück, in his grouping of the lichens in Engler and Prantl's Pflanzenfamilien, indeed gives them a place of their own but nevertheless designates them as fungi parasitically associated with algæ. This is all the more remarkable since Fünfstück very concisely sets forth those morphological, physiological and chemical characteristics of lichens, which clearly indicate their autonomous nature. He refuses to look upon the relationship of fungus and alga as mutually beneficial, and designates it as a special or peculiar form of parasitism ("Eine besondere Art von Parasitismus"). It is furthermore a misapprehension of the expression "mutualistic symbiosis" to interpret it as meaning that the several symbionts are equally benefited. The term simply implies that the several symbiotic components are benefited (which is frankly admitted by Fünfstück) but that one may receive the greater return favor or benefit. There are some botanists who refuse to recognize in this wonderful biological relationship anything more than ordinary parasitism. Such a deduction is possible only when the components or symbionts are considered separately and not in their mutual relationship. For example, in like manner it is possible to reach the conclusion that the domestic animal is injuriously affected through the influence of man, or that civilized man himself is merely a parasitized or degenerate form of the ignorant savage. To speak of the algal (gonidial) symbiont as imprisoned and parasitized is as irrational as to speak of the imprisoned and parasitized horse or cow. It is very true, man uses the milk, the hide, the hair, the teeth, the meat, the bones, the hoof, in fact every part of the animal. It does look like a clear case of the most pronounced one-sided parasitism, but the aspect is changed markedly as soon as we consider both animals, the cow and the man, in their mutual relationship. Had it not been for man, the cow would perhaps not exist at all; as it is, millions of these animals enjoy a life of luxury as compared with the life they would be compelled to lead as independent unparasitized wild animals. Who can then say that the relationship is not mutualistic? By analogy the same argument applies to the alga and fungus in the lichen-group, only here we have a true symbiotic relationship. While it is generally admitted that the lichen components or symbionts may develop and exist independently under artificial conditions, at least up to a certain stage of development, there is no evidence that such is the case in nature. The statement has been made that the algal symbiont may escape from the thallus and vegetate independently on bark, etc., but it lacks proof. Even though that were the case, the fungal symbiont does not exist independently in nature and hence a lichen is an impossibility without

the mutualistic association of alga and fungus. No one has yet succeeded in forming a lichen by associating a true alga (*Cystococcus*) with a true ascomycetous fungus. If this were possible we might reasonably except spontaneously synthetic lichen formations in nature, which is certainly not the case. Lichens invariably arise from preëxisting lichens. Some authorities state that a fungus may attack Nostoc colonies and transform them into collematous lichens but this statement requires verification.

Therefore, it would appear that the most plausible and reasonable attitude to take toward lichen classification is to consider them as a distinct class. This is the conclusion reached after a careful study of the morphology (gross and minute) and ecology of the more important representatives of this very interesting group of plants.

Future experiments may demonstrate that the living inclusions of the cell constitute a symbiotic association of what were once independent organisms which entered into a mutualistic association which has now become so highly specialized that we fail to recognize their ancestral relationships and origin. As already stated, we have not been able to induce any of these living cell inclusions to continue existence outside of the living cell of which they are a part. Some years ago Reinke expressed the opinion that some skilled scientist of the near future would succeed in cultivating chlorophyll bodies in artificial media. We know that Carel and others have succeeded in inducing tissue proliferation in artificial media.

IV. Compound Symbiosis

By compound symbiosis is meant the association of two or more different types or forms of symbioses. Thus we may have two or more organisms mutualistically associated with each other, but forming a common antagonism with the host. It has long been recognized that most of the infections are not simple, but rather multiple. In tuberculosis of the lungs, the bacillus of tuberculosis is not by any means the only infecting organism which is present, other bacteria are present, also yeasts and molds. The leprosy bacillus wlll not thrive unless associated with certain symbionts, as amebæ, body cells, and leprous substance or tissue. The Boas-Oppler bacillus is a fairly constant associate with cancerous tissue and this organism is therefore antagonistic to the cancer, and both are commensal upon the body of the cancerous patient. In *Monostomum bijugum*, a parasitic worm found in birds, it is known that two individuals always occur together. Most abscesses contain from several to many common infecting organisms. The Staphylococcus group acting as the pioneers, preparing the way for the entrance of the other organisms, which feed upon the products resulting from the primary infection.

V. Cytosis

By cytosis is meant certain cell activities which partake of the character of symbioses. They may be classed into autocytoses and heterocytoses. In the former cells derived from and forming a part of the organism enter into cytotic change. In the second instance, the cytotic activity takes place in cells derived from some other organism. The autocytose may be divided into patrocytosis and paracytosis, as follows.

Patrocytosis.—By patrocytosis is meant an increased activity of certain body cells for the purpose of protective immunization and warding off the invasion by pathogenic organisms. The best example is phagocytosis, which will be more fully explained elsewhere. Even more typical are the special cell activities concerned in the formation of healing tissues and in regenerative growths of all kinds, in plants and in animals. Among the higher animals, man in particular, the leucocytes, the lymphocytes, the endothelial and epithelial cells, are chiefly engaged in the patrocytotic activities. In plants the sphærocytes which are most abundant in ripe fruits, form a most important patrocytosis (sphærocytosis), these structures being thrown off from the cytoplasm for the purpose of continuing the life of the plant part after such part has become separated from the mother plant. They also ward off the invasions by the organisms of decomposition. The sphærocytes do not begin to develop abundantly until active cell proliferation (in cambial zone and apical areas and elsewhere) has ceased. These structures evidently continue the life of the cell, after cell division has ceased.

In patrocytosis, certain body cells, that is cells which are normal to the multicellular organism, perform a beneficient relationship with the organism of which they are a part, which beneficient relationship is in every way mutually helpful. The patrocytes occupy a subordinate position toward the organism, but their work is to assist in the maintenance of the state of health. In a way, they bear the same relationship to the organism that children of a family bear to the head of the family in a well regulated family. The patrocytes take their origin in the organism and they are absolutely dependent upon the living organisms for their existence.

Paracytosis.—This is the opposite of patrocytosis. In paracytosis certain body cells assume an antagonistic relationship toward the organism of which they are a part and from which they took their origin. Typical examples are malignant growths of all kinds, such as epithelioma, sarcoma, carcinoma. Here again the epithelial and endothelial cells play an important part. In epithelioma we find an enormously augmented pathologic proliferation of epithelial cells. For some cause or causes yet unknown the epithelial cells refuse to bear a normal relationship to the rest of the body cells. They appear to have become vicious strikers.

They have become the degenerates and perverts among the body cells. Instead of bearing a beneficient relationship toward the cells which gave them origin, they do all within their power to destroy the cells with which they are associated.

Occasionally the leucocytes, the lymphocytes and the endothelial cells of the capillaries go on a strike, not only refusing to continue the performance of their normal functions, but undergoing active disintegration thus bringing about a symptom complex which soon leads to the death of the entire organism, as in purpura hemorrhagica and in pernicious anemia. These degenerative changes appear to have some intimate interrelationship with the sympathetic nerve system. It would appear that the continuous and prolonged suppression of the emotional feelings and sympathies leads to the degenerative cell changes mentioned. No doubt the endocrine secretions are also profoundly altered in these cases. Numerous fatalities due to cellular disintegrations have occurred during the World War among those who for various reasons were obliged to completely suppress or hide their true emotional feelings. The peculiar pathogenic cell proliferation encountered in malignant growths are not induced by the suppression of the emotions. Neither the direct nor the inciting causes of these formations are as yet known.

By heterocytosis is meant a foreign cell proliferation in or upon an organism. Thus cancer tissue may be transplanted upon a mouse. Tissues and organs may be transplanted into widely distinct animals. These interesting cell proliferations are sufficiently common as not to require further explanation as to their nature. They are unquestionably of symbiotic nature.

CHAPTER VIII

BACTERIA IN THE INDUSTRIES. UTILITARIAN BACTERIOLOGY

Because of the fact that pathogenic bacteria received the major attention at the beginning of modern bacteriological study, the general opinion gained credence that all bacteria were harmful or objectionable in some way. This is far from the actual fact. The useful bacteria by far exceed the harmful kinds. Not only is this true, but many of the harmful forms are converted into various beneficial uses. The useful bacteria are altogether too much neglected by the student of bacteriology. In a book of such limited scope it is not possible to mention all of the uses to which bacteria are put in the industries, or the ultilitarian part they play in human economy. The following discussion is intended to indicate the importance of the various microörganisms in some of the human activities,

I. Bacteria in Agriculture

Introduction.—Without bacteria the higher plants and animals could not exist. As is known the carnivorous animals (meat eating) seize upon herbivorous animals (plant eating) as their food supply and the herbivora feed upon the higher plants which in turn obtain their nourishment from the soil. Now, soil is nothing more nor less than a mixture of dead organic matter, bacteria, sand particles, certain chemical compounds, with a variable amount of moisture. The dead organic matter, commonly called humus, is derived from decomposed plants and animals upon which the soil bacteria feed, in the presence of air (oxygen), warmth and moisture. The sand particles are derived from disintegrating rock (the result of bacterial activity, weathering, and water erosion effects), and the chemical compounds, so essential to plant life, are derived from water solutions and through bacterial activity.

It is general knowledge that as soon as a plant or animal dies, it is at once decomposed by the so-called rotting bacteria. This is the ultimate end of all living things. These decomposed plants and animals become mixed with the soil and add to its fertility or productiveness. It is also general knowledge, based upon daily observation, that organic matter which is freely exposed to air and warmth, and in the presence of moisture, undergoes bacterial decomposition. There are indeed many conditions which modify these bacterial activities, such as temperature, air supply,

moisture and sunlight. Most bacteria require an ample supply of air (free oxygen) and these are spoken of as *aërobes*. A comparatively smaller number thrive in the absence of air (free oxygen) and these are called *anaërobes*. The soil bacteria are essentially aërobes, as is perhaps self-evident.

Bacteria are however not the only microörganisms found in the soil. Soil also contains minute algæ, fungi and microscopic single-celled animals (protozoa), to say nothing of earthworms, insects and other higher animals, concerned in certain soil changes of minor importance. The protozoa, algæ and fungi (molds) may be and are, of great significance in crop growing. In a general way it may be stated that most of the algæ, the fungi and the protozoa work antagonistically to the bacteria which are normal to soils. It must however not be supposed that *all* of the bacteria found in the soil are useful or beneficient. Occasionally harmful bacteria develop in certain soils, causing plant diseases and otherwise interfering with plant growth. It is the aim of modern scientific agriculture to so regulate cultural operations as to encourage the *optimum development* of the *beneficient soil bacteria*, reducing at the same time, to a minimum, the development of all *harmful soil organisms* (bacteria, protozoa, molds, etc.).

Incidentally it may be mentioned that soil harbors bacteria and other organisms which play no part in plant growth or in agriculture proper; such as the lock jaw bacillus (*Bacillus tetani*), the anthrax bacillus (*B. anthracis*), the bacillus of malignant œdema (*B. Welchii*), the tuberculosis bacillus (*B. tuberculosis*), the typhoid bacillus (*B. typhosis*), and others. Soils may also harbor the cause of rabies, of foot and mouth disease, the larvæ of hook worm and the larvæ of other intestinal parasites, besides a host of minute organisms injurious to plants and to the lower animals. Swamp lands are the breeding places of the malaria and yellow fever carrying mosquitoes. Proper drainage and tillage of soils tends to check and to reduce to a minimum the development of these highly objectionable soil inhabitants and thus the farmer becomes a most useful worker in behalf of public health and sanitation. Proper sewage disposal prevents dysenteries and typhoid, cholera and many other dread diseases. Every farmer should have a good general knowledge of rural sanitation.

Historical.—Crops have been grown for thousands of years. The earliest cultural methods were crude indeed and at that remote period nothing was known about soil bacteriology, but even in Virgil's time (about 70 B.C., hence nearly 2,000 years ago) agriculture had made some notable progress, for the noted poet in his Georgics, clearly sets forth the value and importance of turning over the soil, the beneficial results of crop rotation and the value of vetch and of other leguminous crop plants

for enriching the soil. Incidentally, it is of interest to know that Virgil's agricultural epic just referred to was primarily intended as a strong plea for turning the minds of the idle rich back to the soil, a plea which was never more urgent than it is at the present time.

The Roman farmers practiced crop rotation, summer fallowing, green manureing, and considered the bean, the vetch and luzerne (alfalfa) as special enrichers of the soil. The farmers of middle Europe early acquired a knowledge of these cultural operations from their Roman neighbors. Red clover soon became known as a valuable enricher of the soil, and has been long used for that purpose by the farmers of France, Germany and England. The Chinese and Hindoos have for thousands of years made use of a pressed bean fertilizer in rice culture. Transfer of a rich soil top dressing to new or arid fields has been practiced for many centuries. In those remote times the value of soil tillage, of soil warmth, of fertilizers, was fully recognized but it is only within recent years that the true significance of these basic agricultural factors has been discovered.

Plant Growth and Bacteria.—The bacteria concerned in plant growth may conveniently be divided into three great groups, as follows:

1. Those that are a part of the soil. That is, those bacteria which are normally present in the soil and which feed upon the organic matter (humus) in the soil, rendering the humus available for the use of plants which may be growing in the soils.

2. Bacteria which live upon and in the immediate vicinity of the roots of plants. These bacteria are essential to the normal development of plants and each kind of plant has its own special kind or kinds of bacterial associates. The bacteria and the host plants form a mutualistic association, that is an association for mutual gain and benefit (mutualism, or mutualistic symbiosis).

Organisms other than bacteria may form such beneficient associations with higher plants. Thus, we find molds in mutualistic association upon the terminal rootlets of oak seedlings and upon the roots of other representatives of the oak family. Some of the soil algæ, under certain conditions, will enter into beneficient relationships with certain plants (mints, calamus, iris, sedges, and other wet soil plants). An alga (Nostoc) forms a mutualistic association with the cycad (*Cycas revoluta*).

3. Bacteria which live within the root tissues of plants. Perhaps every farmer and gardener has observed certain nodules on the roots and rootlets of plants belonging to the bean family, as bean, pea, lentil, soy bean, alfalfa, clover, peanut, cassia, lupine, melilotus, etc. The internal tissue cells of these nodules contain billions of bacteria which have the power of binding the free nitrogen of the air, converting it into nitrógenous compounds which are utilized by the host plants and which upon the death of

the plants reënter the soil, enriching it. Pure cultures of these bacteria have been in use for some time (over thirty years) for the purpose of increasing the crop yield in virgin soils and in soils deficient in nitrogenous compounds. These socalled vest pocket fertilizers are extensively manufactured in the United States, in Canada and in other agricultural countries, some of them bearing special fanciful trade names.

The biological and mutualistic relationship between the three groups of bacteria and the crop plants is very definite. The bacteria of group one may be looked upon as the pioneers, preparing the way for groups two and three. It is they which prepare the raw and as yet wholly unavailable food materials for the use of growing crop plants. That is, they render available to the crop plant, either directly or indirectly, the as yet unavailable or locked foods of the soil. It is they which must contend with the various objectionable organisms found in the soil, such as the protozoa and the molds. The bacteria of group two are more favored by virtue of their position upon and near the rootlets and the root hairs of plants where the moisture supply as well as chemical food materials are more constant. Unless bacteria of group one perform their work properly, bacteria of group two cannot in turn perform their work and a crop failure is the result. Bacteria of group two may be looked upon as the go betweens of the crop plants and the bacteria of group one. The position as well as the work of the bacteria of group three is unique. They receive unusual protection by virtue of their position within the root nodules and they deal directly with the plants with which they are thus closely associated, having no biological relationship to the bacteria of either group one or of group two. They constitute the transfer agents between the free nitrogen of the air and the host plants with which they are associated. It must however be stated that many bacteria of group two, as for example the Azotobacter group (includingal so other organisms of the soil, such as higher fungi and many of the algæ) have the power of assimilating, for the use of the crop plants, the free nitrogen of the air; however free nitrogen assimilation is the special function of the bacteria of group three.

The question as to which of the three groups of bacteria is the most important in crop growing, might be debated; however, all scientists are agreed that the three groups are of the greatest importance in agriculture. Good crops might be matured in soils having a paucity of bacteria of group one provided the necessary chemical materials were supplied artificially. Again, fair crops might be matured in soils having a paucity of bacteria belonging to group two, provided certain other, directly available, chemical fertilizers were supplied; and it is known that members of the bean family will grow to maturity and yield fairly well without the root nodule bacteria, provided the proper nitrogen bearing food materials are supplied. It has

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however been conclusively determined that all three groups of bacteria are essential to the best yield of crop plants.

Bacteria of group one are essentially rotting bacteria, causing the disintegration of dead plants and animals, forming ammonia and other compounds. They are the decomposers of organic matter. Bacteria of group two are essentially elaborators of chemical compounds which the crop plants can use directly as food, in which work these bacteria make use of the materials formed by the bacteria of group one. They are therefore builders up, rather than tearers down, or decomposers; although they also form certain decomposition products. Bacteria of group three possess the remarkable power of converting the free nitrogen of the air into nitrogenous compounds which the crop plant can utilize as food material.

As to form (morphology) most of the soil bacteria are rod shaped, some being comparatively small and others comparatively large, but even the largest do not measure more than 9 microns (a micron being the 1/1,000 part of one millimeter) in length. The smaller forms do not measure over 1 to 3 microns in length. Spherical forms also appear in the soil and upon the roots of plants. Spirally twisted forms (Spirillæ) are fairly common in most soils. As has already been mentioned, certain highly pathogenic bacteria (disease producing) may be found in soils. Old, long cultivated soils are apt to contain the tetanus bacillus (the cause of lock jaw). Pasture lands may be infected with the anthrax bacillus (traceable to cattle dead from this disease). It is believed that certain animals (dogs, coyotes, wolves) get the dread disease rabies or hydrophobia from infected soils, although the usual source of infection (in humans as well as in animals) is the bite of some animal already suffering from this disease.

Soil and Crop Bacteria in Their Relationship to Cultural Operations. It has now been conclusively proven that every agricultural operation which results in an increase in soil productiveness or increase in crop yield, has the effect of encouraging the development of those bacteria which elaborate and set free (render available to the crop plant) those chemical compounds required for the better growth of the crop plants. Turning the soil and making it fine admits air and air (the free oxygen in it) is one of the essentials for the development of soil bacteria. The rays of the sun generate or create warmth and warmth is the second essential to the proper development of soil bacteria. The other essential to bacterial development is moisture and every farmer knows that a certain amount of soil moisture is absolutely essential for the growth of plants.

The activities of soil bacteria may therefore be discussed or stated under three heads as follows:

1. *Soil Moisture and Bacterial Development.*—There are three states or degrees of soil moisture generally recognized. First, that which consti-

tutes the excess water (from rains, overflows, melting snows, surface seepage) which percolates downward between the particles of the soil (pore spaces), due to the influence of gravity. This water which is thus carried downward more or less rapidly by the force of gravity, is called *hydrostatic water*. Very naturally the amount of hydrostatic water in soils depends upon the physical conditions of the soils themselves, more especially upon the porosity and fineness of the subsoils. The hydrostatic water carries with it certain solutes (chemical compounds) and very minute particles (organic, bacteria, colloids, mineral, etc.) and many of these are wholly lost because carried beyond the reach of the crop plants which might have utilized them.

While hydrostatic water is instrumental in the distribution of soil bacteria it is of little significance in the growth and multiplication of bacteria. The crop soils should be in such condition as to allow the hydrostatic water to percolate to the deeper soil strata (two to eight feet) and even in these deeper strata the pore spaces of the soil should only be partially filled with water and never for long periods of time during the growing season, because souring of soils and root rot would likely result, should temperature conditions be favorable. Farmers in arid and semi arid countries are fully aware of the fact that excessive irrigation during the growing season encourages root rot, especially of the deep rooted crop plants. In fact heavy irrigation during the growing season is one of the means employed for exterminating the morning glory and other deep rooted weeds ("drowning out" the weeds).

Hydrostatic soil water is an agricultural essential as it constitutes the storage water upon which the growing plant must draw during intervals of little or no rain fall. The amount of annual moisture precipitation necessary to the successful growing and maturing of crop plants depends upon the nature of the crop plants, the temperature (during the growing season), the physical character of the soil, and upon the methods and means employed for conserving the soil moisture and also upon the amount of atmospheric moisture present. Thus wheat, barley, corn, peas, beans and other shallow rooted short period (six weeks to three months) crops, can be made to yield well with a seasonal precipitation not to exceed four or five inches, provided, of course, that the precipitation comes in one or several volumes shortly before and during the early growing season. An annual rain fall of from fifteeen to thirty inches is ample for the thrifty growth of all kinds of plants. It is perhaps evident that the deeper soils will retain and store more hydrostatic water than will shallow soils. There are a great number and variety of factors which modify the distribution of hydrostatic water.

It is generally known that if one end of any dry porous substance (lamp

wick, piece of rope or string, short piece of wood, etc.) is placed in water, the moisture in the porous substance gradually rises above the level of the water. This is due to what is known as *capillary* action. Soil is a porous substance and the particles composing it (sand particles, bacteria, organic matter, etc.) contain *capillary moisture* derived from the hydrostatic water just mentioned. While hydrostatic water invariably moves downward under the influence of gravity, capillary moisture (or water) moves exactly in the opposite direction, namely upward, or, more correctly in all directions of the capillary influence. This capillary water or moisture forms a continuous film over the particles composing the soil and it is in this moisture that the principal bacterial development takes place. The pores of the soil in which the capillary moisture exists are saturated with water vapor. As the capillary moisture evaporates into the air at the surface of the soil, new supplies are drawn upon from below (the hydrostatic water), until that supply is exhausted, and very soon thereafter the capillary moisture also disappears through evaporation into the air and into the soil pore spaces. From this it becomes evident that the cultural operations should be directed toward the reduction in the loss (by evaporation) of the capillary moisture, which in turn means the conservation of the hydrostatic water supply of the soil. It only need be stated, for the purpose of explaining moisture conservation, that the loss of capillary moisture is completely checked as soon as the air spaces about the capillary or porous substance is saturated with moisture and the escape of moisture from such spaces is prevented. Thus a wick dipped into water, in a tightly corked bottle, will hold its capillary moisture indefinitely. It must however be borne in mind that a complete check in the upward movement of capillary soil moisture would mean a serious interference with the bacterial development as these organisms require continually renewed water supplies in order to take therefrom the soluble substances which are necessary as food materials. The cultural operations should therefore be such as to give rise to an *optimum* (rather than a maximum or minimum) *upward movement* of *capillary water*.

Soils which have lost both hydrostatic and capillary water, still contain some moisture derived from the air and this moisture is known as *hygroscopic moisture*. This varies in amount and is contained in the organic particles of the soil. Hygroscopic moisture is generally not sufficient to permit the development and multiplication of soil bacteria, but it is sufficient to keep bacteria alive as may readily be proven by making plate cultures of long kept air dry soils. In some localities, the hygroscopic moisture may indeed be sufficient to permit some bacterial development in the surface soils, but this moisture alone is inadequate to permit the continued growth of crop plants or of other larger vegetation.

As is known certain plants survive in air dry soils because they themselves carry a surplus water supply upon which they draw during the rainless season. To this group of plants belong the cacti, the palms, the cycads and others. The plants with succulent or fleshy roots, as the radish, beet and turnip, store water for use during seasonal dry spells. In these cases the bacteria living upon the root surfaces, or in the root tissues, continue to multiply.

The essentials of field cultural operations, as far as bacterial development and soil moisture are concerned, may be stated as follows. No farm implement should ever be allowed to operate in the soil layers containing hydrostatic water. All farm implements may work freely in soils with capillary moisture, especially during the early growing season. During the actively growing season the cultivator should keep the soil layers containing the hygroscopic moisture, fine to very fine and should penetrate through the air dry layers into the soil containing capillary moisture, to a depth of several inches. Keeping the top layer of the soil fine reduces the loss of moisture by evaporation.

Numerous scientific tests have been made (Russell. Rothamsted Exp. Station, England, and others) which prove that soils which are partially sterilized by exposing them to a temperature of 60° C. improves the productiveness, due to the fact that the harmful protozoa (harmful because they feed upon the soil bacteria) present are largely killed at that temperature whereas the more resistent soil bacteria survive and continue their beneficent work unhindered by the protozoa. Similar results follow when soils are exposed to certain antiseptic vapors, as of toluene, benzine, benzol, etc., which also destroy the objectionable protozoa without killing the soil bacteria. These undoubtedly beneficial methods of partial sterilization of soils are not practicable on a large agricultural scale. The farmer must therefore employ those methods of cultural operation which will encourage to a maximum, the destruction as well as inhibition of the agriculturally objectionable protozoa (including also other objectionable organisms), and at the same time encourage to the optimum degree the growth and multiplication of the desirable soil bacteria (including also some other organisms). This, as has already been suggested, is accomplished through tillage, drainage, cultivation, etc. Soils which are excessively wet, though otherwise satisfactory, will encourage the water loving protozoa; and moist soils very rich in humus are apt to encourage the development of the soil souring molds. Proper drainage and tillage prevents these troubles.

2. *Soil Aeration and Bacterial Development.*—The beneficent soil bacteria are essentially aërobes (requiring free or uncombined atmospheric oxygen) as already stated, and in order that they may develop to a

maximum degree, the soil must constantly be supplied with air; in other words, the soil must be aerated. This is done by stirring and turning the soil. The stirring and turning operations must however be of such nature as not to bring about any undue waste or loss of soil moisture. As soon as the seeds of the crop plants have germinated and the young plants are growing actively, any deep turning of the soil must cease as this interferes with bacterial activity and furthermore causes undue loss of moisture by evaporation. The ideal crop cultivator should keep the soil (to a depth of from three to six inches) moderately fine, well stirred and should carry new air into the soil. If properly done, the only limit to the number of times a crop plant may be cultivated, is of purely mechanical nature. On an average one cultivation every two weeks during the entire growing season, will perhaps induce an optimum bacterial development, and the most desirable reduction in the rate of the upward circulation of the capillary moisture.

3. *Soil Temperature and Bacterial Activity.*—Not by any means do all soil and plant bacteria develop to an optimum degree at the same temperature. Some of the manure bacteria develop actively at a temperature of 70° C. Some of the bacteria of the ocean water and of the polar regions develop best at a temperature of from 10° C. to 5° C., and even at lower temperatures. The great majority of soil bacteria in the temperate regions develop most actively at a temperature ranging from 12° C. to 25° C. It is interesting to know that while the soil owes its warmth to the heat rays of the sun, the actinic rays of sunlight inhibit bacterial development, especially in the surface soils, and this reduction in bacterial growth in this soil stratum is further increased by the absence of capillary moisture. The maximum bacterial development in soil takes place at a depth ranging from three to six inches. Below that depth there is a gradual decrease in microörganisms, few being found at depths beyond four to eight feet.

In countries having severe winters (November to April), the farmer hastens the warming up of the soil air in the spring, preparatory to crop planting, by turning the soil with a plough. This operation also causes a better distribution of the soil bacteria and admits air. In semi-tropical and tropical countries the preparatory turning of the soil for sun warming purposes, is not so important, but proper soil aeration may nevertheless not be neglected, for reasons already stated.

Bacterial activity results in some warmth generation and deep manuring (hot beds) is practised to force young plants. The heat in silo fermentation may rise to 70° C. The curing of hay and of other vegetable substances results in a rise in temperature. The bacterial activities in the soil do not produce any definitely measureable rise in temperature.

In soils, the maximum bacterial activity dependent upon temperature, is due to the warmth created by the sunlight. At the optimum soil temperature, such plant foods as ammonia, nitrates, phosphates and sulphates, are rapidly formed. It is perhaps self-evident that soils in warm countries furnish more available plant foods than the soils in cold or temperate countries. The production of more plant food due to increased bacterial activity results in the more vigorous growth of higher plants and this in turn means the more rapid depletion of soil moisture. In warm countries having rich soils, but with comparatively low annual rainfall, the conservation of soil moisture is of the greatest importance.

To sum up very briefly, soil bacteria are of the greatest importance in agriculture. The farm cultural operations are for the purpose of encouraging the growth and development of the beneficent soil and plant bacteria to a maximum degree. The top soil must be kept fine for the purpose of checking the evaporation of the capillary moisture. The soil layers holding the capillary moisture must not be too fine nor yet too coarse and must be frequently aerated by means of the cultivator. Wet soil must be properly drained so as not to allow the development of soil souring and otherwise objectionable algæ, molds, protozoa and bacteria. If the soil is too dry, there is a paucity of desirable soil bacteria and the other two groups of plant bacteria already mentioned. If the soil is inadequately aerated, bacterial development is greatly reduced.

Soil Fertility and Bacterial Activity.—Soil fertility is directly dependent upon the available plant food which is present and the fertility is maintained in two ways. By the setting free (by chemical methods) of the as yet unavailable plant foods which exist in the soil, and through the addition to the soil of plant foods in the form of fertilizers. Of the latter, only certain chemical fertilizers are directly available to the crop plants, others must first be rendered available through bacterial activity. It is true, all manures contain some available plant food but this also has been the result of bacterial activity. If follows as a natural consequence that the need for adding fertilizers to soils is reduced in the direct proportion to the increase in the development of the normal and beneficent soil and plant bacteria. How long the fertility or productiveness of a field may be maintained by proper tillage, suitable crop rotation, intelligent summer fallowing, green manuring, including the rational use of microbial crop inoculation, has not yet been determined. It is known that soil has yielded good crops for over one hundred years, without the addition of chemical fertilizers or of manure. There can be no objection raised to the intelligent use of fertilizers, excepting the cost and the labor of applying them. The time honored practice of spreading a top dressing of rich soil, from a fertile field, upon virgin soil (virgin to the crop under

consideration) and upon non-fertile or poor soil, is no longer considered scientifically correct. The transfer of such soils frequently caused trouble and the harm done through the simultaneous transfer of objectionable soil fungi, bacterial diseases and noxious weeds, far exceeded any gain which was derived from the desirable soil bacteria which were also present. Bacterial soil inoculation should be made by means of pure cultures or mixtures of pure cultures, properly activated by means of special culture methods and culture media. Bacterial soil inoculation is at present merely in its initial stages but the results thus far obtained are indeed promising. The results of recent experiments suggest the possibility that each species or kind of plant has associated with it, *via* the root system, certain bacteria which are beneficial and even essential to its growth and development. The farmer of the near future will no doubt inoculate all seed, about to be placed in the soil, with pure cultures of the predominating beneficent bacteria belonging to the crop plant in question.

Soil Colloids and Soil Fertility.—Within recent years the chemists and physicists have made certain very interesting observations regarding the behavior of matter in very finely divided state suspended or dissolved in liquids or in semi-liquids. It has been known for a long time that the socalled soluble salts (chemical combinations of basic elements with acids) when in molecular solution will pass through dialyzable membranes (as parchment, films of collodion, sausage casings, dried and fresh skins of animals, etc.), whereas certain other substances apparently also in solution (usually organic in nature), will not pass through such membranes. All substances apparently in solution but which will not pass through dialyzable membranes are called *colloids*. Recent investigations have shown that all substances, organic as well as inorganic, may be changed or converted into colloids. In other words, colloidality is a universal property of matter. Thus, we have colloidal iron, colloidal gold, copper, silver, mercury, etc., etc. The Hindoos have for many centuries, prepared and used colloidal iron in the treatment of certain diseases, as anemia, and other disorders in which iron compounds appear to be deficient. There is already a vast literature dealing with the colloids of the soils in their relationship to the activities of soil bacteria and to soil fertility. To review, or even to cite the most important contribution to these subjects would be wholly impracticable in this introduction. It is indeed regrettable that those who treat of and discuss the practical phases of cultural operations and the use of soil fertilizers, do not explain, or at least briefly indicate, the colloidal principles involved.

We may therefore, define colloids as very minute particles composed of groups of molecules, dispersed in or held in suspension in various media, hcus as water and other liquids, gases, and even in solids. It is generally

believed that the soil humus and soil colloids are one and the same, but this is not in accord with the facts, as may be gathered from the above introduction. Humus is in part colloidal and all humus has colloidal properties. The same may be said of the mineral constituents of the soil no matter what their composition. The agriculturally active soil colloids are represented by a long list of organic and inorganic compounds (in very minute, mostly microscopic and ultra-microscopic molecular aggregates) combined with more or less water, the water being the chief dispersing medium for the colloidal particles. These soil colloids may be roughly grouped as follows:

1. Silicic acid and the soluble silicates.
2. Aluminum hydroxid and its compounds with silicic acid, represented by the clays.
3. Iron hydroxid and its compounds.
4. Humus. Represented by humus acids, organic matter generally, including the soil bacteria and other microörganisms of the soil.

A fairly good idea as to the nature of soil colloids may be obtained in the following manner. Place a teaspoonful of rich soil in a tumblerful of water and stir well, let stand for a few minutes and pour the supernatant murky liquid into a second tumbler and let this stand for thirty minutes and again pour the supernatant liquid into a third tumbler and let stand for a few hours, decant very carefully; let stand for 24 hours and again decant. You will now have five grades of soil particles. In tumblers 1 and 2 you will find the coarse gravel and coarser sand particles, with a certain amount of very fine matter. Tumblers 3 and 4 will contain the finer sand particles and most of the precipitable colloids, while tumbler five will retain the very finest sand particles and the majority of colloids in solution and in suspension. The contents of tumbler 5 will appear quite murky and will not clear itself entirely even if allowed to stand undisturbed for days and weeks. If this liquid should be entirely clarified by filtering repeatedly through a fine filter paper, it would nevertheless contain much colloidal matter, organic as well as inorganic, as might be demonstrated by dialysis, chemical precipitation, etc. The enormous river deltas (of the Nile and the Mississippi) are colloidal precipitates the result of contact of the salt water with the fresh river waters carrying the colloids brought down from the rain washed mountain sides and the valley lands.

It has been stated that the fertility of the soils is directly proportional to the percentage of colloids present. This is certainly not true. An excess of colloids is as harmful as is a deficiency of colloids. It is therefore correct to state that the fertility of the soils is proportional to the increase of colloids up to the optimum, not the maximum. It has been known for centuries that the winter freezing of very rich soils results in increased

yield, due to the coagulation of some of the colloids. The time honored practice of burning over soils excessively rich in organic colloids (the rice lands of India and other countries) results in increase in yield through a reduction in the organic colloids. As is well known the rich soils of California (adobe soils) are much improved by adding lime which has the effect of coagulating colloids. The addition of sand to heavy rich soils is beneficial because it dilutes the colloids and renders some of them harmless through adsorption upon the sand particles. Adding phoshates to soils rich in colloids is apt to result in souring the soils because of the increased solution of the colloid (by the process of hydration). Following the phosphates with lime will prevent the objectionable changes through the coagulation of the colloids, as already indicated.

As the colloids increase beyond the optimum in wet soils, mold and rotting bacteria gain the ascendency and soil souring follows, and this souring still further increases the rate and degree of objectionable colloidal hydration. It is indeed true that the water content of the soils is regulated through those colloids (organic as well as inorganic) which are hydratable, that is which have a very marked affinity for water. As is well known, gravelly and sandy soils do not hold the rain moisture, nor do they readily draw the moisture from the depths below, unless perchance the sand is exceedingly fine.

One of the important agricultural qualities or properties of colloids is their power of absorption; that is, the power of accumulating and holding upon their surfaces, the various substances with which they are more or less closely associated and which are required by the soil bacteria, such as water and chemicals inclusive of the smallest colloidal particles. Unfortunately, they adsorp noxious gases, soil toxins, bacterial toxins, harmful chemicals, and other agriculturally objectionable substances, with equal facility, should any be present. It is the work of the farmer to so regulate and adjust the cultural operations as to render impossible, or to reduce to a minimum, the solution and adsorption of harmful substances.

The growing crop plant cannot utilize the larger colloidal particles direct. The rotting bacteria, assisted by a variety of enzymes, transform the colloidal masses and larger particles into smaller particles, which in turn are seized upon by the bacteria of groups (2) and (3), converting them into colloidal particles small enough to be used as food material by the growing plant. The efforts of the modern agriculturist have to do, either directly or indirectly, with the formation and transformation of soil colloids, rendering these colloids available for the growing crop plant. The change from mature plant represented by straw, hay, oats, corn etc., to manure, and from manure to available plant food, and from available plant food back into mature crop plant, is one continuous kalaidoscopic

colloidal transformation series, in which water, enzymes and bacteria play the leading role.

Bacterial Soil Inoculation—Bacterial Fertilizers.—By means of thorough soil cultivation and the systematic use of fertilizers we simply encour-

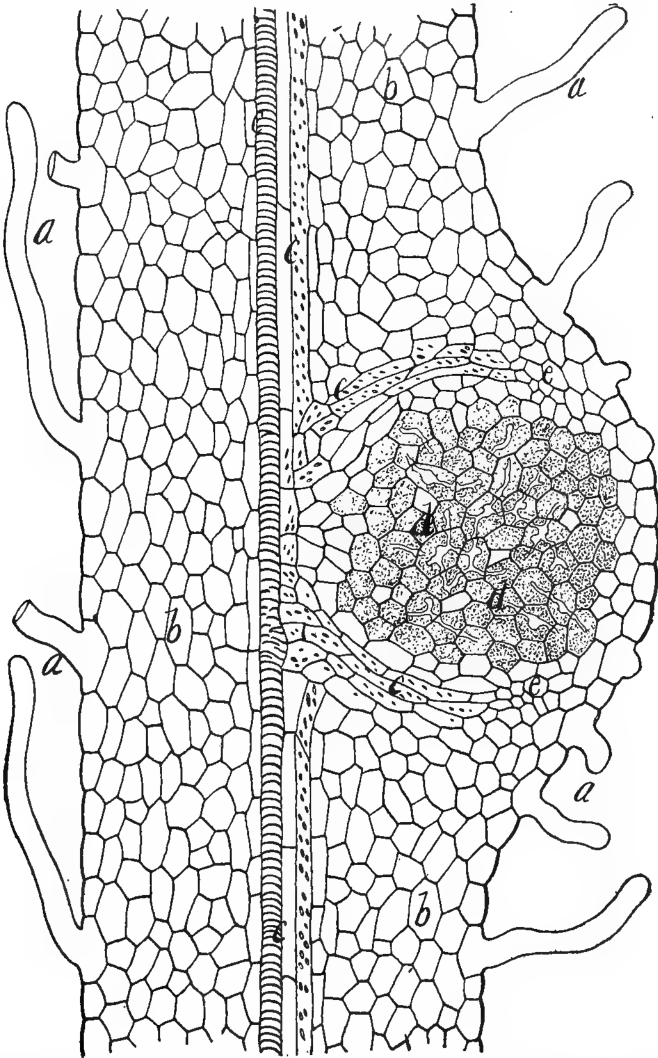


Fig. 50.—Longitudinal section through red clover rootlet, showing tubercle formation due to the root nodule microbe, *Rhizobium mutabile*. The tubercle is only partially developed. *a*, root hairs. These do not develop on the nodule. *b*, the normal root parenchyma. *c*, vascular tissue. *d*, infected area, also showing the infecting strands (*Infectionsfäden*). The cells are filled with bacteria. *e*, apical areas, the growing areas of the tubercle.

age the development of the particular microbes that will set free or render available the food substances required by the crop plants under cultivation. Agricultural bacteriology is beginning to make practical use of certain plant food forming microbes. Of these the free nitrogen-binding microbes are most promising from the standpoint of practical commercial utility, and have received much attention in recent years. The more important species are: *Rhizobium mutabile, Bacillus ellenbachiensis* Caron, *Azotobacter chroococcum; Bacillus subtilis, Bacillus californiensis,* and a few others. Of these, *Rhizobium mutabile,* the root-nodule bacterium of the Leguminosæ, has received most attention.

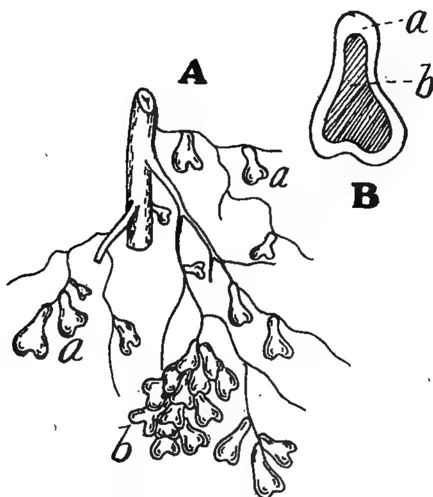


FIG. 51.—Root nodules of sweet clover, somewhat magnified. *A*, rootlets with nodules. *a*, single nodules. *b*, clusters of nodules. These are sometimes very large, consisting of hundreds of nodules, loosely united. *B*, diagram of single nodule. *a*, uninfected area. *b*, infected area.

The first to suggest a plan for practically utilizing the root nodule bacteria (Rhizobia) and to secure letters patent for the process in Germany and the United States, were Nobbe and Hiltner, of Tharand, Germany. Patent No. 570,876 was granted Nobbe and Hiltner in the United States, November 3, 1896. This patented fertilizer for leguminous plants consisted of pure cultures of the several varieties (or perhaps species) of *R. mutabile,* each species of plant, as bean, pea, clover, alfalfa, etc., having the cultures derived from the root nodules peculiar to it.

This commercial preparation was given the name "nitragin," and its efficiency was quite carefully and extensively tested and commented upon by European and American investigators. The consensus of opinion seems to be that it was of doubtful practical utility for agricultural pur-

poses. Some authorities maintained that it was of unquestionable value in virgin soil. In rich and otherwise favorable soil conditions it is of only slight value. It is maintained that nitragin aids very materially in developing and ripening the fruit. As becomes evident from careful con-

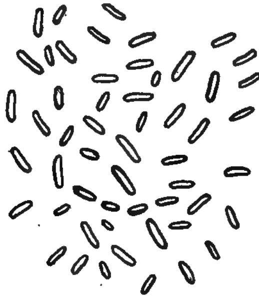


Fig. 52.—Motile forms of *Rhizobium mutabile* as they appear in fresh cultures. They are very small, ½ to ⅔μ in length.

sideration, the value of this microbic fertilizer depends upon whether or not it will cause an increased development in the number and size of root tubercles over and above those which would develop without the presence of this artificial aid. If the soil is already well supplied with rhizobia

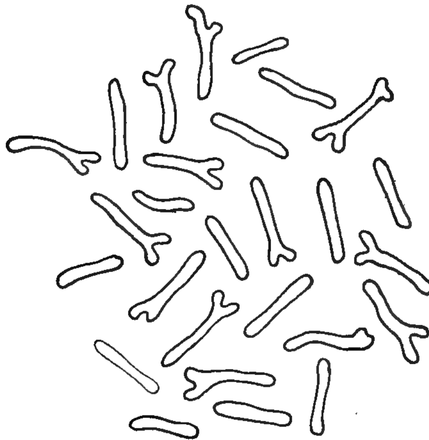


Fig. 53.—Non-motile matured forms of *R. mutabile* as they appear in mature sweet clover root nodules. Most of them show the forked ends. This may be considered the normal form of this organism.

or root tubercle bacteria, as soil would naturally be if the leguminous plants under consideration had been grown in it for one or more seasons, nitragin would in all probability be of little or no value. In any case, the anticipated results have not been fully realized, and nitragin is withdrawn from the market, and is no longer manufactured.

A second and later improvement in the method of inoculating seeds with root tubercle bacteria (Rhizobia) is given by Hartleb in the specifications forming part of letters patent No. 674,765, granted May 21, 1901, at Washington, D. C. Although not so stated in the specifications, it is evident that the Hartleb process is a method for applying pure rhizobia cultures to seed of leguminous plants. Whether the method offers any advantages over the method of Nobbe and Hiltner is questionable. In any case it would prove practically advantageous only under the conditions referred to under the discussion of nitragin. Although the method has been freely discussed and experimented upon in Germany, the fertilizer is no longer

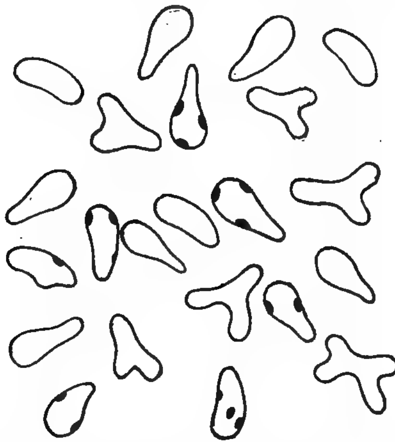


FIG. 54.—*R. mutabile* as it appears in mature nodules of red and white clover root nodules. This may be considered the extreme form variation due to hypernutrition.

on the market. There is on the market a third patented germ or microbe soil fertilizer of German origin, known as "alinit." It consists essentially of a pure culture of the soil bacillus known as *Bacillus ellenbachiensis alpha* or *Bacillus ellenbachiensis* Caron. The germ was first brought to the attention of the agriculturists by Caron, a land owner of Germany, who first isolated it and called attention to the fact that it had the power of chemically binding the free nitrogen of the air. The microbe is said to be closely allied to *B. megatherium* and *B. subtilis*. According to some authorities it is especially concerned in assimilating free nitrogen for gramineous plants. If it is true it may prove of great value to grain growers.

The commercial alinit is a dry pulverulent substance of a yellowish-gray color, with about 10 per cent. moisture and 2.5 per cent. nitrogen. It is evidently prepared by mixing spore-bearing pure cultures of the bacillus

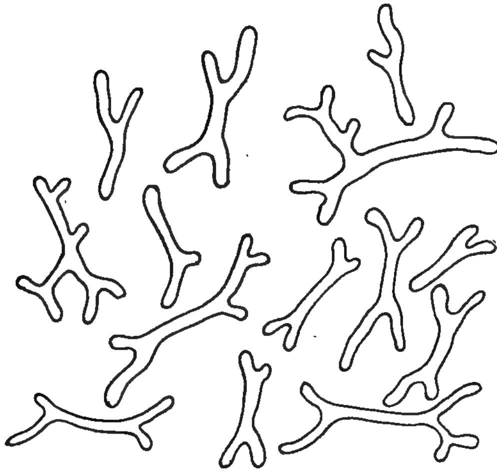


FIG. 55.—*R. mutabile* from the root nodules of *Trifolium heterodon*, showing the extreme form variation due to hyper-growth. The forms shown in Figs. 52, 53, 54, 55 and 56 are simply involution forms of the same species due to differences in environment and host relationship. The chromatin bodies found in the hyper-nourished forms (Fig. 54) are probably reserve products.

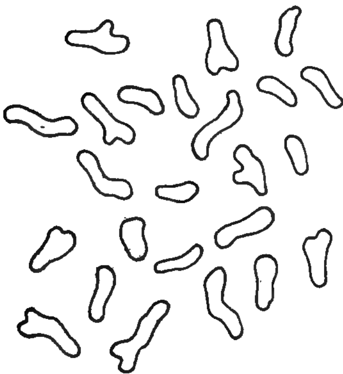


FIG. 56.

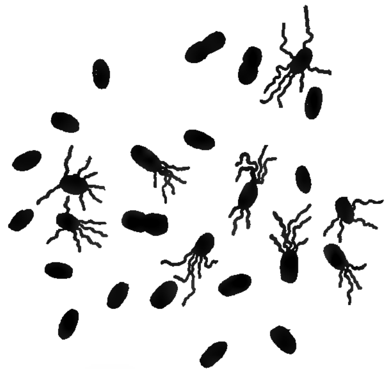


FIG. 57.

FIG. 56.—Involution torms of *R. mutabile* as they occur in artificial culture (beef broth). *R. mutabile* can be cultured quite readily upon a great variety of culture media, showing marked adaptability to variations of food supply and in environment.

FIG. 57.—*Azotobacter agilis* deeply stained. This organism is actively motile as indicated by the pressure of numerous cilia. The closely related species *A. chroöcoccum* is less actively motile. Both possess the power of free nitrogen assimilation to a high degree, especially when cultured in a nitrogen-free medium. The organisms are large (3 to 6μ in diameter) in the comparative sense. *Clostridum pastorianum* is also an active free nitrogen assimilator, but differs from the Azotobacters in that if forms spores, a property which may render it highly valuable in economic agriculture as cultures in the sporulating stage can be kept for a long time while the cultures of non-sporulating bacteria soon die off or lose their potency.

of Caron, with a base of starch and albumen. It is used to inoculate soil either by spreading it broadcast or by sowing or otherwise planting it with the seed. It is not a nodule or root tubercle-forming organism, and does not enter into intimate symbiotic or biologic relationship with plants. Its work is simply that of binding free nitrogen, forming nitrogenous compounds which enrich the soil, thus increasing the yield of any crop benefited by such compounds.

It is known that there are soil bacteria which are more especially active with certain plants or groups of related plants, and this peculiarity has suggested the possibility of isolating them, artifically increasing their potency and using them commercially for fertilizing purposes. It is also true that not all soil bacteria are beneficient. Under certain conditions, pathogenic and otherwise, harmful microbes are present in great numbers and become very destructive to crop plants, causing diseases of roots and other plant organs. *Bacillus californiensis*, isolated from sugar beets and from sugar beet soil, appears to promote the growth of sugar beets, particularly the seedlings. The microbic leguminous fertilizer of the Department of Agriculture, Washington, D. C., is a slight modification of the Hiltner method. The microbic cultures are grown in the absence of nitrogen or nitrogenous compound making them nitrogen hungry, thus increasing their potency to produce nodules when brought in association with germinating leguminous plants. The process is patented in the United States, and free samples have been liberally distributed among farmers for test purposes, but the results reported have been rather variable, and as a whole quite unsatisfactory. The indications are, however, that future experiments will clear up the present difficulties, and some of these so-called vest-pocket microbic fertilizers will no doubt prove highly beneficial.

2. THE MICROBIOLOGY OF WATER SUPPLIES

The following is intended as an introduction to the laboratory methods employed for the examination of water supplies. The usual laboratory methods are chemical and bacteriological. The bacteriological methods usually pertain to plate and tube cultures and have been standardized and are quite generally employed in state and municipal laboratories. Those interested are advised to obtain copies of the methods as prepared and used by the Bureau of Animal Industry. The official methods, that is the methods generally employed in the municipal and state laboratories for the examination of water supplies, are incomplete as to the microbiological examination, and the organoleptic tests. Bacteria are not by any means the only objectionable organisms which occur in water, as will be explained. The microscopical examination of water has received some

attention within recent years. Perhaps the best reference work on this phase of water analysis is that by Whipple (Microscopy of Drinking Water. John Wiley and Sons, 1919) to which the student is referred. An excellent work on water bacteria is that by Prescott and Winslow (Elements of Water Bacteriology. John Wiley and Sons, 1913). Most of the authoritative works on general hygiene contain a brief mention of the microscopical as well as bacteriological examination of water supplies.

Even the most casual observation will make it clear that the study of water supplies forms the very groundwork of sanitary science. Not only is it important to study the water intended for drinking purposes, but also the water supplies used for irrigating purposes, the sewage waters, rivers, lakes, ponds, ditches, water of swamp lands, etc.

A. *Water Analysis.*—Water and food are the absolute essentials to life, and of these two, water is the more important. The water supply must be ample and must be of good quality. Water for drinking, cooking washing and bathing purposes must be free from harmful or otherwise objectionable ingredients. The water supplies intended for drinking purposes must be free from sewage contaminations and must not contain any poisons (chemical, vegetable, or animal) which might prove harmful. The importance of abundant pure drinking water cannot be too strongly emphasized. An abundant supply must be at hand or immediately available at all times. All possible desirable sources of drinking water must be carefully investigated and examined, and the supplies well guarded and none allowed to be used for other than drinking purposes, until that one all-important need is liberally supplied and future requirements assured.

Streams, large and small, lakes, ponds, wells and springs, are the main sources of drinking water. As a rule deep wells and deep source springs furnish the best drinking water, and shallow pools and small streams the poorest, considered from the standpoint of possible harmful contamination. Lakes and larger streams assure an ample supply and the quality may be fair or even very good. Mountain streams fed by melting glaciers and snow, furnish good water.

Four methods are employed in the examination of water, the organoleptic, the microscopical, the bacteriological, and the chemical. Each method has its special merits and all four are essential.

As to the organoleptic tests, water may be wholly colorless or clear; it may be turbid due to fine sand or silt and clay or dirt; it may be yellowish turbid due to fine clay; it may be a yellowish lemon tinge due to diatoms; or greenish due to nostoc, oscillaria and other algæ; it may be brown or flocculent brown due to iron fungi (Leptothrix and algæ); or it may be wine colored due to peat; etc. It may be and should be odorless. Rain

water (from roofs of houses) may have a smoky (creosote) odor or flavor (drained from shingled roofs of houses). Certain algæ (Nostoc, Oscillaria and others) may give rise to very disagreeable odors. Chemicals (permanganate of potassium, hypochlorites, salt, lime) may impart a peculiar taste and flavor. The containers (buckets, barrels, coolers, wood, leather, canvas, etc.) may transmit special flavors. The odor may be garlicky or otherwise disagreeable due to heavy sewage contamination. Pure water is without taste, though it does produce a pleasing gustatary sensation if cool and well aerated. The water in wells near large bodies of salt water (ocean, bay, inland seas) may have a decidedly brackish taste. Alkali seepage may impart a bitter or otherwise disagreeable taste. The water of small mountain streams is usually highly contaminated with decaying vegetable matter and frequently also contains decaying animal matter.

In the comparative sense, the highest contamination of bodies of water prevails at or very near the shore, no matter whether the water is at rest or flowing. The least contamination is found at points farthest removed from the waters edge, the bottom and the surface. Gravitation soon carries bacteria to the bottom, while some algæ tend to accumulate near or at the surface. In the case of flowing water, the gradual retardation of the rate of flow, toward the shore, causes an accumulation of deposits along the outermost edge of the stream. The intake end of the waterpipe of the pumping station should be as near to the center of the water supply as possible.

The following is a tabulation of the more important contaminations found in water supplies.

I. Derived from soils indicating surface waters and surface seepage.
- 1. Vegetable.
  - a. Protococcus group.
  - b. Desmids.
  - c. Diatoms.
  - d. Nostoc.
  - e. Oscillaria.
  - f. Yeasts.
  - g. Molds.
  - h. Spores of cryptogams.
  - i. Pollen grains.
  - j. Decayed vegetable tissues.
- 2. Animal.
  - a. Amebæ.
  - b. Paramecia, Spongilla, etc.

- c. Ova of Nematodes.
- d. Hair of animals.
- e. Insect fragments.
- f. Animal excreta.

II. Sewage Indicators.

1. Kitchen refuse. Vegetable.
   - a. Starches. Cereal, potato, bean, etc.
   - b. Spice elements.
   - c. Vegetable tissues derived from fruits, roots, tubers, bulbs, etc., used as food.
2. Kitchen refuse. Animal.
   - a. Blood corpuscles.
   - b. Oil and fat globules.
   - c. Muscle elements.
   - d. Fibrous tissue elements.
   - e. Ova of intestinal parasites.
3. Bacteria.
   - a. Coccus forms, abundant.
   - b. Diplobacilli, usually abundant.
   - c. *Streptococcus fecalis*, usually present. A positive sewage indicator.
   - d. A positive presumptive colon bacillus test.
4. Other organisms.
   - a. Yeasts and mold may be present.
   - b. Spores of molds and ova of intestinal parasites may be present.
   - c. Motile protozoa.

III. Mineral and inorganic.

1. Oil or resinoid matter derived from vegetable decay (pines).
2. Dirt and clay particles.
3. Sand particles. Coarse and fine. Colorless and colored.
4. Mineral particles and particles of mineral compounds. Colorless and with color. Iron compounds.
5. Diatomaceous earth. Kaolin. Etc.

IV. Amorphous organic particles. These are present in direct proportion to organic contamination.

The analyst should note the following regarding possibly available sources of water supply.

1. The character of the underlying geologic formation.
2. The surrounding vegetation and the animals that dwell in the vicinity.

3. The human habitations near the supply.
4. The source of the water.
5. The available amount of the supply. Cubic contents, or volume of flow per hour.
6. All possible sources of contamination and the nature of the contamination.

A microscopical examination should be made of the shore and bottom mud and ooze at the points of most likely contamination and a record made of the microscopic flora and fauna of the shore. If the body of water is small and is highly contaminated by cattle, horses, or hogs, it must be abandoned for drinking purposes, or the water first purified by the use of some coagulant, by filtration and by boiling. If the body of water is large and the shore is contaminated by animals the water must be taken from a middle point and either boiled or treated with hypochlorites, or other chemicals. Samples should be taken by means of suitable sampling bottles and some of the water thus collected should be centrifuged and the sediment examined microscopically and the findings recorded.

After the completion of the topographical survey, the organoleptic tests, and the microscopical examination, the analyst is then in position to make a definite recommendation as to what is to be done regarding the water in question. His recommendations will be along the following lines.

1. Unfit for drinking purposes and impossible or impracticable to render it potable.

2. Suitable for drinking purposes in the raw state. Safe. No treatment required.

3. Suitable for drinking purposes, after filtering through sand filter.

4. Suitable for drinking purposes, after coagulating and filtering. Sand-alum filtration. Sedimentation and filtration.

5. Suitable for drinking purposes after adding calcium hypochlorite. Hypochlorous acid sterilization of suspicious water supplies.

Additional methods for purifying water supplies are.

1. The use of Pasteur-Chamberland pressure filters. These are impracticable for large volumes of water. May be applicable in homes, public buildings and hospitals.

2. Use of gravity porous clay filters and coolers. Often quite satisfactory where the quantities of water used are comparatively small, as in private homes, stores, schools, etc.

3. Distilling the water. Rather impracticable for large volumes. Used on board ships.

4. Boiling the water for 30 minutes. Very satisfactory for rendering contaminated waters entirely safe for drinking purposes.

5. *Ultra-Violet Rays.*—The high voltage ultra-violet ray mercury lamp has been used quite successfully by the French army in the sterilization of drinking water. The lamps are of quartz which permit the more effective passage of the ultra-violet rays, and are placed directly into the water and are said to have a radius of action of one foot and sterilization is said to be complete in one minute without resulting in any physical changes in the water. The rays are not effective in heavily polluted waters nor in water which is not clear. Heavily polluted waters may first be clarified and partially purified by precipitation (the alumn method) and filtration (through sand, charcoal, cotton, etc.).

6. *Centrifugal Purification.*—The centrifuge has recently come int extensive use for the purpose of clarifying and purifying liquid substances of various kinds, as gelatinous solutions, plant juices, syrups, oils, paints, varnishes, beers, wines, etc. High speed turbine driven machines are now upon the market making from 24,000 to 40,000 revolutions per minute (the Sharples Centrifuge). The Sharples machine is of the continuous feed type which may be operated by hand (25,000 revolutions) or by either steam or compressed air (40,000 revolutions). This machine will render water absolutely clear no matter how heavily polluted, and it is said to remove most of the bacteria. In case of water which is suspected of containing disease germs (typhoid, cholera, etc.) the centrifuging must be followed by chemical or ultra-violet ray sterilization.

The analyst will find details for the application of the sand-alum filtration and the use of calcium hypochlorite in Field Hygiene by Ford and in Sanitation for Medical Officers by Vedder and in other works on sanitation.

The analyst should be thoroughly familiar with the subject of water purification and should assist the sanitary officers in their work. Sterilization of drinking water by means of chemicals has been very carefully worked out by the armies in Europe. The following chemicals have been used.

1. Chlorinated lime (chloride of lime) with 35 per cent. available chlorine gas. Action depends upon the hypochlorous acid formed. Amount used ranges from 1–1,000,000 to 1–25,000. A chlorinated lime containing 75 per cent. available gas is on the market and is preferable to the weaker lime.

2. Sodium hypochlorite is more efficient and also more expensive. Use about the same amount as of chlorinated lime.

3. Sodium bisulphate in tablets, 30 grains to the quart of water, shake,

and let stand for 20 minutes. Excellent for small quantities, as for cavalry men, and for men on the march.

4. Potassium permanganate, 1 grain to the quart, or enough to produce a pinkish coloration. Particularly efficacious against *Bacillus choleræ*.

5. Calcium permanganate is used in Germany to purify water in the canteens (1 grain to the quart). The precipitate which forms must be filtered through plug of cotton or a filter paper cap.

6. Iodine liberated from the mixing of iodide and iodate, in the canteen, has been used by the French army. The salts are put up in red, white, and blue tablets, ready for immediate use.

7. Halazone (*p*–sulphon dichloramino benzoic acid). 1–300,000 is very efficient against typhoid and cholera contaminations. This is cheap and effective. It comes in tablets ready for use.

The following titrating method for determining the amount of chlorine required to sterilize water supplies is used in the army, as reported by Vedder (Sanitation for Medical Officers, Lea and Febiger, 1917).

1. Into a rinsed ordnance cup (1 pint or about 500 cc. capacity) break one tube of calcium hypochlorite and mix thoroughly with a few drops of water. Fill the cup with water to within one inch of the top (500 cc.) and mix well by pouring back and forth by means of a second cup. This solution contains 0.3 gram of available chlorin.

2. Rinse four ordnance cups with the water to be sterilized and fill all four cups with water to be tested. To first cup add 0.2 cc. of the test solution in cup mentioned in (1). To second cup add 0.4 cc., to third 0. 6 cc. and to the fourth cup 0.8 cc. Mix well by pouring back and forth. Let stand for thirty minutes.

3. Into a clean cup crumble a tablet of potassium iodide (or use a few crystals of the iodide salt), add a little starch solution (made by boiling a little corn starch). Pour into this cup the water to which was added 0.8 cc. of the chlorite. If blue color appears, it is an indication that not all of the chlorine has been used up in that mixture.

4. The cup which contains the smallest amount of the chlorite which will give a blue color, contains the percentage of chlorine required to sterilize the water to be used.

5. *Example.*—Let us suppose that the cup to which was added the 0.4 cc. hypochlorite solution represents the smallest amount just producing a blue coloration, hence enough to sterilize one pint. To sterilize 36 gallons (288 pints, the amount in one Lyster water bag) would require 115 cc. of the test solution in cup (1). The pint of hypochlorite prepared would therefore suffice to sterilize a little over four bags of water. In

practice it is customary to use double the amount indicated by the reaction.

The caution to be observed in the use of chemical water sterilizers is to mix them thoroughly into the water and allow them sufficient time to act, at least 20 minutes. The amounts used should be adjusted to the degree of contamination which is ascertained by titration.

It must be borne in mind that filtering material, no matter what kind, soon becomes clogged with accumulated sediment and rapidly developing algæ and fungi. The filtering material must therefore be frequently renewed, in some cases every day and in no case should the interval be more than five days. The analyst should make daily examinations of the accumulated scum as this will convey valuable information as to the impurities in the water and the efficiency of the filter. The filter, if not properly attended to, may itself become a source of contamination. Crenothrix forms are very apt to develop in sand filters, especially if there is a deficiency in oxygen supply.

Water supplies (ponds, small lakes) with abundant filamentous algæ may be treated with copper sulphate (1–1,000,000) before filtration. This method met with much favor a few years ago, but has been quite abandoned and forgotten recently. The copper sulphate can be placed in a gunny sack, fastened to a boat, and distributed by rowing the boat about until all of the chemical is dissolved. 1 part in 4,000,000 has given good results, as far as freeing the water of filamentous algæ and also protozoa, is concerned. After filtration no copper can be found in the water. The filtering material must, of course, be renewed occasionally.

Sedgwick and Rafter have devised a method of making a microscopical examination of water. Desirable quantities of water are run through a graduated cylinder, having a sand plug at the lower open end. In place of this cylinder an ordinary glass or tin funnel may be used. Place a perforated stopper (cork or rubber) at lower end, carrying bent outflow tube. On top of opening in stopper place a bit of cotton or cheesecloth, followed by ignited sand quartz (sands, Nos. 60, 120, 140, as may be desired). On top of the sand place another bit of cotton or cheesecloth. Pour through the desired volume of water (250 cc., 500 cc., 1000 cc., etc.), and then mix the sand (with the cotton and cloth) in 5 or 10 cc. of distilled or pure water and examine microscopically making the desired counts.

The following blank report sheet of a bacteriological examination will serve to illustrate the nature of such analysis in a state or municipal laboratory:

Report of the Bacteriological Examination of Water

Laboratory No............. Date Reported.................
Town or locality...
Source of water..
Location of sampling point...
Collected by.......................................Date.................
Reported to...
Number Bacteria per cc., Gelatin 20° C..............Agar-37.5° C..............
B. Coli......confirmed in......cc. B. Coli Index, approximate number per cc. ....
Turbidity..........Alkalinity as $CaCO_3$ ....Chlorine.........Hardness........
(Results in parts per million)

Condition of sample:
Remarks:

Approved:

....
Chemist and Bacteriologist Director, Bureau of Sanitary Engineering

EXPLANATION OF RESULTS

The laboratory results can be properly interpreted only in the light of a comprehensive field examination of the source of the water by an expert. Laboratory tests can not show whether colon bacilli in the water were derived from the excreta of animals or the much more dangerous sewage carrying the feces of human beings. Neither can the laboratory determine whether a slight pollution at one time would mean a heavy pollution at another, owing to fluctuations in the volume of sewage or to seasonal variations in the amount of water, disturbance of sediment by waves, etc. Careless sampling and growth in transit are large factors.

Certain conclusions can, however, be drawn from the laboratory reports alone. If colon bacilli are not demonstrable in 10 cc. of the water, it may be regarded as safe for drinking purposes at the time of the taking of the sample, as far as sewage pollution and consequent danger from typhoid fever and other water-borne diseases is concerned. If colon bacilli are demonstrable in 10 cc., but not in 1 cc. the water may be looked upon as under suspicion, and a field examination is necessary to determine whether or not it is safe. If colon bacilli are found in 1 cc. of the water, but not in 0.1 cc. the water should be regarded as probably unsafe, and a purification process should be installed if the sources of contamination, animal or human, can not be removed. If colon bacilli are found in 0.1 cc. of the water, or smaller amounts the water should be considered as polluted to such a point as to be unsafe for drinking purposes.

The B. Coli Index is the reciprocal of the smallest portion of water in which the B. Coli group is confirmed. The number of B. Coli in a reasonably safe water should be less than 0.1 cc.

3. BACTERIA IN MILK AND IN THE DAIRYING INDUSTRY

A. General Discussion

Bacteria play an important part in modern dairying, and they are destined to play even a more significant part in the near future. Certain microbes are active in the ripening of cream, butter and cheese. Formerly it was customary to let nature attend to the inoculation of the cheese, resulting in a rather variable product. Now the up-to-date dairy-man

inoculates the cheese with pure cultures of the kind of microbe producing the desired flavor as Roquefort, Bre, Limburger, etc. In time it will no doubt be possible to produce hitherto unheard-of cheese flavors by means of new species, varieties, and strains of cheese microbes. Cream-and butter-flavor bacteria are also used. The souring of milk is due to the omnipresent but illy defined *Bacillus acidi lactici* and other bacteria. Stringy or ropy milk is due to bacterial infection. Under conditions favorable to the development of the organisms, the ropiness appears within from twelve to twenty-four hours after milking, and becomes so pronounced that the milk can be drawn out in long threads or strings.

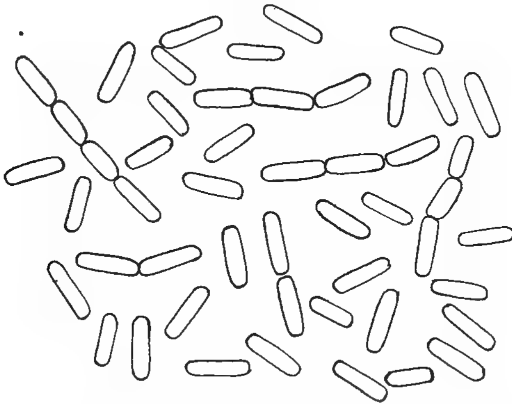


FIG. 58.—Lactic acid bacillus. There is a large group of bacteria, similar in appearance to the lactic acid bacillus, which have the power of forming lactic acid in milk. Some of these are used in pure culture to make the so-called artificial buttermilk. Milk bacteriology is still in its infancy. For so long have we been accustomed to the use of contaminated (filthy) milk that in a recent test made with samples of pure milk and samples of which cow manure was added, 90 per cent. of those who were asked to taste the milks preferred the milk to which the cow manure was added, declaring that it was the only sample which had a "milk flavor."

It is a not uncommon condition of milk in Switzerland, where is is considered specially noxious, but in Holland it has been produced by design for making Edam cheese. Ropiness of milk is caused by a variety of microörganisms, among them being *Bacillus actinobacter*, *B. lactis viscosus*, *B. gummosus*, etc. The microörganism used in Holland for the manufacture of the cheese referred to is known as the *Streptococcus Hollandicus*. The *Bacillus cyanogenus* causes the milk to become blue without coagulating it or rendering it acid. The *Bacillus butyricus* occurs in milk which it coagulates, also producing butyric acid. It is this microbe which develops the rancidity of butter. There are, however, many different species of microbes which produce butyric acid fermentation.

Freshly drawn milk is not germ-free, even under the most aseptic and sanitary conditions and surroundings. As a rule even the milk in the udder contains some germs, in spite of the fact that milk possesses decidedly bactericidal properties. However, the milk from different animals varies in this regard. The bacterial impurities of freshly drawn milk are traceable to the skin of the cow, the dust and filth about cow stables, the vessel containing the milk, and above all to the hands of the milkers. The milker is often the cause of inoculating the milk with disease germs, as typhoid, acute dysentery, diphtheria, scarlet fever, small-pox, and tuberculosis. The medical journals cite cases of typhoid epidemics traceable to milkers who were "typhoid carriers" without actually suffering from the disease. Cows are very susceptible to tuberculosis, and the milk from tuberculous animals has infected thousands upon thousands of children and many adults.

Since milk is an excellent culture medium for a great variety of germs, it is evident that, under favorable conditions, it may be a fruitful source of infections. Serious epidemics of typhoid fever and of diphtheria have been traceable to and exactly limited to the milk route of a certain dairyman. Tuberculous infections of the children in a number of families have been traceable to the milk from a single animal. As a rule mixed milk (that is the milk from many animals) is safer than the milk from a single animal, though this is not necessarily always the case. The milk from animals that are free from disease and that are tested regularly once each year) for tuberculosis, and that are kept under sanitary conditions, is absolutely safe, provided the containers are clean and the milkers and others in the dairying establishment are free from latent or active communicable disease and are cleanly in their habits. The number of germs in freshly drawn milk varies from 1000 to several millions per cc., and is directly proportional (within the limits indicated) to the cleanliness and sanitary conditions of the dairying establishment. The bacterial content of milk from the same source is of course higher in warm and hot weather than it is in cold weather, other things being equal. Certain dairying establishments supply what is known as "certified milk," or milk which is certified by the board of health as coming from animals that are regularly tested for tuberculosis and which are kept under the sanitary conditions imposed by the milk commission or by the board of health, furthermore, such milk must be bottled in sterilized bottled which are hermetically sealed and placed on ice at once and kept on ice until delivered to the consumer. There is, however, a lack of uniformity in the regulations governing the supply of certified milk in different communities. The following conditions *should* prevail:

a. All cows should be healthy, that is, free from diseases of *all* kinds.

The animals should be tested for tuberculosis every six months. As soon as an animal gives a positive reaction for tuberculosis, it should be removed from the herd and killed. Milk from sick animals should not be used.

b. The sanitary conditions and environment of pasture, grazing lands, sheds, stables, etc., should be excellent. The entire water supply should be pure, and all water supplies should be tested chemically and bacteriologically at suitable intervals. All food supply for cows must be wholesome and free from objectionable contaminations.

c. Those employed about the establishment must be free from latent or active disease. They should be tested for tuberculosis, latent typhoid, and should be examined for skin diseases. They must be cleanly in their habits. Before milking, the hands of the milkers and the teats of the animals should be washed with clean warm water and then dried with a clean towel.

d. The containers must be sterilized thoroughly every day, inside and outside. This can be done by thoroughly washing and rinsing in boiling hot water and thoroughly drying, before pouring milk into them.

e. Just as soon as the milk is drawn, it should be bottled (sterilized bottles), bottles capped, hermetically sealed (paraffin), and placed on ice until ice-cold, and delivered at once to the consumer. The bottles should be on ice in delivery, and, even though hermetically sealed, should be kept away from dust and dirt. The bottles should be placed in paper bags so that the driver need not touch them at all. The housewife should take the bottle from the bag and place it in the ice-chest, cellar, or cooler until the milk is wanted for use.

Milk, on standing, should show no dirt deposit. This crude test is a fairly reliable guide as to the sanitary conditions in the dairying establishment and the rules of cleanliness that are observed. It has been shown that the quantity of bacteria in freshly drawn milk is directly proportional to the amount of dirt (sediment) present. A bottle or tumbler full of milk should show no dirt sediment after standing for an hour or longer.

Good cows' milk should have from 3.5 to 4.0 per cent. of butter fat. It is marketed in three forms: Full milk having all of the butter fat, half milk or partially skimmed milk, and skimmed milk. Because of the variability of milk which is partially skimmed, it would be wise to withdraw it from the market. When milk is sold without further specification, full or unskimmed milk is understood. It is unlawful to sell skimmed milk as milk, or without designating it as skimmed milk.

In some countries, as Germany for example, the rules and regulations directed against dairies, dairying and the sale of milk, are very far-reaching, and are strictly enforced by the local health authorities. Specific rules are laid down as to what milk may or may not be marketed, how the cows are

to be kept, what cattle diseases render the milk unfit for use, how cows and milkers must be prepared for the milking process, etc. The use of preservatives is not permitted, because these substances reduce the digestibility of the milk and because their use encourages lax and careless methods in the dairying establishments.

The bovine disease most to be dreaded is tuberculosis. It is very prevalent among cattle, and the milk from tuberculous cows is a serious menace to the health of those who use it, particularly to susceptible (by inheritance children. The most efficient means of safeguarding the public health against this source of infection consists in removing the infected animals from the herd, with a view to disposing of them by slaughter and burial as soon as circumstances will permit. Where this wasteful method has been employed the results have been discouraging, even when the State recompensed the owner in part for the loss of his stock. The government meat inspection regulations now admit the use of meat of slightly tuberculous animals, for it is declared that under such circumstances the thorough cooking of meat is an effective safeguard against danger.

Testing cows for the presence of latent or undeveloped forms of tuberculosis is simple, safe, and should be rigidly persisted in. Turberculin is injected into the neck or shoulder region. If tuberculosis exists there will be a rise in temperature (102° to 104° F.), in the course of from eight to eighteen hours. If the disease is far advanced there may be no reaction, in fact, the reaction is then unnecessary as the indications are already sufficiently positive.

The tuberculin used is prepared from glycerinated bouillon in which tubercle bacilli have been grown from six to eight weeks. The bouillon culture is first boiled for two hours to kill all the living organisms. It is then filtered under pressure through a germ-proof earthenware filter to remove the dead bodies of the germs, concentrated by evaporation, a little carbolic acid added, and it is then bottled for distribution. There is no evidence that its use causes an increase in the rapidity of the progress of the disease in animals already affected with tuberculosis, or that it is injurious to them in any other way. It does not even temporarily injure the quality of the milk.

Preservatives, as boric acid, salicylic acid, benzoic acid, sodium benzoate and formalin, are sometimes added to milk to prevent bacterial development. A very small amount of formalin (1:10,000) is sufficient to check the souring of milk. The others are added in larger amounts (1:1000 or more). These additions are not, as a rule, appreciable through the sense of taste or smell and do not in any way modify the appearance of the milk. In some countries milk preservatives are permissible, in others they are not, and in still others they are permitted provided there is a declaration

to that effect and the amount does not exceed a definite percentage, as provided by law.

In England, a limited amount of certain preservatives added to milk is permissible, the argument being that it is better to supply preserved milk than milk loaded with germs. This argument has its commendable features. In very large, congested cities like London, New York and Chicago, it is impossible to supply the poor with certified milk or milk which can be kept free from excessive germ development until it is wanted for consumption.

Boiling the milk for twenty minutes kills the germs, but unfortunately the boiling temperature produces certain changes which greatly reduce the food value of the milk, besides the germicidal properties of the milk are destroyed, so that the bacterial development is afterward even more active than before. Sterilizing at lower temperature (50° to 80° C.), known as pasteurizing, does not interfere with the nutritive qualities of the milk, but destroys the bactericidal properties, as already mentioned. The process is, however, generally recommended by physicians. A simple home method may be carried out as follows (Roger):

Milk is most conveniently pasteurized in the bottles in which it is delivered. To do this use a small pail with a perforated false bottom. An inverted pie tin with a few holes punched in it will answer this purpose. Punch a hole through the cap of one of the bottles and insert a thermometer. Set the bottles of milk on the pie tin in the pail and fill the pail with water nearly to the level of the milk. Put the pail on the stove or over a gas flame and heat it until the thermometer in the milk shows not less than 65° C. nor more than 70° C. The bottles should then be removed from the water and allowed to stand from twenty to thirty minutes. The temperature will fall slowly, but may be held more uniformly by covering the bottles with a towel. The punctured cap should be replaced with a new one, or the opening sealed with wax or paraffin, or the bottle may be covered with an inverted cup.

After the milk has been held as directed it should be cooled as quickly and as much as possible by setting in water. To avoid danger of breaking the bottle by a too sudden change of temperature, this water should be warm at first. Replace the warm water slowly with cold water. After cooling, milk should in all cases be kept at the lowest available temperature.

It should be remembered that pasteurization does not destroy all bacteria in milk, and after pasteurization it should be kept cold and used as soon as possible.

Rosenau sums up the *pros* and *cons* of milk pasteurization as follows:

Advantages.—The advantage of pasteurization is that it is a cheap and

effective means of preventing the transmission of infectious diseases such as tuberculosis, typhoid fever, diphtheria, scarlet fever, etc., commonly spread by milk.

Disadvantages.—a. Pasteurization promotes carelessness on the farm and dairy, etc. (This may be controlled by proper regulations, inspections and laboratory examinations.)

b. Pasteurization renders milk less digestible. (While it is generally conceded that boiled milk commonly induces constipation, the majority of the evidence plainly indicates that pasteurization has little, if any, effect on the digestibility of the milk.)

c. Pasteurized milk favors the production of rickets and scurvy. (There is no proof to this effect and authorities agree that the danger is slight; and, further, that it may readily be obviated.)

d. By destroying the non spore-bearing bacteria, pasteurization sometimes allows toxic organisms to grow and produce serious poisons in the milk. (On the other hand, these same poisons are more frequently produced in milk that has not been pasteurized, and thus danger may be obviated in pasteurized milk by cooling it quickly, keeping it cold and shortening the time for distribution.)

e. Pasteurization is inefficient as a preservative; the milk keeps only twelve to twenty-four hours longer than otherwise. (This is really no disadvantage, for the quicker bad milk sours, the better.)

f. Pasteurization injures the taste of the milk. (This is not so, if properly done.)

g. Pasteurization increases the cost of the milk. (True, but it is the cheapest safeguard, and the expense of pasteurization is offset by the keeping quality of the milk.)

Rosenau has made extensive tests to determine the thermal death-point of those pathogenic microbes most commonly found in milk. His conclusions are summarized as follows:

Milk heated to 60° C. and maintained at that temperature for two minutes will kill the typhoid bacillus. The great majority of these organisms are killed by the time the temperature reaches 59° C., and few survive to 60° C.

The diphtheria bacillus succumbs at comparatively low temperatures. Oftentimes it fails to grow after heating to 55° C. Some occasionally survive until the milk reaches 60° C.

The cholera vibrio is similar to the diphtheria bacillus regarding its thermal death-point. It is usually destroyed when the milk reaches 55° C.; only once did it survive to 60° C. under the conditions of the experiments.

The dysentery bacillus is somewhat more resistant to heat than the

typhoid bacillus. It sometimes withstands heating at 60° C. for five minutes. All are killed at 60° C. for ten minutes.

So far as can be judged from the meager evidence at hand, 60° C. for twenty minutes is more than sufficient to destroy the infective principle of Malta fever in milk. *M. melitensis* is not killed at 55° C. for a short time; the great majority die at 58° C., and at 60° C. all are killed.

Milk heated to 60° C. and maintained at that temperature for twenty minutes may, therefore, be considered safe so far as conveying infection with the microörganisms tested is concerned.

Evaporated, condensed and dry milk are found upon the market and are extensively used. Sugar is frequently added as a preservative. In making condensed milk, it is evaporated in large pans until it assumes a creamy consistency. Dry milk is prepared by spraying the milk on revolving hot cylinders. The thin film of milk is evaporated to dryness in a moment, and in that state is scraped from the cylinders. Dry milk is a common ingredient of baby foods and invalid foods, and is also very extensively used in the manufacture of chocolate creams. The condensed and dry milks do not keep long in spite of the greatest care in manufacture. The containers and milk must be thoroughly sterilized or pasteurized, and the cans must not be opened until ready for use. Such preservatives as salicylic and boric acid are sometimes added to condensed milk.

It is known that sweet cream yields a very insipid, flavorless butter, whereas cream which has "soured" for a few days yields a pleasant tasting and pleasingly flavored butter, provided the desirable species or variety of bacteria are present. If the souring is continued too long the flavor may be hopelessly vitiated. In the past it was customary to add a small amount of old cream, having a desirable flavor, to a new lot of cream. This mother cream was designated the "starter." It contained the desirable cream-ripening bacteria, mostly of the lactic acid variety. These old-time natural starters are now largely replaced by starters, prepared in the laboratory consisting of pure cultures of certain strains or varieties of cream flavor, producing germs of the lactic acid group. A proper regulation of the temperature is very important in the ripening of cream (60° to 75° F.). It is also necessary to pasteurize the cream before adding the bacterial starter in order to prevent the development of microbes which might interfere with the proper development of the starter microbes. Naturally the use of clean, sterilized utensils and uniformity of methods are all-important, in order that uniform results may be obtained.

Cheese flavors are also due to bacterial action, but not wholly so, as many of the higher fungi, as species of *Penicillium* (Camembert Penicillium) and of *Oidium* (*O. lactis*) also play a very important part as flavor producers. The Roquefort cheese owes its characteristic flavor, in

part at least, to a variety or form of *Penicillium glaucum*. The qualities and properties of some Swiss and soft Belgian cheeses are largely due to *Oidium lactis*. The ripening of hard cheeses (Cheddar, Edam, American, some Swiss varieties, and others) is due exclusively to bacterial action. Cream, butter and cheese are very prone to the attacks of objectionable bacteria and moulds which cause very unpleasant flavors and bitter taste.

It must also be borne in mind that cream, cheese and butter may carry disease germs. Tubercle bacilli have been reported in these food articles, but it has not been demonstrated that they are frequently present. Typhoid infections have been traced to the use of cream, but no case of typhoid fever has ever been definitely traced to eating butter or cheese. Of course, these articles may become infected after manufacture and thus become a possible means of spreading disease.

B. The Bacteriological and Microscopical Examination of Milks

The examination and rating of milk is largely a municipal affair and every city of any considerable size has a Board of Health or an officer who is empowered to enforce the regulation governing the quality of the milk to be sold. Dealers in milk and dairy men are required to produce evidence that they are complying with the health ordinances pertaining to the sale of milk for human consumption. Score cards are provided which the keepers of dairies must fill out. Evidence as to the conditions under which the cows are kept must be furnished. Samples are taken from time to time and analyzed in the city laboratory, which examination is generally chemical and bacteriological. The legal definitions of the different kinds of milk are given and the analysts are required to show whether or not the quality of the milk under examination conforms to the definition. The following statements will make these points clear.

1. *General Statement as to Quality.*—The lacteal secretion obtained from the domesticated cow and from other animals, as goat, mare, and ass, is a highly important food article. The chief food ingredients which it contains are casein, fat, and sugar (lactose). The major bulk is water. Milk happens to be an ideal food for many bacteria and raw milk freely exposed to air undergoes complete bacterial decomposition in a comparatively short time, the rate of decomposition depending on temperature, oxygen supply and degree of contamination. The organisms which may be considered as the normal milk decomposers are the group of lactic acid formers, or the milk sourers. These appear to be omnipresent in the air and upon the earth's surface, entering air exposed milks and by their rapid multiplication soon crowd out other associated air bacteria.

The production of pure milk is all-important and pure milk can be

supplied provided sanitary rules and regulations are strictly observed and enforced.

Temperature and climatic conditions are of great importance in regulating the bacterial contamination of milks. Thus there is in many states and localities, a summer standard and a winter standard. Again, the varied and variable dairying conditions has resulted in the recognition of several grades of milk, as certified, guaranteed, Grade A, Grade B, etc.

The milk from diseased animals is universally recognized as objectionable and no one would knowingly use such milk. Milk also serves as a carrier of disease, such as tuberculosis, various forms of coccic and streptococcic infection, typhoid, dysentery, diphtheria, etc. These are all matters of general knowledge to sanitarians and need not be discussed more fully.

2. *Milk Impurities.*—Under the compound microscope, ordinary cow's milk may show the following elements.

a. Butter fat globules. Variable in size, occurring singly and more or less agglutinated.[1]

b. Casein granules. Very minute and formless, granular.

c. Body cells. Epithelial cells, leucocytes, red blood corpuscles, pus corpuscles.

d. Impurities derived from cow, from cow stable, from cattle feed.

e. Impurities derived from milkers.

f. Impurities derived from containers.

g. Impurities derived from air and dust.

h. Pathologic impurities from the milk yielding animals and from the human associates.

As far as the micro-analytical examination of milk is concerned, no attempt is made to identify the different species of microörganisms which may be present. The essential is to determie whether or not the milk in question is fresh, pure, and wholesome.

3. *General Milk Rating.*—Two distinct milk ratings must be noted. The older rating based upon plate and tube cultures, still operative or applied in most laboratories, and the more recent rating based upon direct microscopical examination. The older method (plating method) gives evidence of the approximate number of living bacteria present only and is quite limited in scope and significance. The direct microscopical examination gives evidence as to the following.

1. Total number of dead and living bacteria present.
2. Body cells of all kinds.
3. Colostrum (fat globules and characteristic ameboid body cells).
4. Dirt and similar impurities present.
5. Butter fat present.

[1] Boiling and pasteurizing causes the fat globules to agglutinate.

The following extracts from the California State Pure Milk Act explains the essentials regarding pure milk.

1. *Tuberculin Test.*—It shall be unlawful for any person, firm or corporation, except in bulk to the wholesale trade, to sell or exchange or offer or expose for sale or exchange for human consumption any milk from cows that have not passed the tuberculin test, until it has been pasteurized by the holding process at a temperature not less than one hundred forty degrees Fahrenheit for twenty-five minutes; provided, that milk for drinking purposes shall not be heated above one hundred forty-five degrees Fahrenheit. It shall further be unlawful for any person, firm or corporation to sell or exchange or offer or expose for sale or exchange any milk products except cheese, into the composition of which any milk enters other than that permitted in this section of this act, to be sold at retail. For the purpose of this act milk shall be construed to include cream.

2. *Uninspected Milk.*—It shall be unlawful for any person, firm or corporation to sell or exchange, or offer for sale or exchange, in any city, country or city and county, in which a milk inspection service, approved by the state diary bureau, has been established, any milk otherwise than as hereinafter provided in this act, and for the purpose of this act, the term "inspecting department" shall be construed to mean the health department of a county or group of counties, city or group of cities, or city and county, maintaining a milk inspection service approved by the state dairy bureau.

3. *Impure Milk.*—All milk, except certified milk, guaranteed milk, grade A milk, and grade B milk, is hereby declared to be impure and unwholesome and must not be sold for human consumption.

4. *Grades.*—For the purpose of this act, milk shall be graded as follows: certified milk, guaranteed milk, grade A milk, grade B milk and milk not suitable for human consumption; provided, that milk not suitable for human consumption shall be plainly so marked.

5. *Guaranteed Milk.*—No person, firm or corporation shall sell or exchange, or offer or expose for sale or exchange, as or for guaranteed milk, any milk, raw or pasteurized the quality of which is guaranteed by the dealer, without approval in writing of the inspecting department, which milk must be of a higher standard than that required for grade A raw milk.

6. *Grade "A" Milk.*—No person, firm or corporation shall sell or exchange, or offer or expose for sale or exchange, as and for grade A milk, any milk that does not conform to the rules and regulations and the methods and standards for production and distribution of grade A milk adopted by the inspecting department.

Grade A milk shall conform to the following requirements as a mini-

mum: if raw, it shall consist of the clean raw milk from healthy cows as determined by physical examination and by the tuberculin test by a qualified veterinarian under the supervision of the inspecting department, and from dairies that score not less than seventy per cent. on the score card adopted by the United States bureau of animal industry, department of agriculture. The tuberculin test must be repeated annually if no reacting animals are found in the herd. If reacting animals are found they must be removed from the herd, and the tuberculin test repeated in six months. All cows are to be fed, watered, housed and milked under conditions approved by the inspecting department. All persons who come in contact with the milk must exercise scrupulous cleanliness and must not harbor the germs of typhoid fever, tuberculosis, diphtheria, or other infectious diseases liable to be conveyed by milk. Absence of such infections shall be determined by cultures and physical examination, to the satisfaction of the inspecting department.

This milk is to be delivered in sterile containers and is to be kept at a temperature established by the inspecting department until it reaches the ultimate consumer, when it must contain less than one hundred thousand bacteria per cubic centimeter. If pasteurized it shall come from cows free from disease as determined by physical examination at least once in six months, by a qualified veterinarian of an inspecting department. It shall contain less than two hundred thousand bacteria per cubic centimeter before pasteurization and less than ten thousand bacteria per cubic centimeter at the time of delivery to the ultimate consumer. Dairies from which this milk is derived must score at least sixty on the score card adopted by the United States Bureau of animal industry, department of agriculture.

7. *Grade "B" Milk.*—No person, firm or corporation shall sell or exchange, or offer or expose for sale or exchange, as and for grade B milk, any milk that does not conform to the following requirements as a minimum: it must be obtained from cows in no way unfit for the production of milk for use by man, as determined by physical examination at least once in six months by a qualified veterinarian of an inspecting department. Before pasteurization such milk shall contain less than one million bacteria per cubic centimeter. After pasteurization it shall contain less than fifty thousand bacteria per cubic centimeter.

Milk for pasteurization must be kept at a temperature established by the inspecting department up to the time of delivery to the pasteurization plant and rapidly cooled after pasteurization to a temperature of fifty degrees Fahrenheit, or below, and so maintained to the time of delivery of the same. Pasteurization shall be by the holding method at a temperature not less than one hundred forty degrees Fahrenheit; provided,

that milk for drinking purposes shall not be heated above one hundred forty-five degrees Fahrenheit.

Such pasteurizing plant shall be equipped with a self-registering device for record of the time and temperature of pasteurization. Such records shall be kept for two months and be available for inspection by any health department, the state veterinarian or any of his agents, or the state dairy bureau. Pasteurized milk shall be marked with the day of the week of pasteurization and must be delivered to the consumer within forty-eight hours thereafter. If milk is repasteurized, it must not be sold except as not suitable for human consumption.

8. *Milk Not Suitable for Human Consumption.*—Milk not suitable for human consumption may be sold for industrial purposes, provided it be heated to a higher temperature than necessary for pasteurization, and delivered in a distinctive container, plainly marked with the words "Not suitable for human consumption," in letters not less than one-quarter inch in length and one-twelfth inch stroke.

The following limit counts are based upon direct microscopical examination and is intended as a guide to analysts.

TOTAL NUMBER PER CC.

Grades	Bacteria	Body cells	Organic particles	Fat globules
Ordinary	50,000,000	500,000	1,000,000	
Certified	20,000,000	40,000	50,000	2,000,000,000
Guaranteed	25,000,000	40,000	50,000	to
Grade A	20,000,000	40,000	50,000	4,500,000,000
Grade B	50,000,000	40,000	75,000	

Canned Milk

The usual laboratory routine in the examination of canned milks is chemical, ascertaining total solids, butter fat, lactose, sucrose, etc. The usual quality standards for evaporated milks, including the standard of the United States Bureau of Chemistry, are based upon chemical composition rather than upon means for ascertaining possible organic contamination. The Bureau of Chemistry Standard specifies that "evaporated milk should be prepared by evaporating fresh, pure milk obtained from healthy cows." The following method will make it possible to determine the freshness, purity, and wholesomeness of, or the bacterial and other contamination in evaporated milk, *after* such milk has been canned, processed, and offered on the market.

While the processing is intended to and does kill all organisms which may be present in the milk, it does not destroy or decompose them, or

render them invisible or unrecognizable under the compound microscope. The microscope will therefore reveal all of the microörganisms, and also other contaminations, which were introduced or which developed in the milk prior to and up to the very moment of the final processing.

The following findings and recommendations pertain to evaporated milks, whole and skimmed, sweetened and unsweetened:

1. The organisms most commonly present are

a. Cocci,
b. Diplococci,
c. Diplobacilli,
d. Streptococci,
e. Bacilli,
f. Tetracocci (Sarcina),

naming them in the order of their relative abundance. Of these groups, the diplococcus, diplobacillus, and streptococcus forms are the most important from the viewpoint of the food bacteriologist.

2. Many of the coccus and diplococcus forms are no doubt derived from the streptococcus forms. Because of the fact that coccus forms may be confused with minute fat globules and nonbacterial organic particles of spherical form, it is suggested that the coccus count be omitted.

3. The diplobacillus forms thus far observed are readily confused with diplococcus forms, because the individual cells are but slightly elongated, the two diameters being as 1 : 1.3. It is therefore recommended that the diplococcus and diplobacillus forms be included in one and the same count.

4. Bacilli are, as a rule, sparingly and irregularly present in evaporated milks. The same may be said of the tetracoccus forms.

5. It does not appear practicable to make body cell counts of evaporated milks, and it is recommended that this be omitted as a laboratory routine.

6. The amount of organic debris present in evaporated milk is quite variable in different cans of a given brand of canned milk. It is, however, recommended that milk be examined as to the relative amount of organic debris present, bearing in mind that the careless or inexperienced microanalyst may confuse casein particles, agglutinated butter fat globules and lactose crystals, with organic debris.

7. Organoleptic testing is of considerable importance in milk examination. Consistency, color, odor, and taste, should be carefully noted.

8. It is also self-evident that the presumptive colon bacillus test should give negative results with all heat-sterilized milks.[1]

[1] It is a notable fact that pasteurization does not always kill the colon bacilli in milk. Numerous samples of pasteurized milk have been found which subsequently contained practically pure cultures of the colon bacillus.

The following recommendations are made:

A. That the quality and purity of sterile evaporated milks be based upon the following findings given in order of their importance:

a. Diplobacilli,
b. Diplococci,
c. Streptococcus chains,
d. Organic debris, other than casein or butter fat.

It is recommended that the diplococcus and diplobacillus forms be included in one and the same count, and that the count be stated as so many pair per cc. Linear groupings of three or more coccus forms are to be counted as streptococci, and the count is to be stated as so many chains per cc.

B. That sterile evaporated milk be declared below standard on the following counts:

100,000,000, or more, pair of diplococci and diplobacilli per cc., or
300,000, or more, chains of streptococci per cc., or
both; with or without the presence of body cells or any considerable amount of organic debris, and with or without bacilli, tetracocci, or other associated microörganisms.

C. That in case of non-sterile evaporated milk, or raw milk intended for the canning and evaporating process, and in inadequately processed canned milk, or milk in imperfectly sealed tins, or milk in unsuitable tins, (tins with "Friction caps," for example), the direct microscopical examination be supplemented by the usual plating, tube, and fermentation tube culture methods.

D. That under organic debris there should be included such organic particles as are distinctly recognizable as other than sugar crystals, casein granules or casein masses, or particles of butter fat. It may include the following substances:

a. Vegetable tissue elements derived from field, soil, stable manure, or cattle feed.
b. Dirt particles.
c. Stringy shreds of albuminous matter.
d. Variously colored (mostly reddish brown), irregular, amorphous, or somewhat crystalline particles of resinoid character, probably largely of vegetable origin (decomposition products).
e. More or less disintegrated and not distinctly recognizable body cells (epithelium, leucocytes, endothelial cells).

These, and other particles of organic debris, are readily observable under the low power of the compound microscope. In homogenized milks that streptococcus chains are often well broken up, becoming largely reduced to diplococcus forms and also to coccus forms.

In the examination of full milks, fresh, evaporated, sweetened or unsweetened, and inclusive of creams, ice creams, etc., a small high speed

(2800 revolutions per minute) hand centrifuge is required. Ice creams, creams, sweetened evaporated milks, must be suitably diluted (1–5 as a rule) before centrifuging in order to facilitate the separation of the bacteria and organic debris and the butter fat. In case of sweetened evaporated milks, the dilution must be sufficient to dissolve all of the lactose and added sucrose. To hasten the solution of the lactose, heat may be employed.

Place 10 cc. of the milk or the dilutions thereof in special two part (1 cc. + 9 cc.) tubes and centrifuge for ten minutes. Remove the 1 cc. end tube, holding the sediment, and mix the contents thoroughly and from this make the bacterial counts, using dilutions as may be required.

The coccus forms and other microörganisms in heat sterilized milks have lost much of their original staining properties. Dead bacteria generally, especially those killed by moist heat, as a rule, react feebly with the usual stains. The streptococcus form found in evaporated canned milk most generally seen, appears to have the morphological characteristics of the *Streptococcus acidi lactici* of Kruse, which is believed to be entirely harmless to man and to be in normal lactic acid fermentation of milk. It is, however, quite immaterial from the standpoint of the purity and freshness of milk, whether the organisms found are harmless or not.

In numerous samples of canned milk where the coccus forms were counted, the numbers ranged from 15,000,000 to 200,000,000 per cc. and over, and the coccus count as a rule exceeded the sum of the diplobacillus, diplococcus and streptococcus counts in the ratio of 4:3.

Based upon the examination of numerous samples of several brands of evaporated skimmed milks, a count of 100,000,000 pair per cc. of diplo. forms, as a rule, corresponds to about 150,000,000 coccus forms, per cc. or a total of 250,000,000 microörganisms per cc. There appears to be no fixed number ratio between coccus and diplo. forms on the one hand and streptos. on the other, hence the suggestion that these groups be given separate rating values.

4. *Municipal Milk Scoring.*—In addition to a score card which indicates the condition of the dairying establishment and which score will indicate whether or not the owner of the dairy is complying with the requirements of the city ordinance, the laboratory analysts are required to make examinations and fill out report blanks, after the following.

A. TABLE FOR SCORING CHEMICAL EXAMINATIONS OF MILK

	Score 100
Total solids;	
13.2 % or more	
For each .1 % under 13.2 % deduct 2 points on score.	
For added water, deduct 100 points.	
If per cent. of butter fat is less than 3 %, deduct 100 points.	
For milk containing preservatives, deduct 100 points.	

B. TABLE FOR SCORING BACTERIOLOGICAL EXAMINATIONS OF MILK

Raw Milk		Pasteurized Milk				
Grade A		Grade A		Grade B		
Bacteria in thousands per c.c.	Score in points	Before pasteurization. Bacteria in thousands per cc.	After pasteurization. Bacteria in thousands per cc.	Before pasteurization. Bacteria in thousands per cc.	After pasteurization. Bacteria in thousands per cc.	Score in points
50 to 100	140	100 to 200	8 to 10	500 to 1000	40 to 50	70
25 to 50	160	50 to 100	6 to 8	400 to 500	30 to 40	80
10 to 25	180	25 to 50	4 to 6	300 to 400	20 to 30	90
under 10	200	under 25	under 4	under 300	under 20	100

Score for raw milk is obtained directly from table.

Score for pasteurized milk is obtained by adding together the score before pasteurizing and the score after pasteurizing.

For raw milk deduct 20 points for the first 1000 colonies of the colon group or streptococci, whichever may be the more numerous, and deduct 10 points for each subsequent 1000.

For milk after pasteurization deduct 10 points for the first 100 colonies of the colon group or streptococci, whichever may be the more numerous, and deduct 2 points for each subsequent 100.

4. THE LACTIC ACID MICROBE AND KEFIR PREPARATION

Within recent years the subject of intestinal digestion and the relationship of intestinal microbes to digestion and longevity has received much attention. Metchnikoff declares that the early senile cell changes in the body are due to the repeated or chronic autointoxications brought about by certain noxious intestinal ferments of bacterial origin which are absorbed into the circulation. Some of these bacteria, especially those found in the small intestines, are beneficial, secreting enzymes which aid digestion, but the enormous quantity of microbes active in the lower large intestine are for the most part injurious, producing putrefactive changes, liberating toxins which when absorbed into the system in sufficient quantity produce the symptoms of ptomaine poisoning.

In order to combat these objectionable bacterial activities, it is necessary to regulate the bacterial development in the large intestine. Lactic acid has long been known as an efficient remedy in the treatment of various intestinal disorders. It is known that the poor of certain European countries who live largely on potatoes and clabbered or thick milk are notably free from intestinal disorders and are remarkably long-lived. It is known that pickles, sauerkraut and sour milk are excellent bowel regulators, in spite of the fact that these foods, the former two in particular are well-nigh indigestible and have little food value. The Arabians have long used koumys as a healthful, life-prolonging article of diet. To this class of foods also belongs the Bulgarian yoghurt and the Egyptian raib.

The ferments of koumys, kefir, yoghurt and raib resemble each other in

that they are mixed, consisting of several lactic-acid microbes or organisms and yeast organisms. These foods or drinks therefore contain lactic acid and a small amount of alcohol.

As soon as it was determined experimentally that the beneficent action of sour milk, thick or clabbered milk and the above-named special preparations was largely due to the lactic acid formed by specific microbes, efforts were made to isolate these organisms in pure culture and to induce them to act in sterile or pure milk. This has been done, and there are now upon the European and American market several patented preparations consisting of the lactic acid bacillus.

Our knowledge of the relative importance of the several organisms which are said to produce the fermentative changes in the milk is as yet incomplete. Bacteriologists have thus far not succeeded in disclosing all of nature's secret processes involved. It is supposed that the microbe of Bulgarian sour milk, the *Bacillus bulgaricus*, is the most vigorous and active of all organisms concerned in the lactic-acid fermentation of milk.

It is not definitely determined whether or not the fermentations of milk induced by the mixed and often filthy "yeasts" employed in making koumys, kefir, yoghurt, matzoon and other similar fermented foods, are superior or inferior to those of lactone and other pure culture milk ferments. It is, however, very evident that the marketed preparations in tablet form give very satisfactory results, as used by pharmacists and in the home. Full directions for using the tablets are found on every package. As is naturally to be supposed, these tablets deteriorate in a comparatively short time and all reliable manufacturers place the age-limit on each package.

Pharmacists can prepare a marketable kefir ferment powder from milk activated by kefir, provided care is observed to guard against outside infection in the several steps of procedure. The following is the method of preparing a kefir powder:

A. *Securing the Kefir.*—The kefir known as kefir grains or kefir seeds may be secured from the large dealers in drugs in New York City or in other large Eastern port cities. The kefir is a solid of a tough gelatinous consistency, brittle when dry, of grayish-yellow color. It is a conglomeration of various organisms, as *Dispora caucasica*, several species of other microbes, a yeast organism, and other undetermined organisms.

B. *Washing the Kefir.*—Place two or three drams of the kefir in a mixture of equal parts of milk and water, enough to cover the kefir. Allow to stand for four hours, decant off the liquid and renew at intervals of about one hour. Repeat this four or five times at a temperature of about 82° F.

This process serves a cleansing purpose and initiates the fermentative

change. The amount used will depend upon the quantity of powder to be made.

C. *Preparing the New Kefir.*—Wrap the washed and softened kefir in a piece of sterilized gauze and place it in one quart of pasteurized milk. Keep at a temperature of 82° F. Allow to stand for from twelve to fifteen hours, until the milk is curdled.

D. *Skimming and Draining the Kefir.*—Remove the cream and drain the curd (kefir) in sterilized gauze until quite dry.

E. *Drying.*—Add (to the drained kefirized curd) an equal weight of sugar of milk, mix, and spread thinly upon sterilized gauze or upon a sterile glass plate and dry in a current of sterile warm air (80° F.).

F. *Powdering.*—Powder the dried mass gently and put up in dry, sterile, one-ounce, wide-mouthed vials, closed with sterilized corks.

G. *Directions for Use.*—Upon the bottles place the following directions for using the powder thus prepared: "Dilute one quart of milk with one-half pint of water, add a pinch of salt and one level teaspoonful of the powder. Set aside for twelve to fifteen hours at a temperature of 85° F. shaking frequently. Use at once or keep on ice."

There are, of course, no conveniences for regulating the temperature in the average household, and the action of the powder must take place at the ordinary temperature of the home. Thus the time required to curdle the milk will vary. The powder should be kept in a cool or cold, dry place. Of course, a small amount of kefirized milk can be used to curdle any quantity of fresh milk without using any of the powder.

The pharmacist should test the kefir which he is about to use in preparing the powder, in order to be certain that it is active in curdling milk. Likewise should he test the powder prepared from it.

The kefir powder above described is similar to, although not identical with, certain microbic lactic-acid ferments found on the market, as the lactone tablets, bacillary tablets, yoghurt tablets, fermenlactyl, lactobacilline and others. These are prepared from pure cultures of species of lactic-acid bacilli, dried and formed into tablets with some pulverulent (starch, milk, sugar) base, ready for use. The milk (in quart bottles) is first pasteurized, a pinch of salt is added and two or three tablets are crushed and mixed with the milk. In a day or so the milk is transformed into an acidulous drink, resembling buttermilk somewhat in flavor, though it is not buttermilk, as is generally supposed.

These tablets have gained in favor within recent years. They deteriorate in time, as already stated, and the time-limit is stamped on each container. Like the kefir, they act more quickly at a temperature of about 25° C.

As may be readily understood, kefir, lactone, etc., will not produce the

characteristic changes in milk to which preservatives have been added; in fact, the failure to produce fermentation is an indication that preservatives are present.

5. MICROBIC PEST EXTERMINATORS

Attempts have been made from time to time to exterminate certain animal pests by inoculating them with some fatal contagious disease of microbic origin. Experiments along this line have been carried on for some time, ever since the causative relationship of microbes and disease was fully established; but it is only within recent years that extensive practical application was made of the use of a few microbic pest exterminators. One of the first to be used with some success was the chintz-bug exterminator. The chintz-bug (*Blissus leucopteris*, also called chinch-bug chink-bug) was a very destructive corn (*Zea mays*) pest of the Central States (Illinois, Kansas, Nebraska, Iowa), causing great damage to crops during certain very dry seasons. Extensive experiments carried on at the University of Illinois and also at the University of Minnesota (Departments of Agriculture) led to the discovery of a microbic disease of this pest which was quickly fatal and which spread very rapidly. The insects, in cages, were inoculated with pure cultures of the pathogenic microbes, and insects in the diseased condition were sent to the farmers with instructions how to scatter them through an infested corn-field. The results were in some instances very satisfactory, and again without appreciable effects. The trouble in the use of this exterminator lay in the fact that the climatic conditions (rainy, damp weather) essential to the spreading of the disease did not generally prevail, and as soon as the climatic conditions were favorable inoculation became unnecessary, as the disease developed without artificial aid and effectually checked further ravages.

Rabbits are one of the very annoying field pests of Australia, and attempts have been made to exterminate them by means of pure cultures of microbes capable of developing a fatal infectious disease among these animals, but the results were quite unsatisfactory.

More recently there have been placed on the market quite an array of mice and rat exterminators of microbic origin under various trade names as ratin, rat virus, azoa, rattite, Danysz virus and mouratus. These preparations consist of pure cultures of bacilli pathogenic to rats and mice, as the *Bacillus murisepticus* and *Bacillus typhimurium*, mixed with some inert base, as corn-meal, oat-meal, etc., forming a coarse powder. Some preparations are in liquid form. They are used by mixing the powder or liquid with moist corn-meal or other food material relished by these animals, and spreading it near their haunts and runs. Fortunately, these substances are harmless to man and animals other than mice and

rats. These microbic rat and mice exterminators have thus far proven to be rather unsatisfactory. They have undoubtedly given excellent results in some instances, and again they have been absolute failures. The tests made by the University of California, and by Dr. Rupert Blue in his famous plague-rat extermination in San Francisco, have given almost wholly negative results. A microbic squirrel exterminator ("squirrelin") has proven entirely unsatisfactory.

When we consider how difficult it is to *prevent* fatal epidemics, it certainly does seem reasonable to suppose that it should be a comparatively easy matter to find ways and means for *disseminating* fatal epidemics, but so far the commercial attempts made in that direction have proven rather discouraging. Further carefully conducted experiments along this line are necessary. It is known that the ravages of certain pests are sometimes suddenly checked by the natural invasion of some pathogenic organisms. This is frequently observed among plant lice (Aphis) and other insect enemies of plants.

6. BACTERIA IN THE TANNING INDUSTRY

The object in tanning leather is to protect it against decomposition and to render it pliable. The various animal hides reaching the tannery are preserved by drying and salting. At the tannery the hides are treated as follows:

A. *Removing the Hair.—Depilation.*—This is done by means of chemicals, as lime or sodium sulphite, or through the agency of rotting bacteria, as *Bacillus* (*Proteus*) *vulgaris* and others. Just which of several species of rotting bacteria is most active in this process has not been definitely determined.

B. *Drenching or Bating.*—After the hair has been removed, the hides are macerated in an aqueous solution of the excrement or dung of pigeons, hens and dogs. These substances set up a lactic acid fermentation due to the microbes contained therein. The active organisms have not been isolated as yet; *Bacillus gasoformans* and *B. erodiens* are perhaps active, but there are also present many yeasts, moulds and other organisms which may have their special effects.

The first part of this process, known as "bating," is initated by bird dung; the second process, known as "puring," is due to the action of dog dung. Attempts have been made to use pure cultures of the active microbes to supplant these filth substances, but so far these efforts have not proven wholly successful.

C. *Tanning.*—The bated hides are next treated in the tan pit (coarse skins) or in bark liquor (soft thin skins), where the souring process takes place. This process is also due to bacterial activity. Our knowledge of

the action which takes place and of the bacteria involved is very incomplete.

Bacteria are important factors in siloing; in curing tobacco, tea and cacao. The flavor of different brands of tobacco is due to different bacteria, and attempts have been made to isolate those producing desirable flavors and to use them in pure culture. It is highly probable that the bouquet of old wines is due to bacterial action. These are, however, matters which require further study. Rotting bacteria are active in paper-making. In the maceration process certain bacteria feed upon and decompose the less resisting vegetable cell-walls, as those of the parenchymatous tissues, the epidermal tissue, etc., leaving the more resisting fibrous lignified tissues as bast and wood fibers. The pulp is then poured on sieves and the rotted or digested portions washed out.

Bacteria are now practically employed in the purification of sewage. This is done in what are known as "contact beds," in which the environment is made favorable to rapid development of those non-pathogenic rotting bacteria which disintegrate the organic substances and at the same time prevent the development of the pathogenic or otherwise objectionable microbes. It is highly probable that this method may be applied to the purification of streams and other large bodies of water.

The possibilities in the practical utilization of bacteria in the arts and industries are promising, and it may confidently be expected that wonderful innovations along this line will be made in the very near future.

7. MICROBIOLOGICAL METHODS IN FOOD, MILK AND WATER ANALYSIS

It is not the province of a work of this kind to enter into the discussion of special methods of analysis. These must be gotten through other sources and channels. The following practical methods are given for the benefit of those who are endowed with sufficient inherent ability to apply them.

1. *Water Analysis. Plankton Examination.*—V. Hensen applied the term plankton to the minute organisms and other organic matter drifting or floating in fresh and in salt water. The term therefore includes bacteria and other microörganisms, inclusive of dead organic matter and of mineral matter. In the more limited sense plankton excludes bacteria; although there is no satisfactory reason why this group should be excluded. The following methods have been used more or less.

a. Hassell Specific Gravity Method.—Let two or three liters of the water to be examined stand in some suitable vessel for twelve to twenty-four hours. Decant all but 200 cc. This remainder of 200 cc. with all sediment, is poured into a conical test glass with rounded bottom and

set aside for six hours. Pour off the supernatant liquid and examine the small amount of sedimentary residue microscopically. In order to reduce to a minimum the growth of the contaminating organisms during the time interval above indicated, the containing vessels should be placed in an ice chest. The method is satisfactory as far as the estimation of inorganic and dead organic water contamination are concerned, but is very unsatisfactory as far as the living contaminations are concerned.

b. MacDonald Gravitation Method.—Into a vessel containing one to two liters of water, place a watch crystal and let stand for twenty-four hours. Siphon off the water, carefully remove the watch crystal with the sediment and examine microscopically. The deposit in the watch crystal represents the amount of material derived from a volume of water equal to the diameter of the watch crystal times the height of the water column in the vessel.

c. Kean Sand Filtration Method.—Run 100 cc. of water through a funnel with a sand plug (clean fine quartz sand). Wash the sand carrying the plankton into a watch crystal by means of 1 cc. of water and examine microscopically.

d. The Sedgwick-Rafter Method.—This is a further development of the Kean method and has been quite extensively employed in the United States. Sand of definite fineness is to be used and 250 cc. of the water are to run through the filter. A specially constructed counting chamber (50 by 20 by 1 mm., hence holding 1 cc. of the sediment) is to be used and the counting is to be done by means of a specially ruled micrometer scale and a ⅔ inch objective. Bacterial counting cannot be done according to this method, neither can the smaller algæ and protozoa be accurately counted.

e. The Dibdin Double Filtration Method.—Run 1 liter of water through filter paper. Wash residue into a clay filter made by plugging the drawn out end of a piece of combustion tubing with a mixture of baked clay and kieselguhr. Filtration is accomplished by means of suction. The sediment (plankton) forms a compact cylindrical layer in the tube and can be measured and the amount of the sediment per liter of the water stated. The sedimentary plug can then be removed and examined microscopically. This method likewise precludes the estimation of bacteria and also some other very minute organisms.

f. Centrifugal Method.—Centrifuge ten to fifty cc. quantities of the water to be examined at a high rate of speed, so as to throw down all matter in suspension, inclusive of bacteria. Decant or pipette off all but about 1 cc. of the water, make up to two or three cc. by adding filtered distilled water, shake or mix thoroughly and make the counts by means of a suitable counting chamber. Should actively motile organisms such as paramecia,

interfere with the counting, they may be killed by adding a drop or two of ether or chloroform, to the centrifuged material. For the purpose of examining and comparing a number of water samples at one operation, the Stewart-Slack centrifuge head will be found very convenient and time saving.

2. *Milk Analysis.*—There are three methods of direct examination of milk in use. Two of these require the use of the centrifuge. The direct microscopical examination of milk will convey information as to quality and purity which cannot be obtained through any of the chemical methods.

a. The Stewart Slack-Method.—Two cc. of milk are placed into glass tubes which are then closed at both ends by means of suitable rubber stoppers. The special centrifuge heads made according to the specifications of Dr. Stewart of the Philadelphia Board of Health will hold twelve such tubes. The tubes are then centrifuged at 2,000 to 3,000 revolutions per minute, for ten minutes. The sediment which has become more or less fixed upon the stopper in the lower end of the tube is mixed with a drop or two of water and smeared upon a slide, so as to cover about four square centimeters of space, allowed to dry and stained with methylene blue. The examination is made with the oil immersion and bacteria, pus cells and epithelial cells counted.

b. The Doane-Buckley Method.—Ten cc. of the milk is centrifuged for four minutes at a speed of 2,000 revolutions per minute. The fat is carefully removed; centrifuged for one minute more and the fat again carefully wiped away by means of a small cotton swab. Pipette off the supernatant fat free milk and mix the sediment with two drops of a saturated alcoholic solution of methylene blue. Let the stain act for a few minutes, assisted by warmth. Make up to the 1 cc. mark of the tube by adding water. Examine for body cells by means of the hemacytometer.

c. The Prescott-Breed Method.—1/100 cc. of a thoroughly mixed sample of the milk is spread on a slide, within the ruled lines of just 1 square cm. The 1/100 cc. of milk is measured by means of a special graduated capillary tube. Both the pipettes and the ruled slides are to be had in the market. However, both can be made in the laboratory by anyone with ordinary mechanical skill. The slide with the milk smear is set aside to air dry, fixed with alcohol and then stained with methylene blue or some other blood stain. The counting is done with the oil immersion, after having determined the area covered by the field of view. Estimations are made of the number of bacteria and body cells per cc. of the milk.

3. *Examination of Tomato Products.*—The industry of canning tomatoes has reached enormous proportions in the United States within

recent years. Much of the material placed in cans and offered in the market is of very inferior quality, being made from tomato refuse, trimmings and rotten tomatoes. Two methods of direct microscopical examination of these products are in use.

a. The Howard or Bureau of Chemistry Method.—The method consists of three parts, as follows:

Mold Counting.—A bit of the tomato product (as catsup, puree, sauce, paste) is placed on the special Howard mold chamber (depth $\frac{1}{10}$ mm.) and covered with a thick cover glass. The counting is done by means of a low power objective, the draw tube being so adjusted that the diameter of the field of view is just exactly 1.83 mm. (therefore an area of about 2.5 sq. mm.). Each field showing mold hyphæ extending $\frac{1}{6}$ of the way across, or more, is counted as positive mold. A tomato product is considered unsuitable for human consumption, if it shows 66, or more, per cent. of the fields of positive mold.

Spore Counting.—The spore count includes mold spores of all kinds as well as yeast cells and the counts are recorded as so many per $\frac{1}{60}$ cubic mm.

Bacterial Counting.—The hemacytometer is used. Only the large "rod shaped forms" are counted, it not being specified whether the smaller forms and the diplobacilli are to be included.

The Howard method has been severely criticized for several reasons. It does not allow for differences in the consistency of the various tomato products. The fact that only "rod shaped" bacteria are counted has rendered the bacterial counting practically valueless as far as the protection of the consumer is concerned, since the distinctively rod shaped bacteria found in tomato products are mostly of the lactic acid forming group, and largely harmless, whereas the coccus forms, streptococcus forms, small diplobacillus forms, to which groups belong many of the filth and sewage types, are not counted. There is also no excuse for the unusual fractional recording of the spores and there should be a distinction made between spores and yeast cells. In those laboratories not under the direction of Federal and State pure food law administration, the Howard method is modified so as to conform to other more scientific methods of foods examination. The defenders of the Howard method declare that the method is purposely simplified in order to suit it to the capabilities of the analysts who are employed to do the microscopical work in the various laboratories. This argument is not worthy of serious consideration. Incompetent analysts have no place in any laboratory, least of all in a laboratory where matters affecting the public health are concerned.

4. *General Method for Making Direct Bacterial Counts.*—The following method is practically applicable in the direct microscopical examina-

tion of food substances of all kinds. Weigh or measure a definite amount of a well mixed average sample, mix, grind or triturate as may be necessary, dilute as may be desired (1–5, 1–10, 1–100), and make the counts by means of the hemacytometer. If the material is to be stained in order to make bacterial counting possible, then place 1/10 cc. of the prepared and diluted material upon a clean slide and spread it out over an area of 10 sq. cm. (2 cm. by 5 cm.), air dry, add alcohol, stain and make the counts by means of the oil immersion objective, *without the hemacytometer*. The pipette must have a free delivery and must be carefully graduated into tenths of one cc. This is essentially a modification of the Prescott and methods as applied to the direct milk count, differing in that larger amounts are used (1/10 cc. instead of 1/100 cc.). It is impracticable to use the smaller amount in most cases, and by using the larger amounts the source of error is correspondingly less. The area of the field of view with the oil immersion lens must be carefully determined. We will suppose that the field of view is 1/50 sq. mm., the dilution used 1–100, and the average number of bacteria in one field of view is thirty, then the total number of bacteria per cc. would be 1,500,000,000 ($50 \times 30 \times 100 \times 1{,}000 \times 10 = 1{,}500{,}000{,}000$).

Those interested in a fuller discussion of the decomposition changes in food substances and the microanalytical methods employed in the examination of food substances should consult The Microbiology and Microanalysis of Foods (P. Blakiston's Son and Company, 1920.)

CHAPTER IX

ZYMOLOGY—FERMENTS AND FERMENTATIONS

1. Introduction.—The terms ferment and enzyme are synonymous. Occasionally the expression "catalytic agent" is used. Unfortunately we are as yet very much in the dark as to the physical and chemical nature of ferments or enzymes as well as the processes comprehended under the term fermentation. The literature on the subject is discouragingly voluminous and correspondingly lacking in clearness and conclusiveness. Of the comparatively recent works, that by Oppenheimer[1] is the clearest and in many respects the most complete. In the following presentation of this subject we have followed this author quite closely.

2. Historical.—As comprehended by the ancients fermentation meant a "boiling" without fire, a "bubbling" a disturbance in organic compounds resulting in a marked change in the appearance of the substance affected. Originally the term applied almost wholly to the activities of the yeast organisms. Alcoholic fermentation was known to the ancient Hindus, Arabians, Greeks and Romans. Centuries prior to the Christian era the Goths, Franks and Teutons made fermented drinks from grain (beer) and honey (mead).

It is noteworthy that no attempts were made to explain fermentation until comparatively recent times. Valentinus (of Erfurt), as late as the fifteenth century, was among the first to offer an explanation, stating that it was a process of purification, probably getting the idea from the fact that in beer and wine fermentation the liquids become quite clear through the settling of the yeast as soon as the process of fermentation is completed. In fact not until the eighteenth century did the subject receive any special attention on the part of chemists, biologists and physiologists. At first there was a tendency to include under fermentation all of the processes or reactions accompanied by visible gas formation or bubbling, and the liberation of odoriferous substances. Putrefaction and fermentation were considered synonymous. The causes of fermentation were supposed to be mysterious vital forces or energies rendered active under special conditions of light, temperature, air supply and contact stimuli. Gradually distinctions were drawn between "spirituous" or vinous (alcoholic) fermentation, "sour" (acid or vinegar) fermentation, and putrefaction. Stahl, and

[1] Carl Oppenheimer. Die Fermente und Ihre Wirkungen. Leipzig, 1900.

others, ascribed fermentation to an internal activity or motion of fermenting substances, resulting in a splitting up of the molecules.

Not until the epoch-making researches of Lavoisier (1789) and those of his follower Gay-Lussac (1815) did we have any knowledge of the part played by the element O in fermentations and in other life processes. Lavoisier explained very clearly the familiar vinous fermentation in which sugar underwent a chemical splitting process, resulting in the formation of alcohol and carbonic acid gas. The name of Liebig (1865) is most intimately associated with the subject of fermentation, as are also the names of Schwann (1837) and Pasteur (1857). Liebig promulgated the theory, which was soon generally accepted, that fermentation was a decomposition process of a chemical nature, which when once initiated in the fermentable substance was capable of being transmitted from molecule to molecule, until the entire mass had undergone a change. Liebig insisted that the fermentation processes were entirely chemical but Dumas, Schwann, Pasteur and others, soon demonstrated that this was not the case, that fermentation was induced by a special organic substance, the ferment, which was formed by living organisms and which had the power of causing a special molecular disturbance or catalytic action in organic substances, resulting in the formation of new compounds.

Since Schwann and Pasteur, a host of investigators have studied fermentation processes, in an effort to determine the chemistry, biology and physiology of ferments or enzymes. We may mention a few of the leading investigators, as Cagniard-Latour (1835), Naegeli (1879), Loew, Hansen (1883), de Bary, A. Mayer, Hoppe-Seyler, Hüfner, Arrhenius, Oppenheimer (1900), Jorgensen (1909) and others. Within recent years the work that has been done on special ferments and fermentation processes and on the commercial use and application of ferments, has indeed assumed colossal proportions. To merely prepare a review of the workers and their work would require many years of careful labor.

It is known that organic substances, in fact all substances, gradually undergo a catalytic change. In the case of minerals and rock formations this change is indeed slow, whereas in organic substances the change is comparatively rapid. The chief influence of ferments is to hasten the catalytic changes in organic substances. Therefore, enzymes do not initiate any catalytic changes which would not sooner or later take place without ferments. This fact has been the cause of much speculation as to the intrinsic properties of enzymes in their relationship to the cells which form them and to the substances which they are capable of catalyzing. The rate of catalysis in substances, even those of an organic nature, without the action of ferments is, however, largely speculation and for our present purpose does not require further consideration.

3. *General.*—From the above it has no doubt become evident that the subject is far from clear nevertheless we may submit certain propositions as being more or less conclusive and which will serve to elucidate some of the more or less problematical statements which follow.

a. As far as is known, all substances which may be designated as ferments, are formed by living plasm within living cells. Ferments may be developed in single-celled plants and animals and in tissues and organs of higher plants and animals.

There is some dispute whether or not enzymes came into existence prior to living matter. Troland and others assert that certain autocatalytic enzymes or protoenzymes came into existence spontaneously in the remote geologic periods and that these greatly increased the chemical changes so essential to the creation of living plasm. There is no way of either proving or disproving the idea and it is a fact that all growth activities manifest the characteristics of enzymatic influence.

b. Since no ferments have as yet been isolated in purity, nothing is known regarding their exact physical and chemical characters and properties. It is, however, generally conceded that they are organic, of an albuminoid nature, and chemically quite complex.

c. Ferments, under favorable conditions, are capable of inducing chemical changes in organic substances, resulting in new compounds which are always simpler in composition than the mother substance. In inducing these changes the ferment itself does not undergo decomposition.

d. To distinguish between organized and unorganized ferments is no longer tenable. All ferments, as far as is known, are organized in so far as they are of living origin.

e. Ferments and the end products of their activities are immediately independent of the vital processes of the cells that produce the ferments. The ferment or enzyme of yeast (zymase), for example, is not necessary to the maintenance of the protoplasmic activity of the yeast fermentation, as alcohol and carbonic acid gas, as they are not used in the metabolic processes of the yeast cell. Nevertheless, the ferments or enzymes appear to be essential to the life of the enzyme forming organisms.

f. Ferments are chemically unstable. They are checked in their activity by low temperatures (10° to 0° C.) and killed by high temperatures (45° to 70° C.).

g. Ferments are only slightly dialyzable, but most of them will pass through porous filters (filter paper, porous clay, etc.), under pressure.

h. Ferments are precipitated by alcohol, though not completely. They are precipitated in proportion to the percentage strength of the alcohol. They are soluble in water, in aqueous solutions of glycerin, in weak acids and alkalies, and in neutral salt solutions. In a general way,

substances undergoing precipitation, carry with them any ferments that may be present.

i. Under ordinary condition the enzymatic action of the ferment is not complete. For example, the zymase does not catalyze all of the sugar in a solution into alcohol and carbonic acid gas. The process can, however, be made to proceed to completion by removing the end products as they are formed (as may be done by means of a dialyzable bag suspended in a stream of water). The reason why the process is not completed under ordinary conditions is because the ferment has a synthetic power, recombining the accumulating end products into sugar. The catalytic process is, however, always much more active than the synthetic process, at least during the earlier stages of the fermentation. Gradually the catalytic process decreases until a stage is reached where the catalytic and synthetic processes are approximately equalized.

j. The queston is often asked why are the cells which form the ferment and the organs in which they are active, not digested or catalyzed by the ferment? While the question is as yet not definitely settled, it is highly probable that the auto-digestion of ferment-producing cells, tissues and organs, is prevented by the formation of anti-ferments or anti-bodies, comparable to the anti-bodies or anti-toxins formed in cells, tissues, and organs, to neutralize the toxins of disease. It is known, for example, that under certain pathological conditions, localized digestion of stomach tissue may take place, as in ulcer. In such cases the anti-ferment is probably non-existent or in some way inactivated, neutralized or destroyed.

k. In some instances it is known that the ferment or enzyme is formed as the result of a pro-ferment or zymogen, activated by a second substance. For example, pepsin is not formed in the stomach cells, but rather in the cavity of the stomach from the pepsinogen which is formed in the mucous cells of the stomach, activated by the free hydrochloric acid present.

l. Considered from the standpoint of their relationship to the cells which form them, enzymes may be divided into three groups as follows: *a*. Those which normally act dissociated from the cells which form them, as ptyalin, pepsin, rennet, diastase, etc. *b*. Those which normally act in association with the cells that form them but which may be isolated and will then continue the fermentation, as yeast ferments; and *c*. Those fernebts which thus far have not been separated or isolated from the cells which form them, as many of the bacterial enzymes.

m. The smallest amount of enzyme will catalyze as much fermentable material as a large amount, provided it is allowed to act for the necessary length of time. On the other hand, it holds that the rate of fermentation is directly proportioned to the amount of enzyme in action.

n. Of equal amounts of enzyme isolated, on the one hand, and left in their natural environment, on the other hand, the latter are by far more active. Just why this should be is not clearly understood; the fact remains that the enzymic product of manufacture is very frequently quite inactive. No doubt the methods of manufacture have a destructive influence upon the enzymes, or it may be that we have not yet learned how to isolate the enzyme properly. Our knowledge of the action of the proferment of pepsin makes it clear that the present methods of manufacturing pepsin are defective in principle. The full strength of active pepsin is found in the stomach secretion, but not in the stomach extract or pepsin of the market.

The earliest students of ferments and of fermentation noted certain analogies between the actions of chemicals and metals in certain states or conditions, and ferments, and it is these analogies which started the controversy as to whether the enzymatic processes were purely chemical, or due to organic activity. The essential condition of the process of fermentation is that the catalyzing or enzymatic agent shall not appear in the end products of fermentation and that it shall remain unchanged chemically. In the usual generation of O, heat is applied to potassium chlorate mixed with MnO_2 resulting in the conversion of the chlorate into the chloride with liberation of O. In this process the MnO_2 remains chemically unchanged, simulating the action of a ferment in that it hastens or accentuates (aided by the heat) the catalyzing process, and does not appear in the end products.

Again, it is known that metals, as platinum or silver, in a finely divided state, will hasten catalytic processes. This is also true of certain metals (gold, platinum, silver, copper, etc.) in the colloidal state, designated as metallic sols. The colloids or sols are prepared by placing the metallic electrodes (of the metals names) into pure water and passing an electric current through them. If the water is not pure, or if it is allowed to become heated, the metal is deposited or suspended as a cloud, and does not form a true colloidal solution. The suspended and finely divided metal particles can be filtered off, leaving the true metallic colloidal solution. Sols thus prepared have the power of hastening catalytic processes without themselves undergoing any chemical change. According to Fischer, platinum sol will decompose (catalyze) hydrogen peroxide with only $1/300,000$ milligram of platinum in 1cc. of water. Metallic sols further resemble true enzymes in that their catalytic action is readily inhibited by a rise in temperature and also in that they are quite sensitive to the actions of toxic agents, as arsenic, strychnine, etc. Fischer suggests that the decomposition of true ferments by heat is merely a physical change and that all ferments are perhaps colloidal solutions and in consequence

exceedingly liable to precipitation and inactivation. The greater sensitiveness to temperatures of true ferments being probably due to the associated salts, for it is known that the sensitiveness to heat, of colloidal solutions, increases with the amount of impurities added. It is known that the comparatively purer ferments are more stable and less sensitive to heat than are those which are comparatively impure. This is a fact well known to manufacturers of such commercial ferments as pepsin, pancreatin, etc. The catalyzing activities of metals (finely divided and as sols) is however not limited to processes of chemical decomposition. For example, in the commercial synthetization of ammonia from free nitrogen and hydrogen, acted upon by the electric spark and subjected to pressure (175 atmospheres) and heat (500° C.), the rate of ammonia production is very materially increased in the presence of certain metals in the powdered form, as uranium osmium, mercury, iron and platinum. The metals remain unchanged and exert their catalytic action for an indefinite period of time. It is also known that the catalytic power of different metals varies greatly. In the ammonia production referred to, osmium is far more active than uranium or iron. In practice the metals giving a maximum yield in proportion to their cost and accessibility, are used, rather than the more active but comparatively rare and expensive metals.

There are many phenomena which are as yet unexplained and which present many of the characteristics of fermentations. It is probable that many of the life processes are controlled and directed by enzymes. The influence of the male reproductive cell is of such a nature. An enzyme-like substance perhaps acts upon the ovum inducing indefinite septation and growth, resulting in a new individual. It would appear that the growth of the body, of its tissues and its organs, is directed by enzyme-like stimuli.

These growth enzymes apparently occupy certain positions in the body and by their oxidizing influence produce or direct the various complex chemical changes (assimilation) which result in the formation and growth of the tissues and organs. Normally these growth ferments are active in such a manner (regulated and inhibited) as to give rise to plants and animals which we designate as normal; but occasionally there is a disturbance or displacement in these enzymes and growth irregularities are the result, such as local and general dwarfism and giantism, atavistic marks and anomalies, supernumerary fingers and toes, duplication of parts, twins, etc., etc. We may assume that the geotropic position of roots and stems and the horizontal position of branches of plants, is controlled by enzymes. Occasionally the normal positions are reversed or changed. It is not uncommon to find large trees, especially in virgin forests, with all branches but one, in the usual or normal position, the exceptional branch having assumed the vertical

trunk position and being tree-like in every respect, excepting that it is devoid of a root system. In some examples of this kind, the lower branches assume the root position, that is they extend downward and the secondary branches assume the more irregular positions of root branches, as compared with normal secondary branches of the tree.

It may, however, be that many of these growth phenomena are not directed and controlled by enzymes but by other substances, for example, the hormones. Hormones are distinguished from enzymes by not being destroyed by heat, up to the boiling point; they are dialyzable and in a general way manifest the characters of chemicals. They occur in the ductless glands, in the ovaries, testes and in other organs. It is known that the removal of the organs named causes very serious disturbances in the bodily functions. The adrenal and pituitary glands are apparently absolutely essential to life as their removal in animals results in death. The removal of the pancreas results in diabetes and the removal of the ovaries and testes produces a very marked change in general metabolism as well as in the mental, muscular and nervous systems, with arrest in sexual development. Some of the bodily secretions containing these hormones[1] are now being used in the treatment of certain pathological conditions, with very gratifying results. It must however, be admitted that our knowledge of the exact composition and function of hormones is as yet not well understood, nor do we know their true relationship to the enzymes.

A ferment or enzyme may be defined as a peculiar energizing substance, formed by living cells with which it is more or less intimately combined or associated but without being vitally influenced in its activities by the vital processes of the cells producing it. This energizing substance or ferment has the power of converting the latent (potential) energies of chemical compounds into kinetic energies, as warmth and light. The new compound or compounds formed always have a lower kinetic energy or oxidizing power than the original substance. The ferment itself remains unchanged during the process. Ferments are specific in their action, that is, each ferment acts upon certain substances only and its activities give rise to constant decomposition products.

Certain ferments (hydrolytic) have the power of taking up moisture and again giving it up to the substance undergoing fermentation, the presence of moisture being necessary to the process. But why the fer-

[1] Among the more important remedial agents of this group are extracts of the parathyroids, the testes, the pituitary bodies, the thymus, the ovaries, the mammary glands, the adrenals and the pineal gland, all of which are now marketed and used with considerable success in more or less specific ailments. These will be considered in another chapter.

ment takes up the moisture and again gives it up is as yet not explained. Ferments are variously influenced in their activities by physical and chemical agents. It is known that certain chemicals which are not normally present in living cells or organisms and which are not component parts thereof, appear to have a stimulating effect upon the life processes of these cells or organisms. For example the spores of *Aspergillus repens* will not germinate in pure water or in inorganic nutrient solutions, or even in peptone solutions, unless some inorganic salt, such as saltpeter, is added. Recent tests made show that radium exerts a very marked influence upon plant growth as well as upon the functional activities of animal cells and organs.

Minute doses of certain toxic agents have a stimulating effect upon the vital processes of lower as well as higher organisms. 1–500,000 parts of mercuric chloride, or the merest trace of iodine, of potassium iodide, of chromic acid or of salicylic acid, have a very beneficial effect upon processes of fermentation. In a general way ferments are less susceptible to the influence of poisonous agents than are the microbes or other organisms which form the enzymes. It is possible through the judicious use of certain antiseptics to kill bacteria and other objectionable organisms without in any way hindering the fermentation induced by associated organisms. This discovery proved of great value in experimenting with and isolating enzymes. The following substances may be used for this purpose—alcohol, ether, etheral oils, dilute acids, salicylic acid, thymol, chloroform, calomel, corrosive sublimate, etc. Many of these germ destroying agents are however not without some checking influence upon the action of the ferments themselves, notably salicylic acid, phenol, thymol and chloroform. Toluol appears to have the least injurious effect upon enzymes.

Ferments influence each other. Pepsin inhibits the action of nearly all other ferments, notably that of trypsin and of diastase. Trypsin again destroys zymase. Pepsin has however only a slight check upon lactic acid fermentation. Weak acids and occasionally also weak alkalies, have the power of converting the inactive proferments into active ferments, which substances Oppenheimer designates as zymoplastic. Other agents as warmth, dilute acids and alkalies, increase the activity of ferments. Still other agencies, as cold, most alkalies, alcohol, etc., inhibit their action while the socalled zymolytic agents (strong acids, heat, etc.) kill them. Other more specific properties of ferments will be given under the description of the ferments themselves. The following classification of the more common ferments will make clear their relationship and will also serve as an introduction to the specific descriptions which follow:

CLASSIFICATION OF FERMENTS

A. HYDROLYTIC FERMENTS

I. Proteid Ferments.

1. Proteolytic ferments (proteases)
   - a. Pepsin
   - b. Trypsin
   - c. Lysins (?)
     - Bacterolysin
     - Hemolysin
     - Cytolysin
   - d. Opsonins (?)
   - e. Papain
   - f. Of ductless glands (hormones) (?)
   - g. Of insectivorous plants
   - h. Of cryptogamous plants (bacteria, higher fungi, etc.)
   - i. Seed ferments
2. Coagulating Ferments
   - a. Rennet
   - b. Vegetable rennet
   - c. Agglutinins
   - d. Precipitins
   - e. Fibrin ferments
   - f. Pectase
3. Starch Splitting Ferments
   - a. Diastases (amylases)
     - Amylase
     - Ptyalin
     - Amylopsin
     - Glycogenic ferment of liver, blood and urine
   - b. Disaccharide (diastase) ferments
     - Maltase
       - of yeast
       - of other cryptogams
       - of animals
     - Invertase
       - of yeast
       - of other cryptogams
       - of animals
     - Trehalase
     - Melicitase
     - Melibiase
     - Lactase

c. Polysaccharide (diastase) ferments
  Cellulase or cytase
  Inulase
  Pectinase
  Seminase
  Cerubinase
4. Glucoside Splitting Ferments (Glucases)
  a. Emulsin
  b. Myrosin
  c. Gaultherase
  d. Rhamnase
5. Fat Splitting Ferments—Lipases (Steapsin)
6. Ammonia Forming Ferments—Urease
7. Lactic Acid Forming Ferments

B. THE OXIDIZING FERMENTS

1. Zymases
2. Oxydases
3. Acid Forming Ferments
  a. Vinegar (acetic acid)
  b. Oxalic acid
  c. Citric acid
  d. Malic acid, etc.

A. Hydrolytic Ferments

The socalled hydrolytic ferments cause the splitting of complex molecules accompanied by the taking up of H_2O. The oxidation processes are of an intramolecular nature, that is, no oxygen is taken up from the outside (air).

I. Proteolytic Ferments (Proteases).—These ferments have the power of splitting albuminoid substances. Nothing definite is known regarding the chemical processes involved for the reason that but little is known regarding the chemical composition of albuminous substances. We must rest content with a partial study of the end products of the fermentative processes. The following are the more important ferments of this group:

1. *Pepsin.*—Pepsin is the albumen digesting ferment found in the stomach of vertebrate animals, though it appears to be wanting in some fishes. Pepsin-like ferments are however also found in insects and other invertebrates. Pepsin occurs furthermore in the intestinal tract, in muscle, in skin secretions, probably in other tissues and organs, and in urine. However the pepsins of different species of animals differ somewhat. It is also noteworthy that the pepsin secretion of one and the

same animal is most variable, depending upon the nature of the food ingested, the stage of the digesting process, the acidity of the stomach, abnormal or pathological conditions as in gastritis, cancer, etc.

The "ingluvin" of the older pharmacopœias and materia medicas, is the pepsin ferment obtained from the gizzard of the domestic fowl which, at one time, enjoyed an extensive use in medicine. The pepsin of the dog is said to be the most active. That of the frog and of cold-blooded animals generally, is less sensitive to cold. For example, frog pepsin is still active at 0° C., while the pepsin of warm-blooded animals is inactivated at 10° C.

Pepsin is not immediately elaborated in the so-called peptic cells of the stomach, rather these cells form a proferment or pepsinogen which in association with dilute free hydrochloric acid, is quickly converted into pepsin.

Pepsin has never been isolated in a pure state. In its comparatively purest state thus far obtained it is a yellowish, brittle, homogeneous mass, soluble in water and dilute solutions of acids, solutions of salts and in glycerin. It is precipitated by alcohol and has the general properties of enzymes. It is quickly inactivated by alkalies. In solutions it is destroyed at a temperature of 55° to 57° C., while in the dry state it can withstand a temperature of 100° C.

Since the stomach cells contain pepsinogen very largely, the washed stomach pepsin extract, as formerly prepared is comparatively inactive. To determine the activity or digesting power of pepsin it is permitted to act upon albuminous substances. Small amounts of pepsin dissolved in dilute solutions of hydrochloric acid (0.10 to 0.20 per cent.) are allowed to act on egg albumen or fibrin. The time required for a unit amount of the pepsin solution to digest a unit amount of the albumen at a given temperature, uniform for all tests, indicates the activity of the pepsin. Grünhagen permits a small mass of fibrin to become saturated with the acidulated pepsin solution and then places the mass upon a filter. The number of drops of digested fibrin which pass through the filter in a given time indicates the digesting power of the pepsin. Mett places small glass tubes filled with coagulated albumen into the pepsin solution in an incubator and notes the amount of albumen digested in ten hours.

The action of pepsin is retarded by chloroform, strong solutions of salts generally, particularly sodium chloride, and by ammonium sulphate. Alcohol below 10 per cent. does not interfere with its action, while beer even with only 3 per cent. alcohol retards its activity very much. Other substances which retard pepsin digestion are wine, saccharin, tea and coffee (due to tannin present), tobacco and strong solutions of alcohol. Weak solutions of acids, small amounts of spices, very minute doses of arsenic,

strychnin and alkaloids generally, and quinin, assist the action of pepsin. Antipyrin and antifebrin and other similar coal tar derivatives retard its activity somewhat while strong solutions of cane sugar (40 per cent.) check its activity considerably. Some authorities ascribe a bacteriolytic power to pepsin, whereas others declare that the apparent germ-destroying power is due to the acid present.

The first change in pepsin digestion is a swelling of the albumens or albuminoids, which action is due to the acid present. The pepsin then splits up the albumens into peptones and albumoses which are diffusible. The peptones are the true end products and differ from the albumoses in that they are more diffusible and that they are not precipitated by acidulated ammonium sulphate, neither are they precipitated by boiling, by acid s or by calcium ferrocyanide. Pepsin digestion is indeed a complex process and the student desiring further information is referred to standard works on physiology and dietetics.

Commercial pepsin is obtained from the stomachs of recently killed hogs. Its digesting power is based upon its proteolytic action upon hard boiled egg albumen. According to the U.S.P., one part of properly prepared hog pepsin should digest at least 3000 parts of coagulated egg albumen. Higher grades are, however, found on the market, such as give 1–4000, 1–5000 and 1–6000 and even 1–20,000.

2. *Trypsin.*—Trypsin is the albumen digesting ferment of the pancreas derived from a proferent, the trypsinogen. It is also found in the small intestine. On its action on albuminous substances it is much like pepsin, though in its behavior with certain modifying influences it is quite different. It is most active in slightly alkaline media (one per cent. solution) though it is also active in neutral and slightly acid solutions. Bile aids trypsin digestion, especially in the presence of lactic acid, also in the presence of hydrochloric acid. Strongly acid and alkaline solutions check its action very promptly while neutral salt solutions increase its activity, especially the sodium salts.

Trypsin is somewhat more resistant than pepsin, in most of its general characteristics and properties it is, however, closely similar. Its digesting power can be determined much in the same manner as for pepsin. Trypsin is especially active in the digestion of casein and of gelatin.

The commercial article pancreatin is a mixture of the enzymes of the pancreas, of the hog, the ox, and of other animals. Its chief value depends upon its peptonizing and diastatic power. According to the French Pharmacopœia pancreatin shall peptonize 50 times its weight of fibrin and convert 40 times its weight of potato starch into sugar. The active ferments of pancreatin are trypsin (protease), amylopsin (amylase), steapsin (lipase) and the milk curdling agent rennin.

3. *Lysins.*—These will be discussed under immunity from disease. Though these cell, germ and blood corpuscle destroying agents found in the serum of the blood and in tissue cells, are not generally classed as ferments, they have many of the characteristics of the proteolytic ferments pepsin and trypsin. Three kinds of blood lysins have been studied, bacterolysins which actively destroy bacterial cells, hemolysins which actively destroy red blood corpuscles and cytolysins which actively destroy tissue cells. They are all specific in nature as will be explained elsewhere.

4. *Opsonins.*—These agents found in the blood and in tissue cells will be more fully described elsewhere. Like the lysins they are specific in action and their enzyme-like nature is rather problematical.

5. *Papain.*—This is a proteolytic ferment found in the fruit of *Carica papaya,* having a very marked action on meats. It was used by the natives of Brazil as an aid in preparing meat. Its action is closely similar to that of pepsin. Papain is a well-known commercial product used in defective stomach digestion and also for the purpose of peptonizing meats. It does not attack living protoplasm hence it is non-toxic. A papain-like ferment is found in the fig, in the pineapple (bromelin), in cucumbers and in other plants.

6. *Of Ductless Glands.*—The ductless glands of the body contain certain substances of an enzyme-like nature which are found useful in the treatment of disease. The commercial articles made from these glands (adrenalin, desiccated thyroid glands, etc.) are fully described in standard works on materia medica and in dispensatories, to which the reader is referred.

7. *Of Insectivorous Plants.*—Certain plants (*Drosera rotundifolia, Nepenthes gracilis, N. hybridus, Dionœa muscipula, Aldrovandia vesiculosa, Utricularia vulgaris* and *Darlingtonia Californica*) secrete a pepsin-like ferment. At one time it was supposed that bacteria symbiotically associated with the insectivorous plant hosts secreted the proteolytic enzyme, but this theory has recently again been questioned. There is, however, no doubt that the plants named have the power of digesting and assimilating animal substances.

8. *Of Cryptogamous Plants.*—Proteolytic ferments are found in some of the fungi as Penicillium, in *Aspergillus niger,* Agaricus and in *Fuligo septica.* Similar ferments are found in many different species of bacteria as anthrax bacillus, cholera bacillus, *Bacillus mesentericus vulgatus,* tubercle bacillus, sarcina and others. Some of these bacterial ferments behave like exotoxins in that they are absorbed into the culture media in which the bacteria are grown. Many bacteria have the power of liquefying gelatine. Some of the yeasts form proteolytic enzymes.

9. *Seed Ferments.*—Proteolytic ferments are widely distributed in

seeds, their function being to split up and render transfusable and assimilable for the germinating seeds, the proteid granules. These have thus far not received any careful study.

The subject of auto-digestion of organs has received considerable attention. Finely chopped fresh organs digested for a time at moderate warmth undergo certain fermentation-like changes resulting in the formation of reducing sugars, leucin, tyrosin, etc., substances which do not occur in living organs or in organs which have been boiled. Bacterial infection is excluded by means of chloroform water (using ten volumes of the chloroform water), also by means of sodium fluoride and thymol, though these latter agents are less suitable than the chloroform water.

Autodigestion proceeds slowly, being a slow decomposition of the albuminous matter. In this digestion, albumoses are formed but not peptones; furthermore, nuclein is split up which is not the case in trypsin fermentation. Autodigestion is no doubt due to a proteolytic ferment which probably exists in the cells but which may be removed from the tissues as its presence has been demonstrated in cell-free extracts. The ferment of autodigestion is probably an autolytic product of tissue cells causing a molecular change in albuminous matter.

2. *Coagulating Ferments.*—The curdling of milk was known in ancient times and is the initial basic process in cheese-making. At one time it was believed that pepsin had the power of curdling milk but that is now known to be incorrect. Berzelius was the first to demonstrate that the curdling of milk could take place without the presence of lactic acid, thus disproving the contention of Liebig that lactic acid combined with the alkali with a precipitation of casein.

a. *Rennet.*—Rennet or chymosin is the milk-curdling ferment used in cheese-making, obtained from the fourth ventricle of the stomach of the calf or sheep. It, however, also occurs in the stomach of all animals. In serious pathological conditions of the stomach (as cancer, gastritis, etc.) it may be partially or even wholly wanting. It also occurs in the intestinal tract and in nearly all tissues and organs.

While rennet has many of the properties of ferments generally, it shows some exceptional peculiarities. In regard to its behavior with salt solutions, it is precipitated by lead acetate only. It is destroyed by bile and by even very weak solutions of alkalies. Dissolved in distilled water it is destroyed upon exposure to a temperature of 40° C. This peculiarity makes it possible to free pepsin from rennet, as pepsin is not affected by this temperature and distilled water.

Rennet is the product of a proferment or rennet zymogen which is secreted by the cells of the stomach, acted upon by the free acid of the stomach. Almost any acid will, however, activate the zymogen, especially

hydrochloric and sulphuric acid. Acetic acid is the least active. Rennet does not occur in the stomach in the absence of free acid though the proferment is present.

Rennet is a highly potent ferment. It will coagulate from 4–800,000 times its weight of milk. It is most active in slightly acid solutions and least active in alkaline solutions. Injecting minute doses hypodermically causes the formation of an anti-coagulating ferment which, when added to milk, prevents coagulation.

b. *Parachymosin.*—The rennet of the human stomach and that of the hog, differ from that of other animals, especially do they differ from that of the calf and sheep. Thunberg found that the rennet of hog pepsin does not coagulate slightly acid milk when neutralized by alkali, but will do so when such milk is neutralized by means of carbonate of lime; whereas calf rennet shows no such difference in behavior. It is therefore presumed that in addition to ordinary chymosin, the pepsin of man and of the hog contains a modified rennet to which the name paracyhmosin has been given. Parachymosin is more resistant to heat than chymosin as it is still active at 75° C., while it is much more susceptible to alkalies. Heating hog pepsin to 70° C. destroys the chymosin and leaves the parachymosin still active, or the parachymosin may be destroyed by means of alkalies too weak to affect the chymosin.

c. *Vegetable Rennet.*—A milk curdling ferment occurs in various plants. *Galium verum* is used to curdle milk, also *Pinguicula vulgaris*, *Drosera* and *Carica papaya*. The seeds of *Witharia coagulans*, a plant found in India and Africa, are used by the Hindus for coagulating milk, their religion forbidding the use of animal substances. Germinating seeds of *Ricinus communis* contain rennet in the form of the zymogen which is activated by dilute acids. Rennet also occurs in figs, artichokes, thistle, and in other plants. The fruit of *Acanthosicyos horrida*, of South Africa, contains a rennet which is said to be soluble in 60 per cent. alcohol. Numerous species of bacteria form milk curdling ferments, among others *Bacillus amylobacter*, *B. mesentericus vulgatus*, *B. prodigiosus* and *B. choleræ.*

d. *Pectase.*—This enzyme causes the coagulation of pectin in plants containing this substance. It is widely distributed among higher plants and also among cryptogams. It occurs in two forms, soluble as in carrots, and in an insoluble form, as in acid fruits generally. The ferment is active in the absence of oxygen and it does not result in gas liberation, and is most active at 30° C. Coagulation is most active in the presence of lime, though the process is also initiated by barium and strontium salts. Boiling or precipitating out the lime, hinders coagulation. Acids destroy the ferment.

e. *Fibrin ferment.*—This ferment causes the coagulation of blood. It occurs in the blood of all animals excepting in "bleeders" in which it is absent, a condition causing much trouble in checking hemorrhages resulting from even very trivial injuries and operations. It is supposed that under certain conditions the fibrinogen is split into fibrin and globulin. The fibrin and globulin can readily be separated, as is done in the manufacture of concentrated diphtheria antitoxin. There are many phases of blood coagulation which are as yet not understood. Those interested will find fuller discussions in modern works on human physiology and anatomy.

f. *Agglutinins and Precipitins.*—These problematical enzymes are found in the blood of animals. The former cause the bacterial clumping as in the Widal test for typhoid fever. The latter cause the formation of precipitates in blood. Both are specific in nature and shall be more fully discussed elsewhere. It is highly questionable whether these agents should be classed as coagulating ferments.

3. *Starch Splitting or Sugar Forming Ferments.*—Under this head are included those highly important ferments which act upon carbohydrates, splitting them up into simpler compounds. The fermentations due to these enzymes are simple hydrolytic processes, analogous to those caused by dilute acids. One of the most common end products is glucose.

The starch splitting ferments are widely distributed in the vegetable as well as in the animal kingdom and they play a most important part in domestic economy. They bear the same relationship to carbohydrates that the proteolytic ferments bear to proteid substances.

The activity of starch ferments is tested in various ways. Complete digestion of starch is indicated by the absence of the iodine reaction. The optical behavior of the substance undergoing fermentation is also an indication as to the nature and identity of the ferment. Of interest is the auxanographic method proposed by Beyerinck. If, for example, it is desired to know if glucose has been formed, the substances is inoculated with a fungus which will develop upon glucose (as *Saccharomyces apiculatus*) but not upon maltose. Fehling's test and other sugar reactions may also be used.

The saccharine substances formed vary in composition in the amount of reduced sugar present, in the character and degree of polarization (rotation), etc.

The different starches as of rice, barley, wheat, potato, etc., do not digest or ferment at the same rate at a given temperature. For example at 50° C. diastase will digest 12 per cent. barley starch, 2 per cent. corn starch and 29 per cent. malt starch; at 55° C., 5 per cent. potato starch, 53 per cent. barley starch and 58 per cent. malt starch; at 60° C., 52 per cent. potato starch, 92 per cent. barley starch and 18 per cent. corn starch.

At very low temperatures diastase has only a very slight effect upon raw potato starch, while barley and wheat starches are quite actively digested.

a. *Diastases.*—These are the true starch splitting ferments and are also called amylases or amylolytic ferments. They are widely distributed in the vegetable and also in the animal kingdom. They are of vital importance to plants and animals. They are the principal ferments in malt, the so-called malt diastase being the best known of all enzymes or ferments. The discovery of malt diastase dates back to 1833, through the investigations of Payen and Persoz, who sought to obtain this enzyme in a pure state by precipitation in alcohol, but in this attempt they were of course not successful. The following are the principal disastatic ferments.

Amylase.—This is diastase proper and acts on starches, changing them into dextrins and maltose. It is very widely distributed in the vegetable kingdom. The stored starch in plants, which is reserve food, must be transformed into a soluble form before it can enter into circulation through the plant tissues in order that it may finally be assimilated for purposes of plant growth and development and the ferment amylase produces this desired solubility.

Ptyalin.—This is the starch ferment of the salivary glands, converting starches into maltose. It is a very active ferment, being most energetic in a slightly alkaline medium and is quickly destroyed by acids. Some investigators declare that very small quantities of hydrochloric acid increase the action of ptyalin. However, 0.015 per cent. acid is sufficient to render it inactive.

Amylopsin.—This is the diastase ferment of the pancreas, closely resembling ptyalin, though it is more active, rapidly liquefying the starch and converting it into dextrin and maltose. It is most active between 30° and 45° C. and is destroyed at 65° C. It is not found alone but in association with trypsin, steapsin and rennin in the commercial product known as pancreatin. The action of amylopsin is reduced by weak acids, but on the other hand, not appreciably increased in the presence of weak alkalies.

Liver Diastase.—The glycogenic function of the liver is due to a ferment which converts soluble starch into glycogen and dextrin-like substances. This ferment is also found in the blood, in urine and in tissue cells.

b. *Disaccharide (Diastase) Ferments.*—These ferments act upon biose sugars, converting them into simpler compounds. The principal enzymes of this group are as follows:

Maltase.—This is very widely distributed in the vegetable kingdom, in different species of Saccharomyces and in other cryptogams. It also occurs in the animal kingdom. It acts upon maltose, converting it into d-glucose.

Invertase.—Like maltase this enzyme is widely distributed in the vegetable and animal kingdoms. It acts upon cane sugar, splitting it into about equal parts of glucose and fructose. The ferment is analogous to maltase.

Trehalase.—This ferment converts trehalose, a disaccharid found in the group fungi and also in a variety of manna (insect manna, trehala or tigala), into two molecules. The ferment is found in species of Aspergillus and in other fungi.

Melicitase.—This ferment is found in several varieties of lower or bottom yeasts, but is said to be wholly absent in all top yeasts. It splits melibiose into d-galactose and d-glucose, in which action it is analogous to lactase.

Lactase.—Lactase acts on milk sugar (lactose) only, changing this disaccharid into d-glucose and d-galactose. The ferment was first discovered in *Saccharomyces kefir* and in *S. tyrocola.* The pancreas contains no lactase but it occurs in the small intestine of young animals, less in the intestine of fully grown animals and is generally wanting in old animals. Lactase is also said to occur in some species of bacilli. The comparative scarcity of lactase-forming organisms explains why lactose is comparatively less liable to fermentation, than is cane sugar, for example.

c. *Polysaccharide* (*Diastase*) *Ferments.*—These ferments split up insoluble saccharine compounds into soluble sugars and are very closely related to the diastases already mentioned. The principle enzymes of this group are cytase which decomposes cellulose which is the chief constituent of plant cell-walls. Inulase decomposes the inulin abundant in many Compositae as in Taraxacum, Inula and in other genera. Pectinase decomposes pectin and thus destroys the products of pectase which gives rise to pectin. Seminase decomposes mannogalactan into mannose and galactose and is very widely distributed in the seeds of higher plants, especially in seeds of the locust, of alfalfa and of fœnugreek. Carubinase is similar to seminase and occurs in the germinating seeds of *Ceratonia siliqua* (St. John's Bread). It converts the polysaccharide carubin into carubinose, which is said to be similar and perhaps identical with d-mannose.

4. *Glucoside Splitting Ferments.*—Glucosides are very important active constituents of many of our most important medicinal plants. These substances are not acted upon by the yeast ferments as maltase, invertase and trehalase, with the one exception of the glucoside amygdalin of bitter almonds.

a. *Emulsin.*—This is the most important of the glucoside splitting ferments. It is widely distributed in the vegetable kingdom occurring in higher plants as well as in the cryptogams. Among the phanerogans

it occurs principally in bitter almonds, in the leaves of Laurocerasus and in the seeds of the Rosaceæ. Among the cryptogams emulsin is found in *Aspergillus niger*, *Penicillum glaucum* and in many other species of higher fungi and also among the bacteria.

Emulsin, also known as synaptase, splits amygdalin into grape sugar, benzaldehyd and hydrocyanic acid, in the presence of water. It is most active at 45°–50° C., and is destroyed at 70° C., although in the dry state it can resist a temperature of 100° C. for several hours. It is destroyed by alkalies whereas acids merely inactivate it. It also decomposes the glucosides arbutin, salicin, helicin, gentiopicrin, syringin, phyllirin, cyclamin apiin and convallarin. It does not act on populin, solanin, hesperidin, convallaramin, convolvulin, digitalin, hederin and quercitrin. Emulsin is said to decompose lactose into glucose and galactose, thus resembling lactase in its action.

b. *Myrosin.*—This is the ferment active in mustard and also in other cruciferous plants. It acts (in the presence of water) upon the glucoside of mustard, sinalbin, converting it into mustard oil, dextrose and sinapin sulphate.

c. *Gaultherase.*—This enzyme was first discovered in Gaultheria and named gaultherase; but it is also present in Betula species, *Spiræa ulmaria*, *S. filipendula*, in *Monotropa hypopitys* and in other plants. It acts only upon the glucoside gaultherin (abundant in *Gaultheria procumbens*). It does not act on salicin or amygdalin, thus differing from emulsin.

d. *Rhamnase.*—A ferment which is said to occur in the seeds of *Rhamnus infectoria* and which acts upon the glucoside xanthorhamnin, decomposing it into rhamnin (rhamnetin) and glucose. Boiling destroys the ferment converting it into rhamniose (a trisaccharid) and other products.

Indigo formation is supposed to be due to the action of a glucoside splitting ferment but this has not yet been proven. It is known that Indican (the glucoside of Indigofera and Isatis species) is decomposed into indiglucin and indigwhite in the presence of chloroform water, whereas boiling destroys such action, this tending to prove that the ferment is not of bacterial origin as was once supposed.

Other glucoside splitting ferments probably exist in plants, as for example in the cucumber. *Ecballium elaterium* contains a ferment which presumably splits up the glucoside elaterin and which may be called eleterase.

5. *Fat Splitting Ferments.*—As is known fats are esters of glycerin. Under the influence of the fat splitting ferments the fats are decomposed into free fatty acids, and glycerin. The fat enzymes have been long

known and are called lipases, steapsins or lipolytic ferments. The best known is the steapsin of the pancreas. It is very sensitive to the action of acids and also to sodium chloride. Steapsin, like that of the pancreas, is also found in the blood and in the liver of various mammalian animals, even in fishes and in insects.

Lipase is also found in the vegetable kingdom, as for example, in the seeds of *Ricinus communis* and among the cryptogams as *Aspergillus niger* and *Bacillus fluorescens*. It causes the decomposition of fats in germinating seeds and in other fat bearing plant tissues and organs.

6. *Ammonia Forming Ferments—Urease.*—These ferments act on exposed urine changing it from an acid to an alkaline body, due to the decomposition of urea into ammonium carbonate. The organisms that form urease are not normally present in urine. They are widely distributed in the atmosphere, producing their characteristic changes in exposed urine. Most of the urease forming organisms belong to the bacteria, as *Micrococcus urinæ* and *Bacillus ureæ*. The latter is highly thermophilous, begin capable of resisting a temperature of 90° C. Many other urine organisms are reported, including cocci, bacilli and sarcinæ. They are all aërobic and require grape sugar, urea, phosphorus, sulphur, potassium and magnesium for their growth.

Certain bacteria are very active ammonia formers. A bacillus which developed on shrimp and also on fish formed a sufficient amount of ammonia to suggest the possibility of utilizing this organism in the commercial manufacture of this gas.

7. *Lactic Acid Forming Enzymes.*—Lactic acid is widely distributed and is most important from the commercial viewpoint. It is the result of the decomposition of a variety of sugars, induced by a great variety of microörganisms belonging to the groups bacilli, cocci, vibriones, and sarcinæ. All agents which inactivate or kill the organisms named also inactivate or destroy the lactic acid enzymes produced by them, as cold, heat (above 60° C.), disinfectants, acids, etc.

In spite of the wide distribution of lactic acid in the animal and vegetable kingdoms and the fact that a great many organisms are known to form or liberate lactic acid, no lactic acid enzyme has as yet been isolated or induced to act independent of living organisms. This has raised the question, does a lactic acid forming enzyme really exist? The presumptive evidence however is decidedly in favor of the existence of such a ferment. It is known for example that some of the lactic acid bacteria when grown on a sugar free medium, for a long time, will when suddenly transferred to a medium containing sugar, not have the power of forming lactic acid. However, on prolonged culturing in sugar-containing media, this lost power is gradually regained. This behavior may be explained on the

supposition of an interruption in the development (by the bacteria) of the lactic acid enzyme. It is furthermore demonstrated that very minute doses of mercuric chloride, copper sulphate and other poisons, will cause an increase in the lactic acid forming function while the power of septation and growth of the lactic acid enzyme organisms is very materially decreased.

B. Oxidizing Ferments

The oxidizing ferments also known as oxidases cause the oxidation of various organic substances. They appear to act as carriers or transmitters of oxygen to the substances undergoing fermentation, though the exact chemical changes involved are not well understood. To this group probably belong a great variety of ferments and fermentation processes, widely distributed in the plant as well as animal kingdoms. Laccase is a ferment concerned in the formation of a lacquer varnish in the lac tree of Asia. Tryosinase found in certain fungi and also in the roots of certain higher plants has the power of oxidizing tryosin which is found in these plants.

Œnoxidase causes the wine disease known as "casse." The wine loses its red color with the formation of a reddish-brown precipitate. It is highly probable that the multitudinous fermentative changes comprised under "ripening" processes, "sweating" processes, aroma and flavor formation in wines, tobacco, cheese, etc., are of the oxidizing variety, besides many of the little understood fermentative changes resulting in so-called "diseases" in commercial products as wines, beers, tobacco, cheese, etc.

C. Alcohol Forming Ferments

The alcohol forming ferments or zymases or yeast ferments proper are by far the most important from a practical commercial standpoint. Zymases act upon sugars splitting these substances into alcohol and carbonic acid gas thus acting upon the end products formed by the diastasse.

Zymases are formed by a great variety of plants and animals, particularly the yeast plants (the Saccharomyces) and Torula species and their varieties. The enzymes of yeast plants may be isolated or separated from the living cells and will continue their fermentative activities independently.

Alcohol fermentation is by no means a simple process. The degree of alcohol production and of by-product formation varies greatly, depending upon a great variety of factors and influences. To enter into a fuller discussion of the details of the fermentative processes and a description of the organisms involved is not essential. We may however mention the fact that the number of sugar bearing substances capable of under-

going alcoholic fermentation is legion. Just how many different species, varieties and forms of yeast organisms and associated organisms are involved in the alcoholic fermentations (natural and artificial) no one knows. In commercial and manufacturing practice (in wine, beer, saké and brandy making, etc.) a distinction is made between upper yeasts, lower yeasts, wild yeasts, etc. Each yeast species having its varieties and forms characterized by special effects produced.

The following are the principal yeast organisms known with the principal fermentative activity of each. Hansen's distinction between the genera Saccharomyces and Torula is based upon spore formation. Saccharomyces form spores (ascospores, usually four in each ascus, rarely eight), whereas Toruly does not. This is perhaps not a practicable distinction.

SACCHAROMYCES

cereviseæ, Hansen (a top yeast)
Pastorianus, Hansen (a bottom yeast)
intermedius, Hansen (top, feeble)
validus, Hansen (top yeast)
ellipsoideus, Hansen (a bottom yeast)
turbidans, Hansen (bottom yeast)
willianus, Saccardo (found in floor)
boyanus, Saccardo (causes turbidity in beer)
logos, Van Laer (bottom and floor)
thermanitonum, Johnson (quick ferment)
ilicis, Gronlund (in Ilex) (bottom yeast)
aquifolii, Gronlund (in Ilex)
pyriformis, Ward (in Ginger beer)
vordermanni, W. & D. (in Arrak)
sakè, Yabe (in saké)
batatæ, Saito (in yam brandy)
cartilaginosus, Lindner (in Kephir)
multisporus, Hansen (a top yeast)
mali Kayser, Kayser (in cider)
marxianus, Hansen (on grapes)
exiguus, Hansen (in beerwort)
jorgensenii, Loschi, (causing turbidity)
zopfi, Artari (in syrup)
bailii, Lindner (in beerwort)
hyalosporus, Lindner (in wort)
rouxi, Butroux (in fruit juice)
soya, Saito (in Soya sauce)
unisporus, Hansen (in dutch cream)
flava lactis, Krueger (in cheesy butter)
hanseni, Zopf (in cotton and meal)
minor, Engel (in bread)
membranæfacions, Hansen
anomalous, Hansen (causing a fruity odor)

saturnus, Klocker (from soil)
acidi lactici, Grotenfelt (a curdling yeast)
fragilis, Jorgensen (in Kephir)
barkeri, Saccardo (in Ginger beer)
ludwigii, Hansen (in oak)
comesii, Covara (millet pedicles)
octosporus, Beyerinck (on dried currants)
mellacei, Jorgensen (top yeast, pleasant odor)
guttulatus, Robin (in rabbit)
capsularis, Schionning (in soil)

Torulas occur in great variety. The *Levure de sel* is a yeast capable of developing in 10 to 15 per cent. sodium chloride solution.

The following are a few products in which there is alcoholic fermentation, in addition also lactic acid formation.

Yoghurt.—This is a Bulgarian sour thick or klabbered sheep's or cow's milk. The milk, as in the other similar fermented drinks, is first boilt, afterwards evaporated to one-half its volume, then cooled to about 45°C. and the ferment (maya or podko assa) added. The maya is simply the dry milk residue from a previous fermentation. The fermented product has a sour aromatic taste. The most important organism in this fermentation is the *Bacillus bulgaricus.* Other bacilli, cocci and yeasts are also present.

Kephir.—Kephir is an effervescent alcoholic sour drink, made from cow's, sheep's or goat's milk, by the Bulgarians as has been explained elsewhere. Several yeasts or torulæ and bacteria are active in the fermentation process. *Dispora* (*Bacillus*) *caucasica* and several species of Streptococci, associated with the Kephir yeast, are the principal organisms found. These organisms no doubt form a mutualistic association resulting in the formation of lactic acid and alcohol in the milk.

Koumiss.—This drink is similar to kephir, made from mare's milk, by the inhabitants of southern Russia and of Siberia. The active organisms are a yeast, a lactic acid bacillus and another species of bacterium which appears to be characteristic of Koumiss and which appears to be active only in the association with the other organisms, thus also indicating a mutualistic association. The fermented milk contains lactic acid and alcohol.

Soya Sauce.—This Chinese sauce or relish is made from the fermented soya bean (*Glycine hispida*). The beans are boiled and mixed with parched wheat meal and acted upon by the fungus *Aspergillus oryzæ.* This is then mixed with salt and water and allowed to ferment, sometimes for a year. The product assumes a rich brown color and a characteristic, aroma. It is then put in bags and an almost clear juice is expresseid which is then further clarified and pasteurized. In the second or long

process of fermentation, several organisms are active, along with *A. oryzæ*, as *Saccharomyces soya*, *Bacillus soya* and *Sarcina hamayuchia*.

Mazun.—This, like kephir and koumiss, is fermented milk, usually of the cow and goat, and is much used in Armenia. The active organisms are a yeast, a bacillus apparently identical with *B. subtilis* and several lactic acid bacteria.

Leban.—This sour, aromatic drink, is closely similar to mazun and is made from boilt buffalo's, cow's and goat's milk, in Egypt. It is said to contain less alcohol than kephir. Five different organisms, evidently mutualistically associated, are active in Leban fermentation; a Streptococcus which coagulates milk and forms lactic acid from lactose, another bacillus, a Diplococcus which ferments glucose, saccharose and maltose; a streptococcus which hydrolyzes lactose and another yeast organism which can ferment glucose and maltose but not lactose.

Ginger Beer.—This is a fermented sugar solution to which ginger is added. The essential fermenting organisms are a Saccharomyces (*S. pyriformis*) and *Bacillus vermiforme*. *Mycoderma aceti* is also present. The two essential organisms are evidently in close mutualistic relationship. The drink resulting from the fermentation of saccharine solution is acid and effervescing. The so-called "ginger beer plant" which is simply a mass or matrix of the active organisms is used to start a new fermentation.

6. Cider Making

Acetic-acid fermentation in wine cider and other fermented alcoholic substances is initiated by the *Mycoderma aceti*, collectively known as "mother of vinegar." This is no doubt a mixed growth, representing several species or varieties of acetic-acid forming organisms. While it is true that nature invariably inoculates the substances named, resulting in the production of vinegar, it is customary to use the top skin or pellicle (mother of vinegar) on vinegar already formed, adding it to new wine or cider in order to hasten the fermentation. As stated, this is not a pure culture representing a single species. In fact, the tests with what were pure species have proven unsatisfactory. The vinegar organisms require an abundance of oyxgen. To supply the necessary oxygen (of the air) it is customary to have the fermentation barrels or casks only about two-thirds or three-fourths full and to leave the bunghole open (generally with a plug of cotton). In Germany a quickened method is much in vogue. The wine or cider is allowed to trickle slowly through a cask filled with wood shavings which are moistened with old vinegar. The wood shavings offer a maximum surface exposure and fermentation is as a result very much hastened.

Occasionally the vinegar loses its acidity. This is due to the invasion of a bacillus (*B. xylenum*) which, in the presence of oxygen, splits up the acetic acid into other compounds. This change can be prevented by excluding air from the containers. Vinegar should contain 4 to 4.5 per cent. of acetic acid (the legal standard).

Beer and Wine Organisms.—We have elsewhere briefly outlined the manufacture of beer and saké. Numerous yeast organisms are active in wine, cider and in other fruit juice fermentations. The student desiring further information is referred to the works by Hansen and Jörgensen, which may be found in any library of scientific books. Cider vinegar and yeast manufacture have been mentioned elsewhere, likewise cheese making and the significance of dairying organisms in the ripening of cream and of butter, etc.

D. Acid Forming Ferments

As is known, dilute alcohol upon standing exposed to air, gradually becomes sour, losing its alcohol more and more. This is due to ferments which act upon the alcohol, splitting it into acetic acid and H_2O. The organisms producing the acid forming enzyme are generally classed with the bacteria, largely in the group *Bacillus*, the principal species being *Mycoderma* (*Bacillus*) *aceti*, *B. pasteurianum*, *B. kützingianum*, *B. oxydans*, and *B. acetosum*. The yeast *Saccharomyces mycoderma* is also capable of forming acetic acid. The vinegar organisms are most active at 25° to 30° C. Very slowly active at 10° C. and killed at temperature but slightly above 35° C. The so-called mother of vinegar consists of an agglutinated mass of vinegar organisms and is used as a starter in the manufacture of vinegar. Thus far it has not been possible to isolate the vinegar ferment as has been done with diastase and zymase.

There are acids of non-alcoholic origin formed by living ferments, such as oxalic acid, malic acid, citric acid and others, which appear to be derived from sugars direct. Citric acid is formed from sugars through the activity of two fungi, *Citromyces pfefferianus* and *C. glaber*. *Saccharomyces hansenii* forms oxalic acid from mannit and galactose, without alcohol formation.

The following table will serve to make clear the relationship of the diastase (starch), zymase (sugar) and alcohol ferments:

Principal foods for enzymes	Starches and dextrins	Sugars	Alcohol
Enzymes	Diastases	Zymases (yeasts)	Vinegar ferments (Mycoderma)
Principal Products	Sugars	Alcohol	Acetic Acid

CHAPTER X

IMMUNOLOGY. IMMUNITY AND IMMUNIZING AGENTS

1. **Introduction.**—Immunity from disease and susceptibility to disease are relative terms. By immunity is meant the power or ability of the living body to resist or prevent the successful invasion by the agent or agents of infection, whether these agents are of vegetable or of animal origin, whether they are the organisms themselves, as bacteria, amebæ, yeast cells, nematodes, etc., or the substances produced by them, as toxins, ptomaines, albumins, toxalbumins, putrescins, venoms and enzymes. The body resistance to the action of chemicals and chemical poisons is generally not classed as immunity, although it is a closely related phenomenon and cannot well be omitted from the discussion.

We know that there is great variation in the immunity of the individuals of the same species to the various agents of infection as well as to the action of the purely chemical poisons of non-living or inorganic origin. A number of individuals exposed to the same infection do not all take the disease. A number of individuals receiving the same dose of poison are not all affected in the same degree or in the same way. It has been known for a long time that the successful invasion of certain infections as small-pox, typhoid, measles, mumps, whooping-cough, etc., immunizes the individual against subsequent attacks. It has also been known for ages that the animal organism may resist gradually increasing doses of highly toxic substances, as arsenic, opium, morphine, tobacco, and alcohol. Even more remarkable is race immunity. Man is immune to most of the diseases of the lower animals, as hog cholera, chicken cholera, and on the other hand most animals cannot be successfully infected by the human diseases, such as measles, mumps, whooping-cough, scarlet fever, and yellow fever. Closely related species may display remarkable immunity differences. For example, field mice are very susceptible to glanders, whereas the common house mouse is quite immune. Jersey cows are less liable to bovine tuberculosis than Holsteins. The Yorkshire breed of swine is less susceptible to hog erysipelas than are other breeds.

Trachoma, measles, poliomyelites, typhus, scarlet fever, rabies, influenza and whooping cough, can be transmitted to monkeys. Smallpox can be transmitted to cows, horses, rabbits, and sheep. Rabies is transmissible to dogs, wolves, cows, rabbits, cats and other animals; malta fever to guinea pigs, mice and rabbits; plague to most domestic animals, to rats, squirrels, and mice. The gonococcus is not transferable to lower animals, whereas the streptococcus, the staphylococcus group and the pneumococcus group are transferable to many of the lower animals. The following diseases of lower animals are transmissible to man; ringworm, favus, scabies, tetanus, anthrax, glanders, actinomycosis, psittacosis (a lung disease of parrots), plague, trichnosis, bovine tuberculosis, foot and mouth disease, and influenza. Many of the lower animals harbor the primary causes of diseases of man without suffering any pronounced or even appreciable inconvenience. Thus the oyster harbors the organisms of typhoid fever and of bacillary dysentery. Mosquitoes harbor the causes of malaria and of yellow fever.

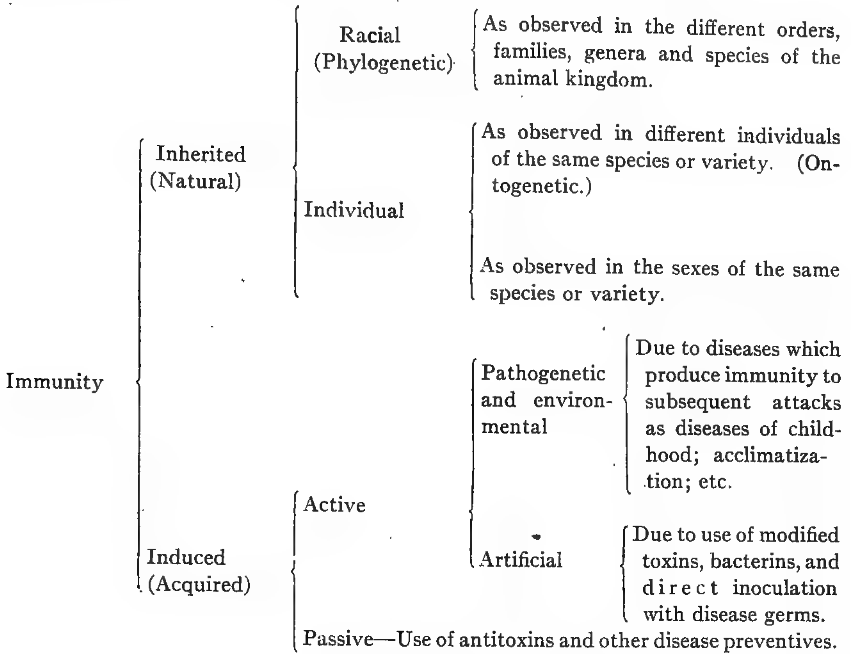
The stable fly carries the primary cause of poliomyelitis and the rat flea harbors the plague bacillus. The differences in racial reactions towards poisons is also remarkable. The hog feeds upon poison oak and is quite immune to snake venom as well as to many other substances which are highly toxic to man. The goat is immune to many infections and feeds with impunity upon many poisonous plants. Herbivora are far less susceptible to vegetable alkaloids than are carnivora. Insects feed upon plants which are highly poisonous to man.

The caucasian is more susceptible to yellow fever than is the negro, whereas the reverse is true as to tuberculosis, smallpox, and syphilis. As compared with herbivora, the wild carnivora are quite immune to tuberculosis and are also far less susceptible to other infections. Some infections are highly humanized, as gonorrhea, syphilis, and cancer.

There are also the peculiarities of age immunity, sex immunity, climatological, occupational and seasonal immunity, etc., all of which require the attention of the physician.

Immunity is very markedly relative. For example, no race of mankind is possessed of absolute immunity to any human disease. The immunity enjoyed by the wild carnivora can be broken down by prolonged captivity, notably the immunity to tuberculosis. A thousand and one factors modify immunity, as lack of food, poor food, fatigue, overexertion, cold, excessive heat, dampness, poisons, habits, occupations, etc., etc.

The several kinds of immunity may be tabulated as follows:



2. **Earlier Theories Regarding Immunity.**—Theories have been advanced from tim to time which were intended to explain immunity. Why the individual and racial variation in the behavior towards infections? Why are certain individuals successfully infected while others escape? Why do some of those who are successfully infected die while others recover? Why do the infections (in the cases of recovery) disappear after a time? The following are some of the theories advanced:

a. *Physiological Resistance of the Body Cells.*—Some thirty to forty years ago, physicians and physiologists had much to say regarding the variable though inherent property or power possessed by the living cells of the body to resist or ward off infection, the so-called physiological resistence of cells. It was thought that as long as the cells of the body were fully or normally active the infecting agents could not find lodgment therein. This theoretical assumption cannot be gainsaid even at the present time, but unfortunately the theorization says nothing and explains nothing. The theory has no visible means of support.

b. *The Exhaustion Theory.*—In order to account for the cessation of successful infections in cases of recovery, it was assumed that the infecting agents (bacteria) used up those body-cell substances which were necessary to the life of the particular infection. It was thought that these cell

substances were of a specific character, each species or variety of disease germ depending upon its specific materials found in the cell. It was supposed that cell exhaustion for dysentery, for example, did not also result in exhaustion for typhoid infection, or cholera infection, or tubercular infection. Like (a) this also was mere theoretical or rather hypothetical assumption and was without adequate scientific proof.

c. *Intoxication Theory. Toxic Destruction Theory.*—According to this theory, the infecting agents (bacteria) were gradually checked in their growth and finally destroyed by toxins or harmful agents which they themselves produced. As is known, the ordinary phenomenon of active combustion (burning) results in the formation of substances actually employed in extinguishing fires, namely water and carbonic acid gas. In a similar manner, so it was supposed, the harmful and destructive typhoid infection (*Bacillus typhosus*), for example, formed certain gradually accumulating substances which were fatal to the continued existence of the infecting agent itself.

Many experiments and tests have been made, the results of which apparently supported the theories (b) and (c) but the experimental evidence as a whole were so faulty and inconclusive that as a result both theories are now practically abandoned, giving way to the more recent statement of immunity and immunizing agents.

3. The History and Development of the Present Conception of Immunity.

1. *General Statement of Immunology.*—In 1890 Behring and Kitasato found that the cell-free blood (serum) of rabbits and of mice which had been artificially immunized against tetanus, neutralized or destroyed the toxic substances of the tetanus bacillus. To this substance they gave the name antitoxin. This was an epoch-making discovery. It led to the finding of other antitoxins or antibodies which are now used in the treatment of disease as will be more fully explained in a subsequent chapter. Antitoxins, like the toxins, possess many of the characters of albuminoids, are quite readily decomposed and are incapable of isolation from the blood or from the tissue cells. Never having been obtained in purity nothing is known regarding their physical appearance. They are readily destroyed at comparatively low temperatures (65° to 75° C.) and by exposure to light and air. They are very sensitive to acids and are best preserved by evaporating the blood sera in which they are contained to dryness in a vacuum at a low temperature and storing in a vacuum, at a low temperature, away from light and in a dry place. Experimentally it has been demonstrated that the antitoxins are intimately combined with the globulins of the blood. This discovery led to the manufacture of concentrated antitoxins by precipitating the globulins with ammonium sulphate, magnesium

sulphate and other salts. Remarkably enough, reactions have been observed which would indicate that antitoxin is not a proteid substance; for example, it is not destroyed (digested) by trypsin.

It has furthermore been found that variably small amounts of antitoxin exist in normal blood; that is, in the blood of animals that have not been naturally or artificially immunized, and also in still lesser amounts in the milk of normal animals. As to the origin of the antitoxins the physiologic evidence points to their formation in the body cells rather than in the blood serum.

Another important discovery was that normal blood could actively destroy (lake) bacteria, and in common with antitoxins, this bactericidal property was found to be specific. That is, serum found to be quite destructive to the typhoid bacillus is not destructive to the cholera bacillus. These germ destroying or bactericidal substances are designated lysins. Ehrlich has discovered that there are in fact three distinct blood lysins; namely, cytolysin, a substance which is capable of destroying (laking) body cells; hemolysin, which is capable of destroying red blood-corpuscles; and bacteriolysin as already explained. By injecting tissue cells, as those of kidney or of some other organ, into an animal, there are developed in the blood of the inoculated animal lysins which will dissolve kidney cells or other organ cells used. If the blood of a bird or other animal is injected into an animal of a different species, hemolysins will appear in the blood of the animal thus injected. This hemolysin is specific, as it will only dissolve or destroy the red blood cells in the blood of the kind of animal of which the blood was used for injecting. An animal inoculated with the typhoid bacillus will produce a bacteriolysin which destroys the typhoid bacillus. Lytic sera become inactive when heated to 55° C. for one-half hour and such sera are said to be inactivated. However, if normal serum is added to the inactivated serum the bactericidal power is fully restored. The bactericidal power of the serum can be greatly increased by the use of highly virulent bacterial cultures, thus producing a serum of high potency. In actual practice, as in the manufacture of bactericidal sera for the prevention and cure of disease, the animal (as horse) is first inoculated with attenuated cultures, then with normally virulent cultures and finally with hyper-virulent cultures of the specific pathogenic microbe. Such sera act by destroying the disease-producing bacteria, but they have no effect upon the toxins produced by the bacteria, thus showing that they are entirely distinct from the antitoxins.

The eminent bacteriologist Metchnikoff made the very interesting discovery that the white blood-corpuscles (leucocytes) had the power of feeding upon and digesting bacteria with which they came in contact. That is the white blood corpuscles, called phagocytes, act as the defenders

of the body against bacterial invasion. This observation by Metchnikoff, fully verified by others, is generally known as the phagocyte theory and the phenomenon is designated phagocytosis. The principle involved in phagocytic activity is well illustrated in the lesser local injuries, as cuts, bruises, abrasions, etc. Normally such injuries are always infected by various germs of the environment, as the several varieties of pus microbes. These invading microbes at once begin their attack upon the tissue cells and blood-corpuscles. The leucocytes which are present begin to feed upon the rapidly multiplying pus organisms but for a time, as a rule, the latter have the upper hand and as a result there is perceptible pus formation ("the laudable pus" of older writers) represented by dead leucocytes gorged with microbes. As the inflammatory reaction becomes more marked, indicated by redness and swelling of the tissues immediately about the injury; increased numbers of leucocytes (phagocytes) are brought to the scene of action and gradually they gain control until finally the invading microbes are all destroyed, thus permitting a rapid and unhindered restoring of tissue cells, recognized as the healing process. This phagocytic action is entirely distinct from the action of antitoxins and lysins, and the three are potent factors in immunity.

The investigations of Metchnikoff and Leishman on phagocytosis paved the way for the discovery of opsonins by Wright. It was noticed that the phagocytic activity was influenced by conditions to be found outside of the leucocytes themselves. Metchnikoff held that the principal part is played by substances found in the serum and in the tissue cells to which he gave the name "stimulins." The purpose of these substances in the tissue fluids have not yet been satisfactorily demonstrated, but Metchnikoff considers their function to be that of acting upon the phagocytes in such a manner as to stimulate them to perform phagocytosis. Wright, Hektoen, Neufeld and others have demonstrated beyond doubt, the presence in the blood of substances which act upon the infecting bacteria and get them ready for the completion of their destruction by the phagocytes. To these bodies Wright gave the name "opsonins" (Latin, *opsono*, I prepare for). That opsonins are not formed in the blood is certain. Experimental evidence seems to prove that they are products of muscular or subcutaneous cellular activity. It is probable that the actual formation of opsonin occurs in the muscle tissues and passes thence to the blood. Wright has demonstrated more or less satisfactorily the presence of opsonins in the blood of animals and humans and by a special technic has measured the relative amount. This measurement is a ratio of the activity of the phagocytes in normal blood and of that in disease, before and after stimulation, determined by the number of bacteria that a single phagocyte will ingest—the so-called opsonic index. This index or ratio is

made intelligible by decimal figures representing the number of bacteria which the average phagocyte will take up. We may assume that one phagocyte in normal blood will ingest an average of 10 bacteria, represented in the index by the figures 1.0, but in disease (chronic) the phagocytes may only take up an average of 3, 6, or other numbers, represented by the figures 0.3, 0.6, etc. After stimulation the phagocytes may take

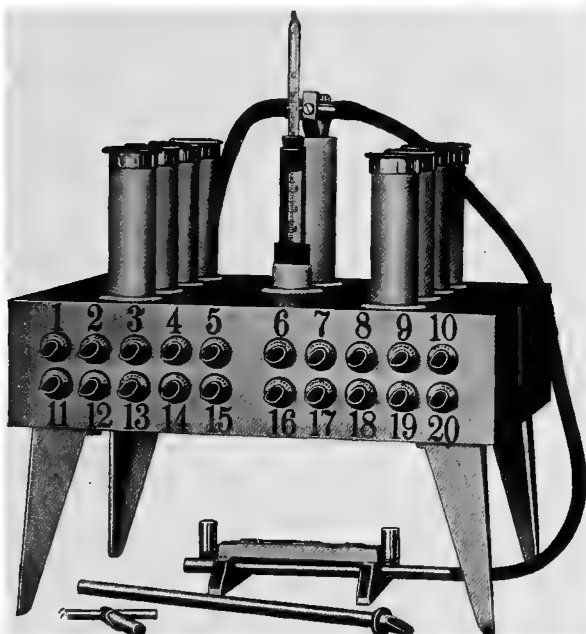


FIG. 59.—Opsonic Incubator. The determination of the Opsonic Index has become so important that these incubators have been made to meet the demands for an apparatus in which twenty pipettes can be incubated at one time and so that any tube may be examined during the progress of the experiment without changing the temperature of the others. There are twenty tubes for opsonic pipettes and an extra tube. The tubes may be easily removed when desired by means of a key which accompanies the incubator. On top there are eight tubes, 22 mm. in diameter, for test-tubes. Each is provided with a nickel-plated cap. The incubator is supplied with thermometer, thermo-regulator, and a two-flame burner, with wire guard.

up 15, 25, or even numbers of bacteria represented in the index by the figures 1.5, 2.5, etc.

Taking the opsonic index of an individual's blood cells for considerable delicate technic. In brief, it is performed by mixing together equal volume quantities (measured in a capillary tube) of blood serum and an emulsion of bacteria and incubating for 15 minutes at 37.5° C. Then making a thin smear of the mixture on a microscope slide, drying and staining, and counting the number of bacteria enclosed in each white blood-corpuscle (50 to 200 cells counted) and striking an average. This average is the

index stated by a decimal figure. The index thus obtained indicates the relative phagocytic power of the individual's blood tested, whether below or above the normal, or normal.

The opsonic index taken in the various chronic forms of bacterial infections is invariably below normal and shows that the phagocytic power is low, and it seems to prove that the chronicity is due to the subnormal phagocytosis. The injection of several millions of devitalized bacteria of the kind causing the infection, induces the formation of the specific opsonin, arouses the phagocytic activity and corrects the pathologic condition. The opsonic method of treatment has been extensively tested through the use of specifically active bacterial suspensions (vaccines, bacterins or opsonogens) which in some instances have given excellent results. It has also been found that substances other than opsonins may increase phagocytosis, as for example, nucleinic acid and collargol.

From the foregoing it becomes evident that immunity from disease depends upon the presence in the body of antitoxins, bacterolysins, and the opsonins which induce phagocytosis. It is furthermore possible to increase the activity of these agents artificially. All three agents are specific in nature as already stated. Ehrlich has attempted to explain the phenomena of immunity according to his receptor or side chain theory (Seitenkettentheorie). This theory, which is rather complex and highly technical, was first used to explain cell metabolism. Hinman's version of the side chain theory is very simple and we give it as follows: As applied to immunity the basis of the theory is the conception of the duplex nature of antigens. An antigen is a substance, of bacterial or other origin, which has the power when introduced into the body, of inducing the formation of specific antibodies. Not all toxins or poisons have this power. For example, strychnin and the toxin of tetanus produce similar physiologic effects, but only the latter is capable of producing an antibody. Ehrlich explains this difference by assuming that strychnin and most other vegetable poisons enter into a loose combination with the cell plasm, analogous to an aniline dye which can be readily dissolved out again; whereas the toxin is firmly bound to the cell, representing in a measure a toxic food-stuff in chemical union with and assimilated by the cell. The atomic combination of the toxin antigen, which represents this chemical union is designated the haptophore group, while the atomic combination of the cell-plasm with which the haptophore group unites is called the cell receptor group. The haptophore group is distinct from the atomic group which produces the toxic or pathologic effects, designated as the toxophore group. These two groups of the antigen (toxin), namely the haptophore group and the toxophore group, act independently of each other and possess different properties. The toxophore group is easily destroyed by heat (60° to 65° C.)

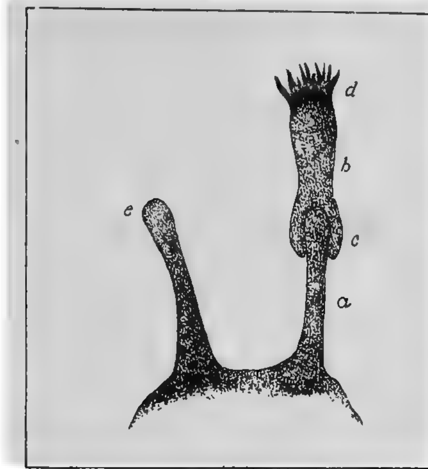


Fig. 60.—Illustrating cell receptors of the first order. A cell receptor (*a*) uniting with the haptophore (*c*) ot the toxin molecule or antigen. The toxin molecule or antigen consists of the haptophore and the toxophore. The toxophore produces the toxic effects upon the cell. *e* is the haptophore of the cell receptor which has the power of combining with the toxin molecule thus neutralizing its possible toxic effects. Free-cell receptors constitute the antibodies, and are ever ready to combine with antigens or toxins, should any be present. Cell receptors and antigen bodies are specific in action. The haptophore of the diphtheria cell receptor does not fit the haptophore of tetanus, for example. Each antigen or toxin reacts with the antibodies fitted to it. (*Journal of the American Medical Association*, 1905, p. 955.)

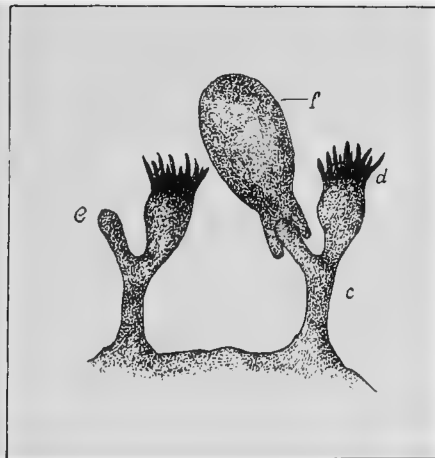


Fig. 61.—Illustrating receptors of the second order, Fig. 54. illustrating receptors of the first order. *c, d,* The cell receptor with a symophore group (*d*) and a haptophore group (*e*) capable of combining with disintegrated bacterial substances (*f*). The zymophore group produces a ferment which acts upon (disintegrates) the bacterial cell or blood-corpuscle, as the case may be, seized upon by the haptophore group. (*Journal of the American Medical Association*, 1905, p. 1113.)

while the haptophore group is not destroyed, retaining the power of combining with the receptor group of the living cell. The toxophore group is not necessarily simple. It may comprise two or more different groups. Snake poison contains two toxophore groups, one agglutinating red blood cells, the other causing its general toxicity. Diphtheria toxin also has two toxophore groups, the one causing the acute symptoms and the other, the toxones with a long incubation, causing the later paralyses and cachexias.

The nature of immunity to these antigens is conceived as follows: The haptophore group is bound to the cell receptor because of a specific affinity. As a result this particular side chain or receptor is lost to the living cell and, following Weigert's law of supercompensation in regeneration, the cell replaces this loss by producing many more receptor groups than were previously present. As in the callus following a fracture there is an overproduction. In this way such a large number of receptors of one type are produced that they become excessive and the cell thrusts them off into the blood and into the fluids of the body. Here they constitute the specific antibodies and, because of their specific affinity, unite with the haptophore group of toxins and prevent their reaching the cell which they thus protect.

Therefore, in antitoxic immunity there are three stages: First, the chemical union of the haptophore group of antigen to the receptor group of the protoplasm molecule; second, the overproduction and liberation of these receptors following this binding; and third, the union of these free receptors or antibodies with free toxin haptophore groups before these can reach the cells to injure them by the action of their toxophore groups. The antigens that are known with their respective antibodies as given by Hektoen are:

Antigens	*Products of Immunization*
Toxins	Antitoxins
Ferments	Antiferments
Precipitinogens	Precipitins
Agglutinogens	Agglutinins
Opsonogens	Opsonins
Lysogens	Amboceptors or lysins
Antitoxins	Antiantitoxins
Agglutinins	Antiagglutinins
Complements	Anticomplements
Opsonins	Antiopsonins
Amboceptors	Antiamboceptors
Precipitins	Antiprecipitins

These antibodies all result from the overproduction of simple receptors, but the protoplasm of cells may form still other cell receptors which are much more complicated and subserve the absorption of more complicated and complex albuminous molecules than those of toxins.

Bacterial clumping or agglutinating phenomena are extremely interesting as well as valuable in the diagnosis of disease. Upon this behavior of bacteria depends the Widal typhoid fever test. If the serum of an animal inoculated with the typhoid bacillus (antiserum) is added to a liquid culture or suspension of typhoid bacilli, the latter cease to move and after a time become aggregated into irregular clumps or masses. The same phenomenon is observed if instead of blood of a typhoid injected animal, the blood of a typhoid fever patient is employed. The reaction is quite specific,

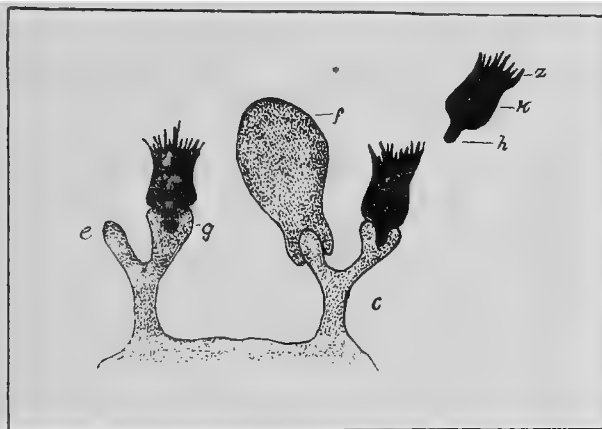


FIG. 62.—Illustrating receptors of the third order, or so-called amboceptors. This serves to explain the action of lysins (bacteriolysin, hemolysin, cell lysins, milk lysins, etc.). The cell receptor (amboceptor) has two haptophore groups, one (*e*) capable of uniting with a disintegrated substance as bacterial cell, blood-corpuscle, etc., (*f*) and the other (*g*) having the power to combine with a complement (*k*). *h* is the haptophore group of the complement (lysin) and *z* the zymotoxic group. Amboceptors, lysin receptors and receptors of the third order mean the same thing. (*Journal of the American Medical Association*, 1905, p. 1369.)

though not absolutely so. That is, similar agglutinating phenomena are produced by related bacilli, as the typhoid bacillus, the para-typhoid bacillus and the colon bacillus. Many other bacteria, beside the colon-typhoid group, are agglutinated by their respective antisera. In addition to diagnosing disease as in typhoid fever (the Widal test gives results even before there are marked disease symptoms), the agglutinating phenomena are useful in the identification of bacteria. The technic while not difficult, calls for many precautionary measures and requires considerable time and care to avoid erroneous conclusions.

In 1897 Kraus found that when the germ-free filtrates from broth cultures of bacteria were mixed with their respective antisera (serum from animals inoculated with the specific bacteria) the formation of a white precipitate occurred. The substance in the immunized serum which causes the formation of the precipitate has been termed precipitin. Simi-

lar reactions are observed with milk and egg albumen, when used with their specific immune sera. These reactions have been utilized to secure evidence in criminal cases. The serum of an animal which has been injected with human blood (humanized immune serum) produces a precipitate when mixed with human blood, even in high dilutions. Like agglutination, the reaction is, however, not wholly specific. For example, humanized animal serum will also produce a precipitate with the blood of higher apes. Dog immunized animal serum will produce a precipitate with wolf's blood, etc.

The chief immunizing agents are the bacterolysins, the antitoxins and the leucocytes (phagocytes) aided by the opsonins. The significance of agglutinins and precipitins in the prevention of bacterial disease is not clear.

Recent observations on drug action tend to prove that some of these remedial agents apparently possess antitoxic and other immunizing properties. It is for example fairly well proven that phosphorus and *Echinacea angustifolia* have the power of increasing the opsonic index in certain bacterial invasions. Sulphide of carbon and silica appear to check and cure suppurative processes, perhaps due to similar activity. Nuclein which is usually derived from yeast, is reported to be decidedly bactericidal and to increase phagocytosis to a marked degree. According to Lloyd, Lobelia, when administered hypodermically, counteracts the toxin of the diphtheria bacillus, being similar in its effects to the antidiphtheric serum (antitoxin of diphtheria). Belladonna is reported to be prophylactic as well as curative in scarlet fever. It is highly probable that as our knowledge of the therapeutic action of drugs develops, there will be a complete revolution in their use as remedial agents.

2. *The Immunizing Agents.*—The following is a brief description of the more important immunizing agents of the body. Other immunizing agents will be given in Chapters XI and XII.

a. Phagocytosis.—As already explained Metschnikoff made the interesting discovery that the white blood corpuscles (leucocytes) seized upon and disintegrated and fed upon bacteria with which they came in contact or with which they were brought in contact. Not only do the leucocytes possess this power but also other body cells, as the endothelial cells of capillaries, and the lymphocytes which are cellular structures of the lymph channels corresponding to and developing into leucocytes upon entering the circulation, certain fixed body cells, more especially the epithelial cells of the intestinal tract and of mouth and throat or upper respiratory tract.

The germ devouring property of leucocytes (phagocytosis or leucocytosis of Metschnikoff) is typically illustrated in an injury to the skin,

as cut, abrasion or other marked injury. In a more or less severe abrasion of the skin there is a tearing of the tissues as epidermis, derma and the lesser arterioles and capillaries, with more or less extravasation of red blood corpuscles and serum. Under ordinary conditions such a wound is always infected, primarily and principally by the staphylococcus group which are present everywhere, less commonly also by the streptococcus group, and occasionally by the tetanus bacillus. The latter being anaërobic is more apt to develop in closed wounds or deep wounds. It is furthermore a spore bearer. These organisms find the serum of the blood and the tissue cell juices a very suitable food supply and an active invasion is thus set up. However, the body defenders, the leucocytes, are at once despatched to the scene of action to repel the bacterial invasion. After the hemorrhage which was the direct result of the mechanical injury, has ceased, there still continues an exudation of blood serum carrying with it numerous leucocytes, and these leucocytes immediately begin the work of seizing upon and devouring the invading bacteria. Vast numbers of the leucocytes are killed and become mixed with the serum exudate, with broken down tissue cells and with bacteria, constituting the *pus*. The dying, bacteria gorged leucocytes, gradually lose the power of ameboid movement and finally assume a fixed spherical form and constitute the characteristic *pus cells*.

Ordinarily or normally the leucocytes gradually gain the upper hand, pus becomes more and more scant, finally ceasing to form altogether. Rejuvenescence of tissue cells begins and finally the damage is entirely repaired and the wound is said to have healed. The source and origin of the cells concerned in regenerative activities is as yet not fully determined. It is known that in the case of the infected skin injuries, numerous leucocytes and lymphocytes migrate to the injured area and are largely concerned in phagocytic activities, but these body cells are also concerned in the healing processes; assisted by the endothelial cells. These several cellular elements constitute the so-called inflammatory lymph which enters into the formation of cicatricial or scar tissue.

The older writers on surgery and pathology (fifty to sixty years ago) distinguished between "laudable pus" and "sanious pus." It was believed that pus formation was unavoidable and when the pus was of a whitish color and creamy in consistency it was a favorable sign as indicating a "normal" healing process and such pus was said to be "laudable." On the other hand, if the pus gradually became watery, blood tinged and foul smelling, it was designated "sanious" and the wound condition was considered unfavorable. We now know that the change in pus formation designated by "sanious" is the result of the gain of the invaders, the pus organisms, more especially the streptococcus group.

The surgeons of today seek to prevent all pus formation. All surgical operations are, and should be, without infection of any kind, hence there is, and should be, no pus formation. Cuts or incisions, which heal without pus formation, are said to show "primary union" or to heal by "first intention," terms which were in constant use when "Listerism" was extensively introduced into surgical practice about 1875, but which are very rapidly falling into disuse, the surgeons of today simply speaking of infected or non-infected wounds, as the case may be.

Occasionally a primary invasion by the staphylococcus group, {*Staphylococcus albus* (white pus), *S. aureus* and *S. citreus* (yellow pus), *Bacillus pyocyaneus* (blue pus, comparatively rare)} is followed by an invasion by the Streptococcus which may result in a more or less widespread infection commonly known as "blood poisoning," accompanied by redness, swelling and pain, with final more or less extended tissue destruction and necrosis.

As to which species or variety of infecting microbe initiates the primary infection depends upon place and opportunity. The staphylococcus group being most common and most widespread, naturally is most likely to be the predominating infecting agent. We may expect streptococcus infection in the presence of abundant decaying organic matter, stable manure, cattle pens, etc. The tetanus infection in old garden soils, in hay, in old stable manure and in the mud and dust of much traveled roads. For example, the extensive infection of wounds by the tetanus bacillus in the trenches of the German-Allies' battle line in northern France (1915) led to the suspicion that infected bullets had been used, until it was found that the old garden and field soils of this region were the prolific sources of the infection.

In the condition known as boils, acne, carbuncles, furuncles, we have active infection by the staphylococcus and streptococcus groups with leucocytic invasion and reaction. There may be infection of the heart (endocarditis), of the joints (rheumatoid), of the pleuræ, of the kidneys and of other organs, especially when the pus organisms gain access to the circulation. An extensive local infection (as in marked and prolonged tonsillitis) may result in the infection of internal organs, often with serious and even fatal results. Localized inflections, as pus pockets in carious teeth, bones, mucous tissues, connective tissues, internal organs, etc., are responsible for rise in temperature and many other systemic disturbances.

Phagocytosis is also marked in living cells outside of the higher animal organism. For instance the amebas and paramecia are active and even voraceous devourers of bacteria and of yeast organisms. It has even been suggested that an active amebiasis of the human body as in amebic dysentery of the tropics and in the very common amebic infection of the mouth cavity (pyorrhea, Rigg's disease), the infecting organisms act pri-

marily as scavengers, feeding upon the multitude of bacteria present. However, that may be, the amebic infection does induce very serious disturbances which were difficult to cure until it was found that ipecac (emetin) was quickly fatal to the amebas of dysentery as well as to those of pyorrhea (enteric pills, Lloyd alcresta ipecac tablets; or hypodermic injections of the active principle of ipecac, emetin).

The amebas found with decaying fruits and other vegetable matter feed upon bacteria and yeast cells. An ameba found in decaying bananas limited its diet almost entirely to yeast cells which it disintegrated very quickly.

In the ordinary conditon of phagocytosis as explained under staphylococcic infection, with the resultant contra-invasion by the white blood corpuscles, there is unquestionable an active destruction of objectionable invaders (the bacteria), brought about by the special body protectors or guardians, the leucocytes, through the assistance of the opsonins, and such a condition may be designated *patrocytosis* as contrasted with the condition as we find it in amebic dysentery and in pyorrhea where the relationship of ameba to the organism invaded (the human body) is harmful, even though the amebas feed upon body bacteria. In malaria for example, we have true parasitism. The invading organism (the malarial plasmodium) does not feed upon or destroy body bacteria and does not render even the slightest service to the human body.

b. Opsonins.—Opsonins have a direct relationship to leucocytosis or phagocytosis (patrocytosis). Wright and others proved that there exists in the serum of the blood and also in the cells of the body, substances which possessed the property of so acting upon and modifying bacteria as to render them more readily seized upon and devoured by the leucocytes. Nothing is known as to the composition of these substances. No one has succeeded in isolating them in a pure state. They are unquestionably proteid in nature combined with the serum and cell proteids of the body. They are specific in their action. That is an opsonin will not act upon different kinds of bacteria. The opsonin which acts upon the *Staphylococcus aureus* so preparing these organisms as to make them more readily seized upon and digested by the leucocytes, will not act upon or prepare the organism which is causative of pneumonia, or any other species or variety of pathogenic organism. Each species, and probably also each variety of pathogenic microbe is acted upon by a special or specific opsonin.

The proof of the existence of opsonins is as follows: If fresh blood is mixed with an emulsion of bacteria and incubated at body temperature for an hour, it will be found that bacteria are within the white blood corpuscles. If we wash the blood corpuscles free from serum (by means

of physiological salt solution and the centrifugal machine) and mix the white corpuscles thus removed from the serum, with the bacterial emulsion and incubate as before, none of the bacteria will be found within the leucocyte. This test however merely proves that the blood serum influences phagocytosis or leucocytosis. In order to prove that the action is upon the bacteria rather than upon the leucocytes, the following test is made. Use the serum free leucocytes as before, but instead of adding the plain bacterial suspension, add to it a blood serum free from leucocytes. Mix this bacterial suspension now containing serum with the leucocyte suspension, incubate as before and it will be found that the leucocytes again contain bacteria. The serum has in some way acted upon (sensitized) the bacteria rendering them capable of being seized upon by the leucocytes. The phenomenon may be compared to the preparation of food by cooking.

Opsonins are specific in action as indicated, are thermostabile, that is they are not destroyed by a temperature of 55° C. Quantitatively they are very variable. They may even disappear entirely from the blood, at least temporarily. Relative quantitative phagocytosis indicates what is known as the *opsonic index*, which may be determined as follows: (Leishman's method). Mix equal parts of the fresh normal blood and a bacterial suspension in normal salt solution and incubate for 30 minutes. Prepare stained slide mounts and obtain an average of bacteria per leucocyte. This will give the normal phagocytic index, given as 1. If, for example, the normal leucocyte count gives an average of ten staphylococci per leucocyte, and a comparative count of a patient afflicted with abscesses, gives an average of 2 staphylococci, then his opsonic index would be 0.20, that is, much below normal.

The opsonic index may be raised by injecting carefully measured quantities of killed cultures (bacterins) of the organisms of the infection. However, satisfactory results have been obtained in a few cases only, as in acne, in pneumonia, in tubercular infections, and in a few other infections. Certain bacterins have given excellent results as preventives, as in typhoid, in plague, in tetanus, and some other diseases.

c. Toxins, Antigens, Toxoids.—Toxins are poisonous substances elaborated by bacteria and other pathogenic organisms, which possess the property of inducing the development of *antitoxin* (anti-bodies, immune bodies) in the serum of the blood and in the body cells. The toxin is the result of the activities of substances formed in bacteria, known as antigens, which give rise to the anti-bodies (antitoxins). That is, the antigen through some form of stimulation gives rise to substances which neutralize the action of the toxins formed by the toxigenic bacteria. Red blood corpuscles and perhaps also other body cells contain antigens. The toxins

have an injurious effect upon the white blood corpuscles, inhibiting leucocytosis (negative chemotaxis). If virulent cultures of the bacillus of anthrax are injected into susceptible animals, they succumb quickly without any evidence of leucocytosis (negative chemotaxis). If the animal thus injected had been immunized against anthrax by means of attenuated anthrax cultures, there would appear large numbers of leucocytes at the site of the injection. If tetanus bacilli and their spores are washed free from toxin and injected, active leucocytoses follows (positive chemotaxis). The interesting observation has been made that the injection of a mixed culture of highly virulent organisms and non-virulent cultures (of the same kind), the action of the virulent form is both hastened and increased. It is suggested in explanation that the leucocytes preferably seize upon the non-virulent forms and have as a result little energy left to seize upon the virulent forms.

Toxins, therefore, are intimately concerned in the processes of immunization. They induce the development of anti-bodies which overcome or neutralize the very substances elaborated by the pathogenic bacteria and they have a markedly checking, retarding or inhibiting influence on leucocytosis. This apparently contradictory action of toxins is not as yet satisfactorily explained. Metchnikoff suggested that immune bodies as well as complement were enzymatic in nature and that both were produced by the leucocytes, thus doing away with the necessity for assuming that the immune bodies, (anti-bodies), were the result of the action of the toxins or antigens. However, it cannot be denied that the anti-bodies are the result of the presence and influence of the antigens or toxins.

The toxins or poisons elaborated by certain animals, as poisonous snakes, resemble the antigens of bacteria in that they are capable of inducing the formation of anti-bodies. The following animal antitoxins are now on the market: *Antivenene* for *rattlesnake poisoning*, anti-toxin for *scorpion* poisoning; and anti-toxins for eel, fish, turtle, wasp and salamander poisons. Most of the animal toxins are not of a simple molecular structure. The toxin molecule of rattlesnake venom, for example, has a distinct toxophore group which give rise to the general toxic symptoms, and a hemolytic group which disintegrates the red blood corpuscles, and the two act quite independently of each other.

True toxins are formed by certain higher plants, as *ricin* by the castor bean plant, *crotin* by the croton bean plant, *robin* by the locust, *abrin* by the jequerity bean plant, and *pollenin* by certain members of the composite family (golden rods, rag weeds, and others). These several toxins when introduced into the body will give rise to anti-bodies which are used to overcome or neutralize the specific toxins (anti-ricin, anti-abrin, anti-robin, anti-crotin, and anti-pollenin).

It is known that tissue cells react more or less specifically with bacterial toxins and these reactions are used for making certain diagnostic tests, such as the skin reaction tests for tuberculosis, glanders, syphilis, diphtheria and typhoid fever.

d. Antitoxins.—The antitoxins are the substances found in the body cells and in the blood plasm which neutralize or destroy the toxins and toxoids of pathogenic bacteria. As has already been explained, when a pathogenic organism is introduced into the body, the lysins destroy the cell membrane, setting free the endotoxins which by their presence stimulate the formation of the antitoxins. The antitoxins are specific in nature. That is, the antitoxin against the diphtheric endotoxin is not active against the toxin of typhoid, or of plague.

e. Anti-antitoxins.—It is known that the normal bodily resistance to disease varies from time to time and that the artificial immunity due to the introduction of specific antibodies gradually wanes and finally disappears. This variability in the action of antibodies is supposed to be due to substances, again specific in character, in the body cells and in the blood plasm, which destroy the antibodies.

f. Agglutinins.—Agglutinins are specific cellular products which cause bacteria to clump or gather into groups, preceded by a cessation in motion in those organisms which possess motility. The Widal typhoid fever test is based upon this phenomenon.

Agglutinins are produced artificially by injecting bacteria into the circulation of various animals. The serum of such animals contains the bodies which give rise to the agglutinating phenomena. Sera can be so highly agglutinative as to produce this reaction in dilutions of 1–100,000 or more. In typhoid patients the agglutinins generally appear after the fifth day and may persist for a long time (several years) after convalescence. By some it is supposed that the phenomenon of agglutination is a preliminary stage in the development of lysins. It is however a fact, that while the bactericidal action of serum is destroyed at a temperature of 56°C., the agglutinating power survives until 62°C. is reached. Bactericidal sera do not interfere with the agglutinating power. These and other observations appear to indicate that the agglutinins are specific bodies independent of the other immunizing agents.

g. Precipitins.—Precipitins are specific bodies which occur in the blood of an immunized animal, as rabbit or guinea pig. If, for example, a rabbit is immunized against human blood (through repeated injections of human blood directly into the circulation of the animal) and the serum of such immunized blood be mixed with a trace of human blood, a precipitate is formed. The reaction is strictly specific, excepting that the blood immunized against a sheep will form precipitate with the blood from the

goat. Blood immunized against the ape will form precipitate with human blood. The test has been used practically in medico-legal and in criminal cases. The details for making the test may be found in the larger works on bacteriology, parasitology and on immunization. The method is given in full in Bacteriological Methods in Food and Drug Laboratories. P. Blakiston's Son and Company. 1915. (Schneider.)

h. Virulency—Aggressins.—The pathogenic bacteria form substances which protect against the defensive measure of the host, however, one and the same species of pathogenic or toxigenic organism is not at all times equally active defensively. In other words, the virulency of bacteria is variable. In the manufacture of diphtheria antitoxin it is desirable to use a strain of the causative organism which is highly virulent, in order to hasten as well as to increase the formation within the blood of the horse, of the defensive or protective bodies. The virulency may be lowered or weakened (attenuated) in various ways. Exposure to high temperatures may accomplish this. In fact the high bodily temperature in fevers is nature's method of reducing the virulency of the invading organism. Again, the virulency may be lowered and completely modified qualitatively by a change of host, as in cowpox. An organism may be entirely harmless and even highly useful in one position, and become highly virulent in another position in the same organism. Thus the *B. coli* is a normal and beneficent inhabitant of the intestinal tract, but should it be introduced (accidentally or by design) into the peritoneal cavity or into any tissue other than the intestinal tract, it may set up serious abscess formation. The virulency of the causative agent of rabies is lowered as well as modified by exposure to dry air. In a general way all agencies which lower the vitality of pathogenic and toxigenic organisms tend to lower the virulency, although there are some notable exceptions.

As it is possible to lower (attenuate) as well as to increase (augment) the activity (virulency) of objectionable microörganisms, just so is it possible to increase the activity of useful organisms. Thus the free nitrogen assimilating power of the Rhizobia group may be greatly increased by growing the organisms upon special media, or we may reduce this power greatly or practically destroy it. To such changes in beneficent organisms we apply the term potency, rather than virulency. We increase or augment the potency of a yeast ferment, and on the other hand we increase or augment the virulency of the diphtheria germ.

The subject of increased virulency of pathogenic organisms has received a great deal of attention within recent years. Bail and others made some interesting observations which may be summarized as follows: By injecting pure cultures of tubercle bacilli into the peritoneal cavity of a guinea pig, a rapidly fatal tuberculosis is produced. If the peritoneal

exudate from this guinea pig is sterilized and injected into a second guinea pig, together with some tubercle bacilli of the kind used in the first animal, the second animal will succumb much more rapidly than the first, usually within twenty-four hours. If the sterilized exudate alone is injected, nothing will happen; and if the tubercle bacilli alone are injected the tuberculosis will develop within a few weeks, as in the first animal. It is assumed that the peritoneal exudate contains a substance formed by the tubercle bacilli which has the power of greatly increasing the virulency and this substance has been called aggressin. Heating the exudate to 60°C. causes a further increase in the virulency of the aggressin, and it was found that small amounts of the exudate were relatively more virulent than larger amounts, and Bail assumes that there are two substances in the exudate, one which is thermolabile preventing rapid death, the other thermostabile, favoring rapid death. It is assumed that a bacterolysin is formed, which acting on the bacilli, liberates the endotoxin which paralyzes the polynuclear leucocytes, the mononuclear phagocytosis being undiminished.

i. Anaphylactic Reactions.—Anaphylaxis is the opposite of prophylaxis. It indicates a state of susceptibility or rather hypersusceptibility to certain substances when brought in biological contact with living cells. The term was originally used to indicate a condition of hypersusceptibility to diseases generally. More recently the term anaphylaxis or anaphylactic shock, has been very largely applied to the hypersusceptibility toward horse serum. The investigations into anaphylaxis were prompted by the comparatively common cases of serum sickness (rash, urticarias, enteritis, etc.) and the comparatively rare sudden collapse and death, following the use of diphtheria antitoxin. These classic investigations proved that the anaphylactic reaction, or state, depended upon the following essentials:

First.—Injecting into the experimental animal (as rabbit or guinea pig) a dose of some non-toxic protein, which sensitizes the animal specifically to this particular substance.

Second.—An incubation period of from eight to fourteen days, followed by

Third.—A second injection of the same protein, at the close of the incubation period. The anaphylactic reaction appeared almost immediately (collapse and death).

Fourth.—The sensitization developed by the initial dose may endure for months and even for years, in fact may endure for life and may be transmitted to the offspring.

The experiments also proved that the anaphylactic condition can be developed toward a great variety of substances, animal, vegetable and

even mineral. The sensitization or anaphylactic reaction phenomena are extremely variable in kinds as well as in degree. The following is a brief summary of the subject:

Disease is nothing more nor less than an anaphylactic reaction to the proteins of the causative agents, as bacteria and protozoa. The reactions indicate that nature is endeavoring to overcome the toxins formed, developed or generated. It is presumed that no one will take a disease unless first sensitized to the specific causative agent. What is generally known as the incubation period in disease corresponds to the sensitization period in anaphylaxis. Recovery from disease simply means that the characteristic reaction (as manifested by the symptoms) has been successful. If death is the outcome, it means that the reaction was exhausted, paralyzed or broken down.

Hypersusceptibility to certain foods, more especially to roe, fish, shellfish, eggs, milk, cheese, rhubarb, strawberries, tomatoes and cereals, is fairly common. The symptoms usually appear soon after eating and vary in kind as well as in degree, depending upon the nature of the food and the degree of susceptibility to it. There may be urticarias, erythemas, prickly heat, spasms, asthmatic conditions, respiratory difficulties, fever, gastrointestinal disturbances and occasionally sudden and complete collapse. Physicians have noted that eczema is frequently caused by certain foods, as excess of fats and starch. The so-called cyclical vomiting of children is supposed to have its origin in food susceptibility, associated perhaps with an inherited neurasthenic condition.

It is theoretically suggested that man was originally hypersensitive to all foods and as a result of the anaphylactic reaction the specific antibodies or protective bodies were gradually developed, finally establishing full prophylaxis toward those substances which we now recognize as harmless and wholesome foods. The reason why we cannot use the deadly night-shade, or nux vomica, or aconite, or tobacco, as foods is because we are still in a state of hypersusceptibility toward these plants. There are many marked racial differences in food hypersusceptibility. The goat and the hog will thrive on vegetables which are highly toxic to man. The existence of an anaphylactic reaction toward a given substance, as strychnine, aconite, curara, etc., indicates an effort to establish immunity. If the amount of ingested food substance for which immunity is not yet established is excessive, the reaction may be wholly exhausted with disastrous results. Anaphylaxis is nature's warning against over-indulgence in a food for which prophylaxis is not yet fully established.

Anaphylaxis which is developed parenterally, that is by bringing the reacting protein or other substance in direct physiological contact

with body cells, without exposure to enteral enzymatic action, is quantitatively as well as qualitatively different from the anaphylactic conditions following the administration of the reacting substances *per os*. The fluids and enzymes of the digestive tract modify profoundly the anaphylactic reaction. It is true, similar enzymes exist within the general body cells, but here they are not associated with activating elements. The enteral solutions and enzymes break down (catalyze) many, in fact most, of the organic compounds, rendering them anaphylactically harmless.

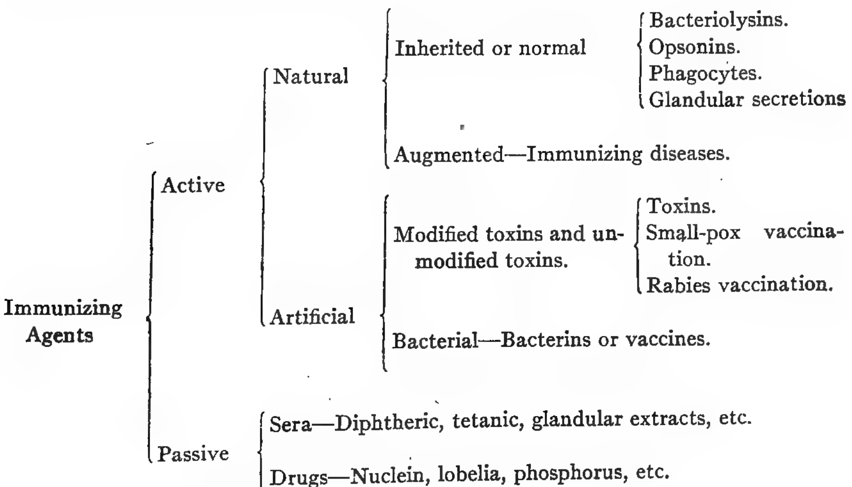
Some very interesting observations have been made in regard to anaphylactic skin reactions with food substances. If a trace of a given food substance or an aqueous extract thereof is briskly rubbed into a small skin abrasion, hypersusceptibility or anaphylaxis toward the food will be indicated by circumscribed redness which develops quickly and disappears in a short time. The redness (inflammatory process) is simply a local anaphylactic reaction. Such tests have been made with a great variety of raw, partially cooked and completely cooked foods. The skin reaction is simply a localized anaphylactic shock. If the same substance were injected into the general circulation, a general bodily reaction might follow.

Skin reaction tests along the lines above outlined could no doubt be made of great practical value to the diagnostician, the clinician, in forensic medicine, in criminal investigation, in the study of dietetics, in food investigation, etc. It would be possible to determine hypersusceptibility, not only to foods, but to a variety of other substances. The physician makes use of a number of recognized skin reactions, as in typhoid fever, in diphtheria, in tuberculosis, in glanders, in syphilis, in gonorrhea, in hay fever. Not only may the existence of the disease be ascertained by such tests, but the degree of susceptibility to such diseases may be ascertained. This latter information would prove of great value as indicating the diseases against which any existing hypersusceptibility should be overcome by appropriate means, as perhaps avoiding as much as possible the chances of exposure to such diseases. Tests determining the susceptibility to drug action would be a means of selecting proper dosage. In fact, experiments with non-toxic plant extracts administered parenterally is opening up a new system of therapeutic activity. The essentials in this newer therapy are based upon the assumption that certain wholly non-toxic plant substances which are enzymatic in nature, as chlorophyll, lipoids and vitamines, act biologically in splitting up foreign proteins, as bacteria, pathological deposits and formations as in cancer, in goiter, in syphilis, in gout, in rheumatism, etc. It is claimed that excellent results have already been obtained from the intravenous and intramuscular administration of plant extracts and metallic colloids

in cancer, in goiter, in chronic eczema, in tuberculosis, in hay fever and in other intractable diseases.

An animal (as rabbit or guinea pig) which has been sensitized to any specific substance, as human blood, deer's blood, egg albumin, casein, cow's milk, goat's milk, horse serum, specific plant extracts, poisons of many kinds, etc., will show a skin reaction toward the specific substance for which it was sensitized. One and the same animal may in fact be sensitized to three or more substances at the same time and will show a separate and distinct and specific reaction for each substance. The specificity fails or rather merges or blends with many closely related substances. Thus a guinea pig sensitized to human blood will react toward the blood of the ape. Other interacting sensitizations develop toward rat and mouse, dog and wolf, horse and ass, sheep and goat. Organ specificity is also highly interesting and gives some apparently contradictory results. For instance, a guinea pig sensitized to cerebral extract of a rabbit will react toward the cerebral extract of widely different species, but will not react toward other tissues.

It may indeed be possible to sensitize one animal toward a large number of substances and such animal may then be employed for making skin reaction tests with any one or all of the substances in question. Thus the physician could use this animal for diagnostic purposes; the criminologist to ascertain whether or not a given blood stain was of human origin; the food analyst to determine whether or not the albumen is from duck's eggs or from hen's eggs; the pathologist for the purpose of ascertaining the malignancy or benignancy of a tumor; the bacteriologist to determine the group relationship of a given microörganism; the toxicologist to ascertain the identity of a poison, etc.



CHAPTER XI

SEROLOGY—THE MANUFACTURE AND USE OF SERA AND VACCINES

The most wonderful recent discoveries in the science of bacteriology pertain to the relationship of pathogenic germs and the serum of the blood of susceptible animals. As already stated blood serum has bactericidal properties (see lysins), but it is often not sufficiently active to destroy certain invading germs (pathogenic) and the disease manifestations, due to the toxins liberated by the germs, gradually develop. The bacterial toxins are of two kinds, those which escape from the bacterial cells and are soluble in the surrounding media, entering the system by absorption; and those which remain within the germ cell and are set free only on the breaking up of the bacterial cells. The former are the toxins proper or exotoxins, the latter are called endotoxins. As already explained the toxins cause the development within the serum of the blood of certain substances (antibodies), which neutralize or overcome the effects of the toxins and which are called antitoxins. Investigators hoped that experiments would prove that every pathogenic germ would cause the development of a corresponding antitoxin which might be used in the treatment of the disease. This hope has not been realized. Of the numerous experimentations with antitoxins only one has thus far proven entirely satisfactory, namely, the antitoxin of diphtheria. Several others have proven more or less useful, as will be explained later, but they are far from satisfactory.

The antitoxins act by neutralizing the bacterial toxins of the disease, and not by acting upon and killing the germs themselves. In this regard the antitoxins or antitoxic sera differ from the antibacterial or bactericidal sera, which act by preventing the development of the bacteria. This distinction and difference is not generally understood. The bactericidal sera have, however, thus far proven quite unsatisfactory in the treatment of disease. They are not standardized by units as are the antitoxins. The dose is by volume, from 10 to 50 cc., and even more, usually given hypodermically. The sera are produced by injecting toxins or the toxic germs (artificially cultured) into the animal, as the horse. As a rule the first injections consist of dead germs; finally, living germs of different virulency may be used. By this means a tolerance is established. The serum obtained from animals thus immunized is used in the treatment of disease, its action depending upon its bactericidal properties. There is a

group of sera known as composite, which give evidence of being a decided improvement over the simple sera. They are called composite because they have the peculiar qualities of two distinct forms of immunity—for example, diphtheria-immune horses may be used in the subsequent bacterial inoculation, which gives the resulting immune-serum a double content of a corresponding antibacterial body and of diphtheric antitoxin. This subject is as yet entirely in the experimental stage. It is also known that one kind or type of immunity has some influence not only upon other immunities, but also upon other diseases. The antitoxin of diphtheria, for example, appears to act as a cure or prophylactic against pathological conditions other than diphtheria.

We now come to a third class of substances used in the treatment of disease, namely, the bacterial vaccines, also designated bacterins and opsonogens (Ohlmacher). The term vaccine (from *Vacca*, a cow) is appropriately applicable to the small-pox remedy, but is entirely inapplicable to these newer agents. Either bacterin or opsonogen is a suitable name.

Bacterins are simply suspensions of dead pathogenic germs which are used in the treatment of disease. A homologous or autogenous bacterin is prepared from germs taken direct from the patient and is used in treating the same patient. A heterologous bacterin is one which is derived from a source other than the patient under treatment. A mixed bacterin is one in which the germs (of the same species) used are derived from several sources. The manufactured bacterins (heterologous) ready for use by the physician are called stock vaccines or stock bacterins.

The following is a tabulation of antitoxins, toxins, antibacterial sera and bacterins found upon the market and used by physicians and veterinarians.

1. For Human Use

A. *Antitoxic Sera or Antitoxins.*

- Antidiphtheric serum.
  - Liquid or usual form.
  - Concentrated form.
  - Dry form (official in some pharmacopœias).
- Antitetanic serum.
  - Liquid or usual form.
  - Dry form.

B. *Antibacterial Sera or Bactericidal Sera.*

- Antistreptococcic serum.
- Antipneumococcic serum.
- Antimeningitic serum.

Antityphoid serum.
Antidysenteric serum.
Antigonorrheal serum.
Antiplague serum (Yersin's serum).
Antianthrax serum.
Scarlet fever serum (Marpmann's serum).
Antituberculous serum (antituberculins).

C. *Bacterins or Opsonogens.* (*Vaccines.*) (Homologous or autogenous, heterogenous and mixed.)

Staphylococcus.
S. pyogenes albeus. } used singly or mixed.
S. pyogenes aureus. }
S. pyogenes citreus. }
Streptococcus.
Gonococcus.
Typhoid.
Typhoid (Shafer's mixed bacterin).
Colon bacillus.
Neoformans bacillus.
Pyocyaneous bacillus.
Bubonic plague bacillus (Haffkine's plague vaccine).
Tuberculins.
Tuberculin, old (T. O.).
Tuberculin residuum (T. R.).
Tuberculin precipitate (T. P.).
Bacillus emulsion (B. E.).
Bacillus filtrate (B. F.).

D. *Toxins (modified).*

Small-pox vaccine.
On ivory points.
In glycerinated tubes.
Dry form.
Hydrophobia vaccine.
Erysipelas and prodigiosus toxin. (Cancer and other malignant growths.)
Antivenine. (Snake toxin.)

2. For Veterinary Use

A. *Antitoxic Sera or Antitoxins.*

Antitetanic serum.
Influenza serum. (Intravenous use.)
Hog cholera serum.

B. *Antibacterial Sera or Bactericidal Sera.*

Antistreptococcic serum.
Canine distemper serum.
White scour serum.

C. *Bacterins.*

Anthrax.
Mallein.
Tuberculin.
Blackleg.
Blacklegine.
Blacklegules (pill form).
Blacklegoids (pill form).
Hog cholera.
Fowl cholera.
White scour.
Texas fever.

The above substances resemble each other in that they are organic and of complex chemical composition. They gradually deteriorate and finally become worthless, some sooner than others. Even the comparatively permanent kinds will not retain their full activities more than a few months, though they may still be sufficiently active therapeutically after eighteen months, or even longer. They should be kept in a cool dry place, away from light. Turbidity in those preparations, which are clear when freshly prepared, indicates that decomposition changes have set in and that they are unfit for use. Many of the bacterins are normally turbid and nearly all of them have some slight color and odor.

Thus far only a few of the substances above tabulated have proven entirely satisfactory in the treatment of the particular disease or diseases for which they are intended. This is but to be expected since their use is very largely based upon theory. Theory and practice have ever failed to develop along exactly parallel lines. Science is however fortunate in being able to assert that in the antidiphtheric serum we have practically a specific for the cure of diphtheria, provided it is used in time and given in sufficiently large and sufficiently frequent doses. The antitetanic serum has given excellent results particularly as a preventive, as has also the antistreptococcic serum. Of the bacterins the Staphylococcus has given excellent results in the cure of actual pathologic conditions. Some of the others have proven less satisfactory and in many cases their great usefulness lies in their preventive rather than curative powers.

We will explain very briefly the manufacture of a few of these substances only, as the methods are quite closely similar for like agents. The

following is a brief outline of the manufacture of the marvelous remedy for the treatment of the dread disease of childhood, namely diphtheria.

1. **Antidiphtheric Serum**

A. *Selecting and Testing the Horse.*—Ordinary, normal, non-pedigree horses are preferred, purchased under a guarantee of soundness. Even though purchased under such a guarantee the animal is kept under observation for a few weeks and tested for glanders by the mallein test. No animal is retained until it is proven that there is no latent or active disease present. The animal is well housed and well cared for during the entire time, under conditions as sanitary as it is possible to make them. All laboratories are also regularly visited by a U. S. Government inspector, who reports his findings to Washington.

B. *Preparing the Toxin of Diphtheria.*—Pure cultures of a selected strain of the diphtheria bacillus, possessed of a high potency, virulency or toxicity, are made in liter flasks containing beef bouillon. The original bacilli thus used are taken from some patient suffering with diphtheria, and by means of isolation methods all foreign microbes are rejected or excluded. After the culture is several days old or when a maximum amount of the toxin has been formed and deposited in the bouillon, the bacili are killed by adding 0.25 per cent. of trikresol. The bouillon with the dead bacilli is filtered. The clear filtered substance constitutes the toxin which is injected into the horse for the purpose of developing (in the horse) the antitoxin of diphtheria. The virulency or potency of the toxin varies and is tested on guinea-pigs and compared with the U. S. Government standard. The highiy toxic race or strain of germs is perpetuated in the laboratory by daily transfers to new culture tubes. In this manner the bacilli are maintained for a long time, several years or longer. However, even with the greatest care the race finally deteriorates, weakens or undergoes a change in potency and it becomes necessary to secure a new stock culture.

C. *Developing the Antitoxin of Diphtheria in the Horse.*—Twice weekly the horse is given (by hypodermic injection into the flank region) gradually increasing doses of the toxin of diphtheria. The rule is to give enough to produce a marked reaction. For a day or two the horse is sick with diphtheria, then recovers as the increased antitoxin in the blood (serum) of the animal neutralizes the toxin. This is continued for from four to six weeks when a maximum amount of antitoxin has presumably developed. The last dose of toxin is several hundred times greater than the first.

D. *Bleeding the Horse.*—A sterilized canula or trochar is inserted into the jugular vein, after the neck has been thoroughly washed with soap and water, shaved and rinsed with a 5 per cent. solution of carbolic acid. The blood is drawn off into sterilized liter tubes, which are plugged with cotton.

From nine to twelve liter of blood are taken from the horse at one time and the bleeding is repeated four or five times at intervals of about six months. The punctured wound is closed by keeping an artery forceps in position for a short time.

E. *Securing the Serum.*—The blood tubes are set aside until the clot has formed and settled to the bottom. The clear serum is siphoned off into a large flask, 0.25 per cent. of trikresol is added as a preservative and

FIG. 63.—Bleeding the horse after a maximum amount of the antitoxin of diphtheria has been developed in the blood. The animals pay but little attention to the operation.

to kill any germs that might be accidentally present, and then filtered through several thicknesses of filter paper, under pressure (suction). The perfectly clear, sterile and germ-free serum constitutes the antitoxin of diphtheria and is ready for use as soon as it is standardized and put into suitable containers.

F. *Standardizing the Antitoxin of Diphtheria.*—Since the antitoxic valence of horse serum as above described varies somewhat, it is necessary to determine the quantitative value in order that physicians may know what amounts to administer in the treatment of diphtheria. The standard unit of strength now adopted by all civilized countries is the so-called

Ehrlich unit, which is the amount of serum (antitoxin of the horse) which will just neutralize one hundred times a fatal dose of toxin when administered to a guinea-pig, weighing approximately 250 grams or one-half pound. The U. S. standard is prepared in the biological laboratories of the U. S. Public Health Service at Washington, and every manufacturer of diph-

FIG. 64.—Sterilized liter tubes into which the blood drawn from the horse is placed. The top, covered with sterilized cloth, is connected with the canula in the jugular vein of the animal.

theric antitoxin in the United States is supplied with standard units from this laboratory. The method of procedure is approximately as follows: Eight containers (test-tubes) are set out in a row and numbered or marked serially. Into each tube is poured just one hundred fatal doses of toxin (fatal to a 250 gram guinea-pig, determined experimentally), and a graded amount of the serum to be standardized, so that the first tube has, in all

probability, not enough antitoxin to neutralize the one hundred fatal doses of the toxin, and the eighth tube has, in all probability, a great excess of antitoxin. The contents of one tube is injected into a guinea-pig, thus requiring eight pigs. The animals are marked and kept under close observation. The first, second and perhaps third die, showing that not enough serum was added to neutralize the toxin. The fourth pig just recovers, showing that the amount of serum added to the fourth tube was sufficient to neutralize one hundred fatal doses of the toxin. This

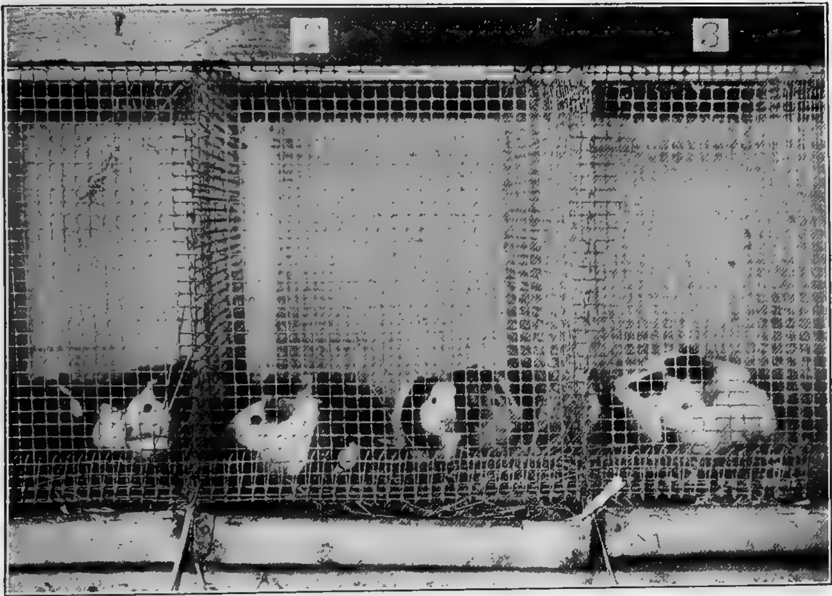


Fig. 65.—Guinea-pigs in wire cages. These lively little animals are used in testing the virulense of the diphtheria toxin which is injected into the horse and also for the purpose of standardizing the antitoxin. The reasons why these animals are preferred are wholly biological and physiological. They propagate rapidly, are easily kept, easily handled, and respond (biologically) to the tests applied.

amount of serum (antitoxic) represents one unit. From this amount or unit the quantities to be put into the containers are determined. 500, 1000, 2500 and 5000 unit quantities are put up, for the convenience of physicians. 500 to 1000 units constitute an immunizing dose, given to those who do not have diphtheria, but who have been exposed to the disease. The larger doses are curative. The rule is to give large doses, repeated as often as may be necessary. 1000 units are usually employed as immunizing doses; 3000, 5000 and 10,000 unit packages for curative doses.

2. Concentrated Diphtheric Antitoxin

While the chemical nature of antitoxin is not known, it has been determined that it is united, in some way, with the globulins of the blood. The attempts to isolate antitoxin have resulted in the manufacture of a refined or concentrated antidiphtheric serum which is used quite extensively

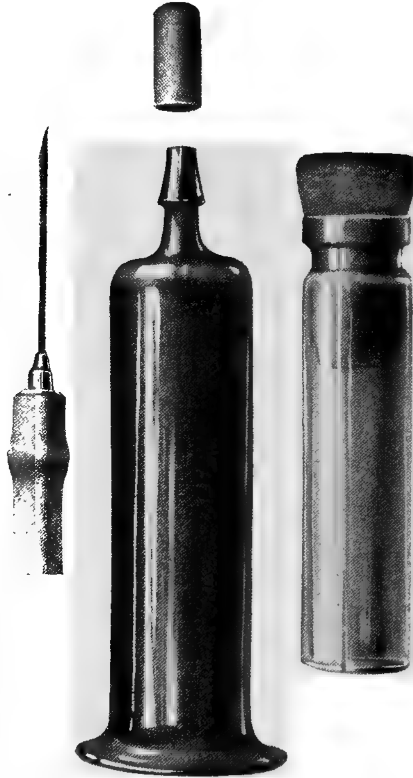


FIG. 66.—Container with diphtheria antitoxin, supplied with hypodermic needle, piston, all ready for immediate use by the physician. The plunger is simply a homeopathic vial with rubber stopper. (*Cutler Laboratory.*)

though it does not meet with the unqualified favor accorded the antidiphtheric serum. The process of manufacture is as follows:

a. The antidiphtheric serum is saturated with ammonium sulphate which precipitates the globulins (containing the antitoxin) in the form of a white mass. It is then filtered and the filtrate rejected.

b. The precipitate left on the filter is redissolved in water and this solution is again treated with ammonium sulphate as in (a). The object in redissolving in water is to wash the globulins.

c. The second precipitation product is treated with a saturated salt solution which dissolves the antitoxin globulins. The solution is then filtered.

d. To the filtered solution 2.5 per cent. of acetic acid is added which again precipitates the globulins on the filter paper where it is partially dried by means of filter paper and towels pressed upon the mass.

e. The partially dried material is placed in a dialyzing bag and suspended in a water current, for several days. This removed the salts by osmotic action and at the same time the globulins enter into solution within the bag.

f. A preservative is added to the liquid which is then passed through a Berkefeld filter. Some physiologic salt solution is also added. This is the final product.

g. After being tested bacteriologically to make sure that it is not contaminated, it is standardized as described under diphtheric serum.

The above process removes the following non-active substances: serum albumins, lecithin, cholesterin, traces of bile salts and acids, blood salts and the non-antitoxic globulins. The dosage of the concentrated antitoxin is less than that of the non-concentrated serum and it keeps longer. For the manufacture of the concentrated diphtheria antitoxin the returned serum is generally employed, that is serum which has exceeded the time limit of use.

3. Antitetanic Serum

This is prepared similarly to antidiphtheric serum. The tetanus bacilli are grown in bouillon, in the absence of oxygen, since tetanus germs are anaërobic. The growth is then killed, filtered out and the clear toxic, germ-free bouillon filtrate is utilized in the immunization of the horse. Small doses, usually mixed with some antitetanic serum, are administered at first and gradually increasing the amount as the horse can stand it until large quantities are given, even as much as 700 or 800 cc. After some months the horse is bled in the same manner as for antidiphtheric serum, the serum is separated and bacteriologically tested in the same way.

The unit of tetanus antitoxin is that quantity of antitetanic serum which is necessary to completely neutralize 1000 fatal doses of tetanus toxin for a 250-gram guinea-pig.

Antitetanic serum has not been a marked success as a curative agent. Its greatest usefulness appears to be as a prophylactic, for which purpose it should be given early, as soon as the injury (cut, gunshot wound, abrasion) has occurred.

The following are the more important antibacterial sera. A fuller description of the processes of manufacture is omitted as that is a matter of no special importance to the pharmacist. Furthermore, manufacturers do not, as a rule, disclose full details of manufacture.

4. Antipneumococcic Serum

This serum is obtained from horses immunized against the Pneumococcus and is employed in the treatment of pneumonia and other infectious disease in which this germ is present. The dose is about 10 cc. repeated several times a day, given hypodermically. The serum must be kept in a cool dark place. When a tube is opened the contents should be used within twenty-four hours, sealed temporarily with sealing wax, paraffin or sterile wadding. This serum has not proven very satisfactory, though it is safe and worthy of a trial. (See pneumonia.)

5. Antimeningococcic Serum

Antimeningococcic serum is obtained from horses which have been immunized with cultures of *Diplococcus meningitidis intracellularis*, beginning with dead cultures, then using living cultures and finally with autolysate. Its use is said to have met with considerable success in the treatment of cerebro-spinal meningitis, when injected into the spinal canal in doses of 10 cc., repeated daily. The serum acts as an antitoxin, it increases phagocytosis and also acts as a bactericide. It should be used early in the course of the disease.

6. Yersin's Serum (Antiplague Serum)

Yersin's serum is made by injecting horses, first with dead plague bacillus cultures (*Bacillus pestis*) and finally with the living organisms. It has been used with varying success in plague epidemics. Large doses (30 to 50 cc.) should be administered (hypodermically) early in the course of the disease. Its chief value is, however, prophylactic. The liquid form of the serum may also be used for intravenous injection. The dry serum is said to keep indefinitely and must be dissolved before using.

7. Bacterins

a. Ordinary Bacterins.—The bacterins are still, so to speak, on trial. Some have given excellent results while others are wholly unsatisfactory. The preference appears to be for autogenous bacterins. The majority of physicians are, however, compelled to use the so-called stock bacterins, or the manufactured bacterins ready for use, for the reason that few physicians have the time or the equipment to prepare the homologous or autogenous bacterins. The method of preparing a homologous bacterin may be outlined as follows:

a. A tube, flask or plate with the suitable culture medium (agar or gelatin) is inoculated with the germs taken from the patient and incubated, until a maximum development has taken place, about twenty-four hours.

b. The growth is separated from the culture medium by means of a

sterile physiological salt solution and a platinum wire loop. The salt solution with the bacteria is transferred to a sterile test-tube which is then sealed in a flame.

c. When the tube is cool, it is shaken vigorously so as to emulsify the bacteria in the salt solution.

d. The tube is opened and about one drop is removed with which to make the blood-corpuscle count, to be explained later. The tube is again sealed in the flame.

e. The tube is now placed in a water bath (opsonic incubator of special construction for this work) at a temperature of 60° C. for a sufficient length of time to kill the germs; one hour is usually adequate. This constitutes the bacterin and is ready for use as soon as it is standardized. Usually some preservative is added when the tube is opened and before the bacterin is injected (0.2 per cent. lysol, 0.4 per cent. trikresol, etc.).

f. From the above it must be evident that no two preparations contain the same number of germs per cc. and hence the physician cannot know how many dead microbes are injected at a dose. Therefore the necessity of standardizing the bacterin, which is done as follows:

g. Mix one part of freshly drawn blood with one part of the bacterin (taken from the tube in d.), add two or three parts of physiological salt solution, and spread evenly on a slide. Examine under the microscope and determine the number of microbes per cc. in terms of the number of red blood-corpuscles per cc. This is done by making numerous (10 to 20) counts of red blood-corpuscles and microbes. Knowing that there are 5,000,000,000 red blood-corpuscles per cc., it is then a simple matter to compute the number of microbes per cc. in the bacterin under consideration. The count thus determined divided by the number of bacteria desired for one dose, indicates the number of times the bacterin is to be diluted. This is very clearly illustrated in a chart prepared by Houghton, shown in Fig. 67.

The number of bacteria administered per dose depends upon the therapeutic effects to be produced, the kind of bacterin used, the nature of the disease and the condition of the patient. The rule is to start with small doses, gradually increasing them in such a manner as to secure a maximum of positive opsonic phases with a minimum of negative opsonic phases. In round numbers the dosage ranges from 5,000,000 to 50,000,000 bacilli, represented by varying quantities of the bacterins.

b. Sensitized Bacterins.—The ordinary bacterins effect protection against disease in two ways. a. Stimulating the formation in the body cells and in the blood serum, the specific amboceptors which will increase phagocytosis. b. Stimulating the development of the specific antibodies which will neutralize the specific bacterial toxin (endotoxin). It takes

from five to ten days to develop the immunizing effects, and their use was accompanied bytmore or less severe local as well as systemic reactions

Red Cells	Bacteria
16	12
14	8
9	5
17	15
16	10
20	9
11	10
19	13
15	9
6	5
17	14
8	6
18	11
18	17
22	10
18	8
14	16
14	10
18	16
18	20
308	224

308 224 5000.
X = 3636
$\frac{3636}{400} = 9$

FIG. 67.—Counting the bacteria in standardizing bacterins. This chart shows the count of bacteria and of red blood cells in twenty successive fields of the microscope. The number of red cells counted (308) is to the number of bacteria counted (224) as the number of red cells per cubic centimeter in normal blood (5,000,000,000) is to the number of bacteria per cc. in the suspension (3,636,000). This count (3,636,000) divided by the count desired in the final dilution(400,000,000) gives the number of times (9) this suspension must be diluted to bring it to the desired dilution. (*Parke, Davis & Co.*).

(anaphylaxis). These undesirable qualities are said to be avoided or overcome by the use of the so-called sensitized bacterins or sero-bacterins.

The sensitized bacterins differ from the ordinary bacterins in that they are pre-charged or saturated with the specific amboceptors, thus when injected hypodermically or intravenously, the body is at once stimulated to form the specific antibodies (anti-endotoxins). They are prepared as follows, using typhoid sero-bacterin as an example. Twenty-four hour pure cultures of the *Bacillus typhosus* are placed in normal salt solution (0.85 per cent.). The mixture is thoroughly emulsified and filtered into a centrifuge tube and to it is added immune goat's serum (that is, blood from a goat which has been immunized against typhoid) and let stand for twenty-four hours at a temperature of 24° C., with frequent shaking. Saline solution is added, shaken, and centrifuged for 5–6 minutes. The supernatant liquid (saline solution containing most of the excess of immune serum) is drawn off. More saline is added, shaken and again centrifuged, and the supernatant liquid again drawn off (saline solution containing the last trace of the excess of immune serum). The reason why the excess immune serum must be drawn off is because experience has demonstrated that it would interfere with the development of the active immunization by the bacterial antigen. The bacteria left in the cylinder of the centrifuge are now said to be sensitized by the immune serum and constitute the so-called sensitized bacterin or sero-bacterin, or in this particular case, the typho-serobacterin. Some bacteriologists are of the opinion that the living sensitized bacteria should be used, whereas others are of the opinion that the dead bacteria are just as effective and their use is not accompanied by the possibility of spreading active infection, although this is not likely as far as the typho-serobacterin is concerned. (This organism developing in the intestinal tract and not hypodermically.) It would appear that the present tendency is to prefer the killed bacteria. For the purpose of killing the sensitized bacteria, heat or phenol, or other antiseptic, is use. The bacteria are then counted by the Wright methods (explained elsewhere) and standardized suspensions are made for use as a preventive of typhoid and also as a cure. At the present time it is customary to inject a trivalent typho-serobacterin, consisting of the sensitized *Bacillus typhosus* and of *Bacillus paratyphosus* A and of *B. paratyphosus* B. Definite numbers of the sensitized bacteria are injected at a dose, from 125,000,000 to 2,000,000,000, suitably suspended in saline solution. Most of the sensitized bacterins are used for the purpose of developing active immunizations rather than as cures, although some of them have proven quite effective in certain chronic stages of disease. The sero-bacterins possess the following properties and advantages over the ordinary bacterins.

1. They produce quick active and lasting immunity, which begins within 24 to 48 hours after they are introduced into the system.

2. There is no negative phase (opsonic) as in the use of the ordinary bacterins. The negative phase (represented by aggravation of symptoms and reduction in phagocytosis) following the use of the old bacterins is due to the fact that the bacteria take up specific antibodies (amboceptors) from the blood of the patient.

3. As compared with the ordinary bacterins, the local reaction (pain, congestion, erythema, etc.) are greatly reduced, and the general or systemic reaction (fever, headache, etc.) is practically eliminated.

The work on sensitized bacterins is recent and much of the research which led to the present perfection of these preparations must be credited to Besredka, Ehrlich, Metchnikoff, Gay, Theobald Smith, Gordo, Meyer and Babes. Sero-bacterins have been extensively tried out during the world war, in nearly all of the armies, particularly the trivalent typho-sero-bacterin. As the result of the use of this remedial agent typhoid fever has become non-existent in the army, once the deadliest foe. The U.S. army statistics show that the typhoid case rate has fallen from 3.03 per thousand in 1909 to 0.009 per thousand in 1914 (or a reduction of 98 per cent.); and the mortality rate fell from 0.28 per thousand in 1909 to 0 in 1913. Certainly a convincing showing.

The following are the more important sero-bacterins now in use.

Acneic. Autogenous or polyvalent stock preparation. Used in acute as well as in chronic cases.

Asiatic Cholera.—Univalent. For preventive immunization.

Bacillus coli.—Univalent, autogenous, or polyvalent (strains). In fistula, local infections, catarrhal jaundice.

Influenza.—Polyvalent. In influenza, catarrh, colds. Prophylactic.

Gonococcic.—In chronic cases.

Meningococcic.—Preventive immunization.

Pertussic.—*Bacillus pertussis.* Preventive and as a cure.

Plague.—Active and rapid immunization.

Pneumococcic.—Univalent and polyvalent.

Pyorrheic.—Polyvalent. In bacterial pyorrhea.

Staphylococcic.—Autogenous and polyvalent. Treatment.

Streptococcic.—Treatment of erysipelas, infections, abscesses.

Typhoid.—Univalent and trivalent. Immunization and treatment.

8. Tuberculins

The tuberculins are of special interest as they give great promise in the successful treatment of tuberculosis. The different kinds have their special use. Their manufacture is briefly outlined as follows:

A. *Tuberculin Old* (T. O.).—This is the original Koch tuberculin or Koch lymph and is a concentrated bouillon culture of the tubercle bacillus,

which has been filtered to remove the germs. It is a toxin solution and not a bacterin proper.

B. *Tuberculin Residuum* (T. R.).—This is prepared by grinding the dried tubercle bacilli, extracting with water, centrifugalizing, discarding the supernatant liquid, regrinding the sediment, which is first allowed to dry, and mixing with glycerin and water. It is thus a suspension of pulverized tubercle bacilli in an aqueous solution of glycerin. The grinding process is tedious and requires much time. The tuberculin is standardized so that 1 cc. will represent 10 mg. of the dry culture.

The supernatant liquid, after centrifugalizing, is sometimes drawn off, instead of rejecting, and constitutes the upper tuberculin (T. O.) (Obere Tuberculin). These two tuberculins (the T. R. and the T. O.) differ in therapeutic value and in physical properties.

C. *Bacillus Emulsion* (B. E.).—This consists of pulverized tubercle bacilli suspended in 50 per cent. glycerin and is standardized to contain 5 mg. of solid matter per cc. It differs from T. R. in that the supernatant liquid (T. O.) is not drawn off.

D. *Tuberculin Precipitate* (T. R.).—This is obtained from old tuberculin by precipitation with alcohol, drying and pulverizing the precipitate. It is used in making the Calmette eye-test. (See tuberculosis).

E. *Bouillon Filtrate* (Tuberculin Filtrate B. F.—Denys Tuberculin). The tubercle bacillus cultures are passed through a Berkefeld filter to remove all germs. The filtrate is preserved with trikresol.

9. Small-pox Vaccine

Small-pox vaccine is not a true toxin nor yet a true bacterin. Its value in the eradication of small-pox has world-wide recognition. The following is the manner in which small-pox vaccine is prepared.

a. *Selecting the Animal.*—A young heifer (five to ten months old) is selected, tested for tuberculosis by means of tuberculin. The animal is observed for a time to make sure of general condition of health; is well fed and well cared for, under conditions as sanitary as it is possible to keep them.

b. *Inoculating the Animal.*—The heifer is strapped securely to a framework, back down; the udder region is cleansed, shaven and cross marked (scarified) with a sharp scalpel. The cuts are just deep enough to cause the escape of serum, not actual bleeding. This scarified surface is then inoculated with glycerinated small-pox virus taken from a patient. When the inoculated material has had time to be absorbed the animal is righted again and cared for under as aseptic conditions as possible. In time (six to seven days) pustules form over the entire inoculated area. The virulent virus from man conveys the disease to the animal, but in its

passage through the animal it becomes modified, losing in virulency, yet capable of producing immunity as the result of a mild intoxication (vaccinia).

c. *Removing the Scab.*—The animal is again fastened to the frame. The inoculated surface is washed and dried. The thick scab which has formed over the inoculated area is then removed and triturated with 50 per cent. glycerin. This constitutes the small-pox vaccine.

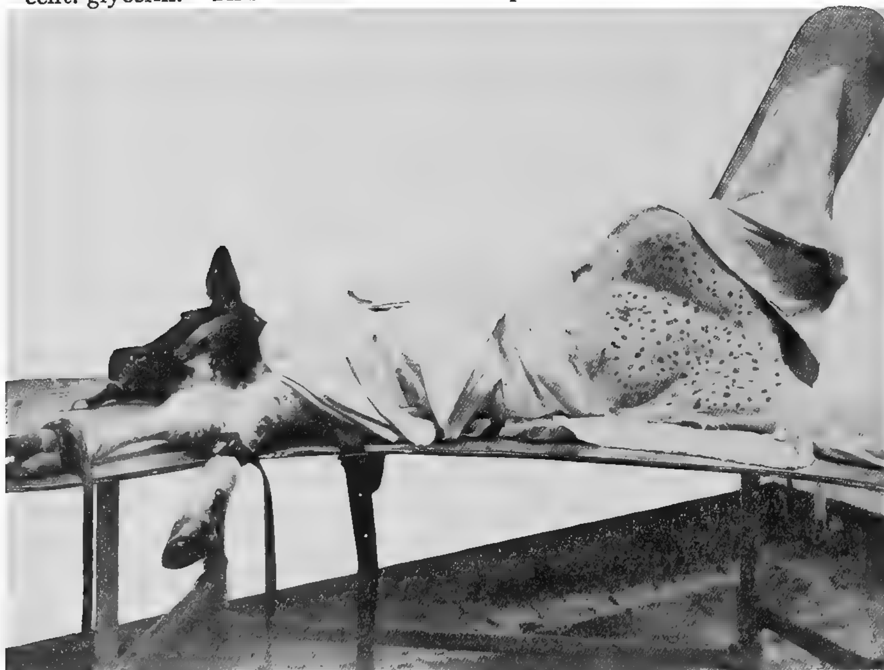


Fig. 68.—Showing the heifer strapped to the frame, preparatory to removing the vaccinia scab from the area which was scarified and inoculated with the small-pox virus. The scab patches show dark.

d. *Aging or Ripening the Vaccine.*—The fresh or raw vaccine is not used as it contains various living microbes. It is acted upon by the glycerin added, for five or six weeks. The virus is tested bacteriologically during this period, and as soon as no more colonies appear it is ready for use.

e. *Preparing for the Market.*—The vaccine is now put into small glass tubes and marketed as glycerinated tube virus. The vaccine should be kept in a cool, dry place. It deteriorates gradually and the time limit of usefulness is stamped on each package.

The old time ivory tips are still on the market and are preferred by many physicians. A dry bulk form of the virus is also marketed. The manner of the use and the action of the virus are universally known. As

now prepared the remedy is absolutely safe. No ill effects ever follow its use. Of the millions of persons inoculated within recent years, there probably has not been a single instance of bad effects which could be traced primarily to the vaccine virus itself. A small-pox vaccination is not nearly as likely to produce ill effects as the customary hand shake. In fact the latter operation does occasionally spread an infection.

10. Hydrophobia or Rabies Vaccine

Pasteur's hydrophobia virus is obtained from the spinal cord of rabbits, inoculated with the virus from a dog suffering with rabies. The inoculation is made into the dura mater of the spinal cord. The rabbit dies in about two weeks. A second rabbit is inoculated from the first, which dies even sooner, showing that the toxin gained in virulency in its passage through the first animal. This is repeated until finally the animal dies in six or seven days after inoculation. Beyond this the virulency of the poison cannot be increased and this constitutes the *virus fixe* (fixed or unchanged virus) of Pasteur.

The spinal cord of the rabbit dead of *virus fixe* is dried in a glass cylinder with potassium hydrate. The cylinder is placed in a cool dry place and each day small bits of the cord are cut off and placed in a vial of glycerin. At the end of fourteen days the virus is no longer capable of producing hydrophobia in rabbits, but the animal inoculated with it can withstand the thirteen days virus (which was preserved in the glycerin) and so on down the scale, until finally the rabbit can withstand the *virus fixe* without experiencing serious effects.

In man it is customary to begin the treatment for rabies (or suspected rabies) with the nine day cord (hypodermic injections of the cord emulsions) and to give each succeeding day a virus one day stronger, until finally the *virus fixe* is injected without producing untoward symptoms. The individual thus treated is now able to withstand the much weaker virus from a dog or other animal suffering from rabies. As the result of this mode of treatment the mortality rate from rabies is now less than 1 per cent. (Ravenel). Those bitten by dogs (or wolves, skunks, cats) suffering from rabies or suspected of suffering from rabies, should cleanse, cauterize and disinfect the wound at once, and then immediately proceed to a Pasteur Institute and submit themselves for treatment. The earlier, after infection, the treatment is begun the more likely will the results be satisfactory. The vaccine is, however, now so prepared as to make home treatment possible. The graded doses of the virus put up in sterilized ampules are ready for immediate use by the family physician.

11. Phylacogens

Phylacogens are sterile aqueous solutions or suspensions of metabolic substances or derivatives generated by bacteria grown in artificial culture media. The bacteria are then killed and filtered through clay filters. Each specific phylacogen consists of equal parts of the products of the infection and a pure culture of the principle infecting organism. Their use is based upon the idea of Dr. Schafer who holds the opinion that most, if not all infections, are mixed, instead of simple, and that the organisms associated with the chief infecting organism play an equally important part in the disease and in developing the immunization. A number of these preparations have been tried out in practice but the only one which appears to have met with any considerable success is the one for rheumatism.

12. Mixed Bacterins. Polyvalent Bacterins

These have been sufficiently explained under sensitized bacterins. It would appear (as indicated under 11) that certain infections are normally multiple. In such cases it is manifestly unreasonable to expect best results from a univalent bacterin, made from one of the several infecting organisms. Unless it can be proven experimentally that one particular organism in a multiple infection is the primary cause, it is to be assumed that the best effects are to be obtained from a bacterin made up of all of the infecting organisms, combined in about the same proportion as they occur in the infection. Such mixed bacterins have been tried out in erysipelas and in cancer, but apparently without any considerable success.

CHAPTER XII

ADENOLOGY. THE ENDOCRINOUS GLANDS AND THEIR EXTRACTS

Adenology is the science which treats of glands, their structure, function and uses in the animal economy. The functional activities of the glands with ducts have been known for a long time, but it is only within recent years that the ductless glands have received serious attention. Sajous proposed the term *hemadenology* for the science which treats of the ductless glands, which translated into English means the science of blood glands. The German scientists spoke of the ductless gland as Blutdrüsen or Blutgefässdrüsen, because of the fact that these glands were highly vascular and formed certain substances which were poured into the blood circulation. The term *endocrinology* is also much used meaning the science of internal secretions, the secretions of the ductless glands and also of the duct glands which pass into the general circulation directly, being so designated. Since products derived from glands with ducts are used medicinally, the term hemadenology is not suitable, and the terms adenology and endocrinology are etymologically more correctly applicable.

The recent investigations in glandular functions have demonstrated that the subject of immunology is intimately bound up with the activities of the glands and of the body cells. It is now known that the duct glands not only secrete substances which leave the gland by way of the duct, but certain other illy defined though important end products of cellular activity, which pass directly into the blood circulation by way of the ultimate capillaries; that is, these glands also secrete true endocrine substances, similar to those secreted by the ductless glands. From this it would appear that the dividing line between duct gland and ductless gland cannot any longer be sharply drawn, at least not from the viewpoint of functional activities.

The study and use of glands and of glandular secretions is not by any means recent. As early as 600 B.C. testicular extracts were used in the treatment of obesity. The keen interest in overcoming the excessive accumulation of adipose tissue was occasioned by the fact that those good senators of Sparta and also of Rome, who through a life of indolence and gluttony, had become excessively obese were liable to be haled before a committee and threatened with dismissal from an easy and lucrative job unless they reduced decidedly and forthwith. The modern interest in internal secretions dates back about thirty years, and is clearly traceable to

the earlier efforts of Brown-Sequard. Brown-Sequard was a keen investigator endowed with a fertile imagination. He assumed that all tissues gave off or secreted substances to the blood which were essential to life and which were peculiar to each kind of animal. He made the mistake of taking the public into his experimental confidence with the result that his work was made so ridiculous through the lay press that he became entirely discouraged and all that he did was discredited and soon almost entirely forgotten, to be again revived within two decades.

Until about thirty years ago, the physiologists gave practically no attention to the ductless glands, merely mentioning them and suggesting that they were functionless vestigial remnants of once larger and functionally active glands. This idea based on ignorance had the effect of encouraging surgical removal of the supposedly useless structures, for little or no cause. Today, for example, the removal of the tonsils has become a craze, equalled only by the wholesale extraction of teeth and the snipping of vermiform appendices. Dr. Williams (in the Practitioner, Jan. 1915) makes this terse and truthful statement which is equally applicable to the operative procedures above indicated. "The truth is, these operative procedures (in Grave's disease) represent the heroic application of loose conclusions from insufficient data."

The work done on internal secretions since 1889 has demonstrated the following:

1. All of the glands, those with ducts as well as those without ducts, secrete substances which are thrown back into the circulation and which are more or less essential to the normal functioning of the body.

2. Some of the glandular secretions are absolutely essential to life, as those of the suprarenals, the parathyroids and the pancreas; while others are not essential to continued life, as those of the testes, the ovaries, the spleen and the tonsils.

3. The functional activities of most of the glands, if not all of them, is interrelated, and again the activities of all of the glands are interrelated with the functional activities of all of the somatic body cells and with the germatic cells. That is, any serious disturbance of any one gland is apt to react upon the activities of the other glands and upon the body cells. On the other hand, the disturbance of the function of the body cells will react upon the activities of the glands.

4. A lessening of a glandular function, or of the functioning of body cells is apt to be compensated by an increased or otherwise modified functioning of some other gland or glands. Law of compensating bodily functions.

The glands in general secrete substances which influence the activities of the bodily organs. Chemical substances of this kind, which stimulate

the functional activities of organs are called *hormones*. Shäfer called attention to the fact that apparently some glandular products inhibited or checked or retarded the normal functional activities of organs, to which the name *chalones* has been applied. The chalones may be compared to a balance wheel, tending to regulate the normal growth and functioning of tissues and organs. Without the chalones there would be hyper-function due to the unchecked hormones.

The function of a gland may be excessive, or lessened, or abnormal being neither excessive nor subnormal. Thus we have a hyper- a hypo- and a dys-function of a gland or of glands. Recent investigations and observation have shown that the dys-function, to a lesser degree also the hyper- and hypo-function of glands, modify profoundly the resistance to infections. In other words, the glands are of the greatest importance form the viewpoint of immunology. This makes it clear why the glandular extracts and secretions have recently come into use in medical practice. The results of their use have in some instances been marvelous, whereas in other instances the therapeutic effects have been almost nil. It may be stated, however, that the therapeutic application of glandular extracts is as yet in its infancy and is based almost entirely upon empiricism. The following is a brief review and summary of the science of adenology.

1. The Glands with Ducts

1. *The Liver.*—This is the largest gland of the body and it is essential to life. The glycogenic function of this gland is familiar to students of physiology. It gives off two substances within the cells, namely glycogen and urea, which are poured into the blood for the purpose of general nutrition or for elimination. Gay has recently isolated a substance from animal livers and also found abundantly in certain mussels (the abalone of the Pacific Coast), to which he has given the name "taurin" and which promises to be a curative agent in tuberculosis. The liver is one of the organs which is usually quite free from tuberculosis and the supposition is that it contains a substance (taurin) which prevents tubercular infection. The laboratory experiments on tubercular guinea pigs have been very promising.

2. *The Spleen.*—Apparently the spleen is not essential to life as has been shown by animal experiments. The chief changes following extirpation are enlargement of the lymphatic glands and a hyper-function of the red marrow of the long bones. The spleen has been credited with giving rise to leucocytes and is supposed to be the grave yard of the red blood corpuscles. The true function of the spleen is not yet known.

3. *The Pancreas.*—The pancreas is absolutely essential to life, as its removal results in death, preceded by a pronounced glycosuria or diabetes

mellitus, with the accompanying symptoms of polyuria, great thirst and hunger and some acidosis. The experimental evidence indicates that the pancreas, in addition to its usual function in digestion, secretes a substance which is essential to the normal bodily metabolism of sugar, which substances (secretins) are probably formed in the so-called island of Langerhans.

The following statements pertaining to the endocrine secretions of the testes, the ovaries and the mammary glands are taken from a series of popular lectures on biological products by Parke, Davis and Company.

4. *The Testes.*—The part played by the testes as internal secreting organs is inversely shown by what takes place after castration. The castrated rooster experiences a shriveling of the comb, wattles, and spurs, the character of the voice is changed, the neck and tail feathers are poorly developed, and there is an excessive deposit of fat and he becomes more docile.

Castration of food-producing animals, especially cattle, is often performed because of the increased tendency toward fat deposits, and the change in the consistency of the muscular tissues produced, this rendering such tissues more suitable for food; and the operation makes the animal more docile and quiet.

The best opportunity of determining the effect of castration on man has been afforded by the custom in certain Eastern countries of thus multilating harem guards. This practice is also resorted to to some extent by a religious sect in Russia, the "Skopzen;" and in Italy it was formerly not an uncommon procedure to castrate male singers during childhood in order that they might retain the juvenile tone and fibre of the voice. If the operation is performed in early life it results in an absence of sexual power and in infantile development of the external genitals. Castrates do not possess the courage, passions and aspirations of normal men, and they appear to be lacking in the higher artistic endowments. They are said to be tricky, revengeful, and cruel. Their intellectual abilities are not impaired to any considerable extent, as many eunuchs have been men of more than the average intelligence.

While the existence of an internal secretion of the testes is definitely established and its far-reaching importance clearly recognized, the use of testicular preparations in therapeutics has never achieved any great degree of success. Many years ago the first attempts to apply products of this kind were made, and it was claimed that such treatment brought about an increase in physical and mental vigor. Subsequent investigations have not made this claim good.

5. *The Ovaries.*—We have abundant evidence of the importance of the ovary as an internal secreting gland. We know, for instance, that there

is an intimate relationship between the ovary and the menstrual function. When the ovaries are completely removed, menses are terminated. Presumably the institution of menses is due to some change in the glandular function of the ovary at puberty, and it is noteworthy that at this time also the development of the distinctively feminine characteristics of form and feature take place, these being stimulated, it is thought, by the ovarian secretion.

The changes which occur at the menopause, both in the physical and mental characteristics of the woman, suggest that here, too, we have a marked alteration in the character of the ovarian activity; apparently there is a cessation of that phase of the function which is instituted at puberty.

It was formerly believed that the internal secretion of the ovary was elaborated by the corpus luteum. The corpus luteum in the non-pregnant state degenerated and shrinks up in a very short time, but if the ovum has been impregnated it persists for several months. It is now known that the corpus luteum is not the sole source of the secretion of the ovary, and by many it is believed that it is not even the more important one. One thing is certain, however: the corpus luteum does represent an active element of internal secretion.

Both corpora lutea and desiccated ovarian glands (ovarian substance) have been extensively used in the treatment of natural as well as artificial "change of life." The best results have been obtained in the treatment of artificial menopause—by "artificial" meaning cases in which for some reason it has been necessary to remove the ovaries completely or in part. In other words, these products are of advantage in cases where an insufficient amount of ovarian tissue is present to adequately supply the physiological demands of the body. The symptoms commonly occurring are often completely controlled.

Ovarian treatment has been used with some success in infantilism of the genital organs, but is must be borne in mind that such conditions are often associated with pituitary disease. The vomiting of pregnancy is occasionally relieved by ovarian treatment, too, but as a rule this condition responds better to the use of suprarenal extracts.

6. *The Mammary Glànd.*—It has not been definitely established that the mammary glands have a function other than the secretion of milk, but there is considerable reason to believe that they elaborate some substance which influences ovarian activity; certainly there is no question as to the intimate relationship existing between the ovaries and mammaries. It is interesting to note, however, that the striking development of these glands during pregnancy is quite independent of the ovaries; if the ovaries be completely removed from pregnant animals, the mamma-

ries develop just the same and lactation is not interfered with. The same phenomenon has been observed in women from whom for some reason or other it has been necessary to remove the ovaries during the gestation period.

It has been claimed by some investigators that the development of the mammaries during pregnancy is dependent upon secretory activity of the placenta ("after-birth"). They have urged in support of this theory that mammary development persists in pregnant animals, regardless of the death of the fetus, just as long as the placenta remains. Furthermore, placental extracts have been reported to be active stimulants to lactation. Other evidence seems to support the assumption that there is some substance derived from the fetus which stimulates the mammaries, and the injection of fetal extracts in normal animals brings about an enlargement of the mammary glands. Possibly both factors are involved.

Therapeutically, the importance of a product made from the mammary gland seems to lie in its effect in neutralizing excess ovarian secretion, and this has resulted in a preparation of this character being applied to the control of menstrual excesses having their origin in over-functionating of the ovaries. A still more interesting application of mammary gland treatment has been in fibroid tumors of the uterus. On first consideration such a therapeutic measure looks preposterous, but the theory is that fibroids have their origin to a large extent in uterine congestion. Several investigators have reported that the use of mammary extracts has resulted in an arresting or disappearance of these fibroids, and while the evidence thus far produced is by no means conclusive, the possibilities of such treatment warrant further study.

2. The Ductless Glands

The ductless glands are without ducts, they are small in comparison with the duct glands and occupy well protected positions in the body. They consist mainly of epithelial cells which are in close relation to the walls of capillary blood vessels and lymphatics, and in some instances, if not all, under the control of the cerebro-spinal nerve system. By reason of their relationship to the blood circulation, they have been called vascular glands and blood glands. Some recent observations would indicate that the sympathetic nerve supply has a very intimate relationship to the ductless glands as well as to the epithelial cellular structure of the capillaries.

While there is much which is as yet undetermined with regard to the ductless glands, there is much that can be said with some degree of definiteness. They most certainly secrete substances which are necessary to the proper or normal correlative functioning of organs. They contain

protective and defensive measures against disease. They regulate the function of ovulation, of sex development, of pregnancy, of muscular tonicity, of vascular tonicity, of adiposity, the growth of tissues, sugar metabolism, glandular activity, etc., etc. Although the glands are far apart in the body, there is nevertheless a functional intercommunication between them. They have furthermore a compensatory interaction, an altered function in one gland being balanced by the functional activities of one or more other glands. Again, a serious dys-function of one gland may upset the functional activities of the others.

Every cell of the body may be likened to a ductless gland capable of taking on some one or other of the functional activities of the glands, and all of the functionally active body cells do coöperate with the glands in the maintenance of the bodily functions. The significance and importance of the intelligent use of ductless gland products are clearly set forth by Sajous as follows.

"What are termed 'backward childern' aggregate, judging from the proportion shown by the public schools of Philadelphia, 318,000 in the public schools of the United States. As this does not include children who are too young to attend school, an estimate of one million of backward children of all ages in the whole country, would be nearer the true figure. These children, usually deemed merely deficient in capacity of spontaneous attention and memory and believed to show no evidences of degeneracy, possess in many instances stigmata which point directly to impairment, through heredity or local lesions, of the ductless glands, and due in many instances to one or more "children's diseases." We may witness one or more signs of hypothyroidism or larval myxedema, with mental torpor, hypothermia, and perhaps a little pallor as only signs; or close examination may elicit a mild form of cretinism, with slightly stunted growth, a pug nose, thick lips, a somewhat harsh skin—children who often show decayed teeth and a predilection for tonsillitis.

A lower grade still of these (often redeemable) degenerates show defects of speech and ideation and deficient capacity of spontaneous attention. Left untreated, such subjects usually drift to the category of "idiots," a blind devotion to tradition having associated these unfortunates with "heredity"—a fit companion for "idiosyncrasy" as a cloak for ignorance. Hemadenology will do much to tear asunder the clouds which hover over this great question. Indeed, through the efforts of eugenists to protect future generations, the unfortunates of our own generation are increasingly exposed to injustice. This will cease when the prevailing tendency to overlook the flood of light which modern contributions to our knowledge concerning the ductless glands have thrown upon heredity will inspire the labors of these well meaning scientists.

In contrast with these defectives are the cases of infantilism, characterized by the persistence of the physical and mental characteristics of childhood, but without idiocy or dwarfism. The miniature men of the Lorain type and the defectives of the Mongolian type with their slanting eyes, bulging foreheads, are examples of this class, due in most instances to defects in the upbuilding of the organism, a process in which all the ductless glands take part. Still another, though rarer, type is the infantilism of pituitary origin—obesity with feminism in the distribution of fat, the nates, thighs, and breasts especially, but with deficient development of the sexual organs, a moon face, and weak mentality.

Crime presents aspects which also belong to the field of the hemadenologist. All the types described above are usually docile, the exceptions being some cases of the pituitary adiposogenital type, and show but little if any predilection for vice. Among those recorded as imbeciles, who show deficient intelligence and loquaciousness, abnormally good memory, untruthfulness, arrogance, and maliciousness, may sometimes be discerned types, which owing to the landmarks of defective development resulting from imperfect balance of ductless gland activities, point to links between the latter and crime. To seek these associations, restore normal equipoise in the production of hormones, thus insuring normal metabolism in all tissues, particularly the osseous and central nervous systems when it is still time, offers broad avenues of hope for the redemption of some of these unfortunates from the drifts of iniquity.

Insanity likewise claims the attention of the hemadenologist. The psychoses of exophthalmic goitre and myxedema, and the idiocy of microcephaly due to inadequacy or absence of the adrenals, are familiar examples. Dementia præcox, which is stated to initiate twenty-five per cent. of the cases of insanity harbored in our asylums, is increasingly being shown to be closely related to perverted action of the same glands, various types of the disease being represented by a corresponding number of forms of abnormal glandular action. Such being the case, we are brought to realize the many directions in which abnormal activity of the ductless glands may affect mentality. Beside the enfeeblement of the mind characterized by unequal weakening of the faculties, emotion, judgment, self control, etc., we witness impulsive actions, flightiness, catalepsy, automatic obedience, vergiberation, mutism, delusions, hallucinations, etc. The field is thus prolific in its opportunities for the elucidation of many of the complex problems with which psychiatrists are confronted in respect to the genesis of psychoses.

Obesity in all its forms normally falls within the scope of hemadenology. Beside the familiar varieties due to overuse of carbohydrates, defective oxidation, etc., there are types which, as is well known, are due to defi-

cient thyroid activity, which entails from my viewpoint, impaired activity of all other ductless glands. In children we may have also the *adipositas cerebralis* of Fröhlich, in which general obesity occurs with defective development of the sexual organs and impaired intelligence—due to deficient pituitary activity. Closely allied genetically with these cases, are those showing the adiposogenital syndrome of Launois, very similar to Fröhlich's, but without impairment of intelligence. The *adiposis dolorosa* of Dercum, in which there is obesity, general or localized in areas, with pain, spontaneous or paroxysmal, is also ascribed to impaired activity of certain ductless glands. Still another type, symmetrical lipomatosis, is characterized by the presence of masses of fat, often tender or the seat of spontaneous pain, symmetrically in the axillæ, groin, or other regions, but oftenest about the neck. Finally, the obesity of pineal deficiency may be mentioned as another example of the close relationship between the ductless glands and obesity.

Falling to the lot of the hemadenologist also are the abnormalities of growth, several of which, even in individuals in apparent health, are manifestations, active, latent, or extinct, of some morbid process. In acromegaly for example, we may have general enlargement of the body, especially of the extremities and face; the lips, nose, and chin are more or less prominent and there is general increase of massiveness of the frame. Individuals presenting such a type are not uncommon; in these, as well as in certain very tall subjects, temporary lesions of the pituitary, awakened by some acute febrile process, may have caused the acromegalic process to proceed far enough to provoke the appearance of its most salient phenomena—all incapable of retrogression, after the causative morbid process in the pituitary proper has disappeared.

Resembling such cases at times are those of adrenal tumor, some of which cause premature development so marked in rare instances that a child of eight years may attain the size of an adult. The *adipositas cerebralis* of Fröhlich and the adiposogenital syndrome of Launois also suggestive of acromegaly in some cases, are deemed extremely rare because the fully developed morbid process is alone taken as standard. Here and there, however, the trained eye of the hemadenologist may discern the stigmata of these disorders, and oppose, through compensative, regulative or inhibitive measures, their evil trend.

Stunted growth as clearly belongs to the domain of the hemadenologist. This may follow, also irrespective of any other abnormal effect, the infections of childhood, especially where the thyroid, thymus, and adrenals had been the seat of lesions. In the complicated types there is, beside the dwarfism of cretinism and its congerers, Mongolian and Loraine nfantilism, the victim of achondroplasia, of fetal rickets

mortification is intensified by the fact that unlike that of the other types, his mind is as alert as that of the normal individual. His large head, saddle nose, short and bowed legs, prominent abdomen, and marked lordosis, bespeak little indeed in favor of a medical science which cannot check the development of such deformities in their incipiency.

Myxedema, cretinism, and other classic disorders of the various ductless glands obviously belong to the field of the hemadenologist. It should be borne in mind, however, that in their larval or mild forms they constitute in many instances, the so-called rebellious cases met with in general practice. The sufferer of larval hypothyroidism, for example, may show little else than occipital or interscapular pain and cold extremities and yet resist all the antirheumatic or antineuralgic measures that a century may have suggested. Unrecognized, such patients sometimes contribute to their physician's diagnostic acumen by becoming frank cases of myxedema—when organotherapy arrest both the latter and the rheumatism. Lying behind tetany, *paralysis agitans*, and osseous disorders are, it is believed, lesions of the parathyroid glands—which thus become, as does the thyroid, elucidative factors in obscure though relatively commonplace disorders.

Much the same remarks apply to larval Addison's disease. While more or less bronzing characterizes the latter, we often meet in pale children, neurasthenic adults, and premature seniles, the typical signs of this condition, asthenia, sensitiveness to cold, cold extremities, hypotension, weak cardiac action and pulse, anorexia, anemia, constipation, etc., but without bronzing. Acute febrile diseases, pneumonia, diphtheria, typhoid fever, etc., may bring on a similar state by exhausting the adrenals, the patient dying after a period of weak heart, low blood pressure, asthenia, a tend ency to fainting, prostration, etc.,—an issue which a few timely doses of adrenaline in saline solution would have prevented. Excessive activity of the adrenals is another cause of death in children seldom recognized. Here the work of the hemadenologist will become elucidative and life saving.

Goitre and exophthalmic goitre, the bulk, as it were, of the cases witnessed by the hemadenologist, need his special intervention, to eliminate at the earliest moment, that of the surgeon. While in no way discrediting the value of operative procedures in appropriate cases, my own experience confirms that of Leonard Williams in condemning promiscuous resort to the knife. This applies also to many cases of ordinary goitre. Many patients subjected to operation could have been cured by medical treatment, thus preserving for them a useful organ. We must not lose sight of the fact, however, that much work remains to be done to establish the precise limitations between operable and inoperable cases—a line

or research which the hemadenologist should carry to an early termination.

The thymus in various ways claims the attention of the hemadenologist. Its temporary existence associated it with development and particularly with that of the brain and osseous system. Idiocy, thymic asphyxia, and *status lymphaticus* are doubtless but a few of the disorders the thymus may awaken. Adenoids, enlarged tonsils, and rickets are kindred conditions which greater knowledge concerning the functions of the thymus will tend greatly to elucidate. Rickets and stunted growth belong to the same category.

The reproductive organs present features which distinctly belong to the domain of the hemadenologist. What knowledge of the functions of the internal secretions of the testes, ovaries, and corpora lutea has already been garnered, has contributed much to our therapeutic resources in conditions which formerly found us relatively powerless. The observations of Brown-Séquard to the effect that the energy of the nerve centres and cord is stimulated and that the individual is endowed with physical, moral, and intellectual characteristics of sex by means of orchitic injections, denote a wide field of usefulness, beside the treatment of sexual impotence. Menopause, physiological and post-operative, amenorrhea, and kindred disorders of the female studied adequately from this new viewpoint cannot but prove fruitful."

The following is a brief summary of the functional activities and uses of the ductless glands.

1. *The Tonsils.*—These are two almond shaped bodies lying one on either side at the entrance to the fauces. They are most prominent in childhood, beginning to reduce after puberty. These glands have been utterly neglected by physiologists and practically nothing is known regarding their function, either actual or problematical. Though neglected by the physiologists and merely located in space by the anatomists, they have not been neglected by the surgeon. The rule seems to be to remove every tonsil that can be removed, whether there is reason for so doing or not, and this despite the fact that many after effects of operations on childern have been most serious. The surgeon tries to explain these cases by stating that it was the fault of the operation, the tonsil being only partially removed, leaving a decayed and infected root, others that the ill consequences should not be laid to the operation, but rather to the delay in having the tonsils removed. In many directions there is manifest a tendency to some hesitation about removing tonsils that are sound or that are only slightly infected. Various suggestions have been offered as to the function of the tonsils, all of which are largely guessing. One is that they protect against infections to both the respiratory tract and the

digestive tract. Another is that they retard the sex development of the child, for which assumption there appears to be some justification in the fact that sexually precocious children frequently have small or rudimentary tonsils. This suggestion appears to be negatived by the fact that the removal of the tonsils in young children does not hasten or increase the sex development or give rise to any signs of sexual precocity.

2. *The Pineal Gland.*—Also known as pineal body and *epiphysis cerebri*, was supposed to be the vestigial remnant of the Cyclopean eye. This is a very small pine cone like body projecting from the third ventricle. In early life it has a glandular structure and reaches its greatest development at about the seventh year. After this period and more especially after puberty it loses its glandular appearance and gradually dwindles, degenerating into a fibrous tissue. In hypo-function of this gland and also in dysfunction, in children, there is accelerated development of the reproductive organs with attendant mental precocity. The inference is therefore, that the gland secretes a substance (a chalone) which inhibits growth and more especially restrains the development of the reproductive glands. Total extirpation of the gland is not fatal.

3. *The Pituitary Body or Gland.*—Lies in the *sella turcica* of the sphenoid bone and is usually described as consisting of two parts, the larger anterior lobe of distinctly glandular structure, and a much smaller posterior lobe of nervous origin and composed of neuroglia cells and fibers. Total extirpation of this gland, or of its anterior lobe alone, results in death, preceded by lowering of blood pressure, of temperature, feeble and slow respiration, unsteadiness of movement, muscular twitching, lethargy and coma. Occasionally there is also glycosuria. Removal of the posterior lobe alone was without marked effects. The secretions of the anterior lobe stimulate the growth of the skeleton and associated tissues. Hyperfunction (hyperpituitarism) in children gives rise to giantism, and in adult life to that special growth of long bones designated by acromegaly. A deficiency of secretion gives rise to infantilism, to excessive fatty tissue formation (*adiposis dolorosa*), sexual inactivity with actual atrophy of the sex organs, loss of hair, disturbances of nutrition, reduced mental activity, etc. The hyper-pituitarism of adults manifests itself by the elongation of the long bones of the extremities, the hands and feet, increased angularity of the skeletal structure, the tongue is thickened causing a thick speech, mentality is lessened; with a characteristic curvature of the spinal column (cervico-dorsal kyphosis with a compensatory lumbar lordosis), there is thickening of the skin, the chin projects and the lower teeth generally project beyond the plane of the upper teeth; there is increased thirst and occasionally glycosuria.

4. *The Thymus Gland.*—This gland is largest during embryonal

development and begins to dwindle at the end of the second year, having almost entirely disappeared at the age of puberty. It lies in the neck on the front and sides of the trachea, consisting of two lobes. The chief function of this gland appears to be the stimulation of embryonal and early infantile development. Occasionally this gland persists during adult life, in which cases it is accompanied by excessive lymphoid development, known as myxœdema. Thymus gland extract has been used empirically in the treatment of hyperthyroidism, in hemophilia, in rickets, in tuberculosis, and in infantile marasmus and atrophy. It has also been recommended in cancer.

5. *The Thyroid Glands.*—This gland consists of two lobes situated in the neck in front of the trachea. This is perhaps the best known of the ductless glands and has received a great deal of attention on the part of special investigators and clinicians. The congenital absence of this gland or an arrested development in infancy, is followed by defective mental as well as physical development. The body is small and more or less irregular in contour, the face appears swollen, the eyelids puffy, nose broad and flat, protruding tongue (or tendency to protrude), abdomen swollen, short extremities, mental dullness and stupidity amounting to idiocy in many cases.

Degeneration of the thyroids in the adult is followed by a complex group of symptoms, the most prominent of which are thickening of the skin due to hyperplasia of the subcutaneous connective tissues of an embryonic type rich in mucinoid material, to which the term myxœdema is applied. As the result of these changes in the skin, the face becomes broadened, swollen and puffy. The features become irregular, coarse and expressionless. The mind becomes clouded, memory is defective, dementia follows terminating in idiocy.

The general conditions due to hypothyroidism are designated by the terms myxœdema, cretinism and *cachexia strumipriva.* The numerous cases of mental and physical degeneracy in Crete are due to the intermarriage of those afflicted with hypothyroidism, and from which the term cretinism is derived. Both the cretins as well as the form of degenerates designated as mongols (Mongolian and Lorain types of degeneracy) are due to inherited or early hypothyroidism.

In excessive secretion of the thyroids (hyperthyroidism) there arise the symptoms designated under goiter and exophthalmic goiter (Graves's disease, Basedow's disease), represented by rapid action of the heart (tachycardia), active pulsation of the arteries at the base of the neck, protrusion of the eyeballs, fine tremors of the hands, and a more or less changed mental state or condition. The excessive enlargement of the thyroid glands resulting in the symptom complex laid to hyperthyroidism,

in adults, is by some stated to be due to the drinking water supply, but this has not yet been satisfactorily demonstrated as a fact.

The thyroid hormone is an organic iodine compound (colloidal iodine) called iodo-thyrin or thyroiodine), said to contain from 0.33 to 1.00 per cent iodine. This is a very stable compound and has been used with some success in hypothyroidism. It would naturally be contraindicated in hyperthyrodism.

6. *The Parathyroid Glands.*—These are supposed to be accessory thyroid bodies and lie near the latter in the neck to the front and sides of the tracheæ. The parathyroids as well as the thyroids are essential to life, as total extirpation is followed by death. By some investigators the parathyroids are looked upon as immature thyroid tissue and there is no doubt as to the close functional relationship of the two glands. Extirpation of the parathyroids is followed by the symptoms designated by the term *tetany*, namely restlessness, excitability, muscular tremors, which before death, develop into convulsions, rigor and complete exhaustion. According to Macallum the symptoms above cited may be relieved at once by the intravenous injection of a soluble calcium salt. In fact the prompt effects are most striking and has suggested the use of calcium salts in dys-function and hypo-function of the parathyroids.

In hypothyroidism of adults there is disturbed nerve function, mostly sympathetic, worry, loss of mental balance, excitability, etc.

7. *The Suprarenal or Adrenal Glands.*—As the name implies, these glands, two in number, are associated with the kidneys, one each lying just above the kidney. They are flattened, more or less triangular and weigh about four grams each. An enormous amount of work has been done in regard to these glands and our information concerning their functional activities is fairly complete. Brown-Sequard demonstrated that removal of these glands resulted in death almost immediately (within a few hours to possibly two or three days). The symptoms preceding death are great prostration, muscular weakness and diminished vascular tone. The symptoms resemble those of Addison's disease which is now known to be due to pathological lesions of these glands. One of the active principles of the adrenals is the animal alkaloid epinephrine, now a much used medicine and fully described in all texts on materia medica. Epinephrine does not however represent the full physiological action of the gland.

In hypo-function of the adrenals there is a feeling of lassitude (Spring fever), liver spots appear and there may be slight general pigmentation. In decided dys-function of the adrenals the symptoms deepen, there is great loss of activity, the skin becomes decidedly darkened and bronzed, which discoloration is extended to the mucous membranes, there is loss

of appetite, reduced tonicity of muscle and of the nerves, disturbance of the digestive function, low blood pressure, subnormal temperature, and feeble respiration. A very common complication of Addison's disease and of dys-function generally, is general tuberculosis which is believed to have its origin in the adrenals. This led to the supposition that these glands secreted an antitoxin or antibody against tuberculosis.

The correlative activities of the thyroids and the adrenals are very definitely demonstrated. The secretions of the adrenals constrict the blood vessels (capillaries) while the thyroid secretions dilate them, adrenals retard digestion while the thyroids promote this function, thyroids increase the heart action while adrenals retard it.

PRESERVATION AND STORAGE OF BIOLOGIC PRODUCTS

Biological products (sera of all kinds, vaccines, bacterins and glandular extracts, etc.) are organic in nature and are readily decomposed. Some kinds of biologics are more readily decomposed than others. Some retain their physiological and therapeutic properties for comparatively long periods of time, provided they are properly stored (as diphtheria antitoxin), while others deteriorate quite rapidly even when kept under ideal conditions (as lutein, pollen extracts, and antirabic vaccine).

The chief factors which hasten the deterioration of biologics are:

1. Sunlight, More Specifically the Actinic Rays. *Therefore, biologics must be kept in the dark.*

2. Air, the Oxygen of the Air. The manufacturer protects most of the biologics against this factor by placing the suitable amounts in hermetically sealed containers. Under no circumstance are containers to be opened until the preparation is to be used, and then only by the physician, under proper conditions.

3. Temperature. Biologics are very susceptible to *temperature changes.* It is claimed that freezing does not cause deterioration of the products and apparently this is well substantiated by numerous observers. It would appear, however, that a biologic which has been frozen for a time will deteriorate more rapidly should it subsequently be exposed to the unfavorable conditions of light, air, etc., as compared with a biologic which had not been frozen but otherwise similarly kept and exposed.

All biologics are quite rapidly decomposed and rendered useless by higher temperatures (60° C. and up). *Therefore,* all biologics should be *kept on ice* all of the time, *until wanted for use.*

4. Moisture. Many, but not by any means all, biologics are in containers which exclude outside moisture. Such biologic preparations as are in the form of dry extracts or dry vaccines, or tablets and pellets, should be kept away from moisture. A safe rule to follow is to *keep all*

biologics in a *dry place.* The keeping and storing of biologics may be summarized as follows:

Keep in a dark place at a uniform temperature near freezing.

Biologics are rapidly coming into use more and more. Most of them are in the nature of emergency remedies, *desired at once and full therapeutic effects expected without fail.* Only too frequently does the physician fail to get the expected effects, simply because the particular product was rendered inert through improper storing. Only a small percentage of practising pharmacists know what biologics really are and how they are prepared and why they must be kept thus and so. In many of the outlying rural districts in particular, it is altogether too common practice to overstock or to keep biologics until they are entirely worthless. Particularly does this apply to smallpox vaccine.

It would be most desirable to establish conveniently located storage stations for biologics, in charge of experienced and expert keepers. This store room should have double walls on all sides with intervening air spaces, should be suitably lighted, well ventilated, and provided with artificial refrigeration (to take the place of the ice chest). The slight differences in temperature of the different parts of the store room, should be utilized to the best advantage by the keeper. In lieu of such central stations, the pharmacist must provide the proper storage. During the summer months (in states where high summer temperatures—from 70° F. to 110° F.—prevail) the biologics may be kept in a special compartment of the soda fountain refrigerator. Certain products may be stored in the cellar or basement. Some are kept on the store shelves, or behind the prescription counter, although this is objectionable for reasons already given.

How long will biologics keep? Very naturally this question cannot be answered definitely, as so much depends upon the varying conditions already mentioned. In a general way no biologic product should be used which is two years old, or more, and very few become worthless in less than one month's time. The following is a list of the more important biologics, giving them approximately in the order of their keeping qualities.

1. Diphtheria antitoxin. Coagulose.
2. Other sera—antistreptococcic, antimeningococcic, antigonococcic.
3. Dry glandular extracts and powdered glandular substance.
4. Bacillary tablets, lactone tablets, tuberculin tablets, and other dry bacillary cultures.
5. Ordinary bacterins, tuberculins.
6. Prophylactic bacterins. Sensitized bacterins.
7. Smallpox vaccine, antirabic vaccine, luetin, mallein, etc.

8. Normal serum against hemorrhage. Hemoplastin?
9. Liquid pollen extracts.

Hydrated biologics (liquid proteins, protein sols) deteriorate more rapidly than dehydrated biologics (dry proteins, protein gels), and on first consideration it would appear desirable to use these products in the dry state, but since they are to be administered hypodermically or intravenously, they must be in condition for immediate absorption and assimilation (hence, in the form of protein suspensoids or sols). A protein which has been dehydrated, that is, which has been changed from a sol to a gel, is often not readily reconverted into a sol. Furthermore, the physician cannot as a rule, take the time to prepare it properly.

Every pharmacist and also every physician, should be familiar with the consistency, color and odor of the biologics, in their normal state. The physician should, for example, not use adrenalin which has become pinkish in color, the normal being colorless. Glandular extracts deepen in color with age and develop an animal odor. Normally clear preparations which have become turbid or flocculent, or which show a precipitate, should not be used. It is true that the manufacturers observe every precaution to insure against mishaps and all products are tested and examined before they are sent out, yet the physician as well as the pharmacist should be qualified to judge of the quality and purity of the preparations.

CHAPTER XIII

YEASTS AND MOLDS

The organisms commonly designated as yeasts and molds, though not belonging to the bacteria (Schizomycetes), are of the greatest importance in human economy and play a most active part in life. Some of them are most beneficent while others are very injurious to health. The yeast organisms (Saccharomyces) cause the alcoholic fermentations in saccharine

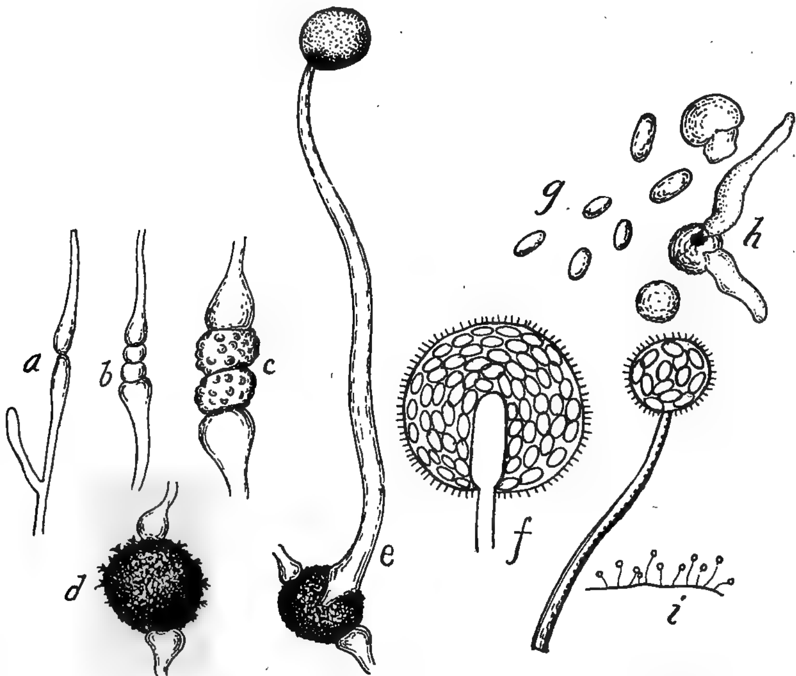


FIG. 69.—Development of *Mucor mucedo*. *a, b, c, d,* stages in the formation of the zygospore; *d,* mature zygospore; *e, f,* endospore formation; *g,* endospores; *h,* germinating spore, this develops and finally gives rise to new zygospores; *i,* mucor slightly magnified. This mould is found on stale bread, damp leather, gloves, etc.

solutions. Many of the mold group cause skin and other diseases. They all belong to the plant division fungi. The more important species may be grouped under three orders, as follows:

I. Phycomycetes. (Zygomycetes.) Zygospore formation.

1. *Mucor corymbifer.*
2. *Mucor mucedo.*

} Both are found in tissue infections.

3. Other species of Mucor are reported as causing pathologic conditions in man and in lower animals. Some are the cause of fatal infectious diseases in such household pests as the common fly. Others attack fruits, as pears, figs in particular, leather goods as gloves, etc.

II. Ascomycetes. Spores formed in asci (sacs).

1. Saccharomycetes—yeasts proper.

a. *Saccharomyces cereviseæ.* This name is applied to many species or varieties of yeasts concerned in fermentation processes, as in beer, wine and saké making.

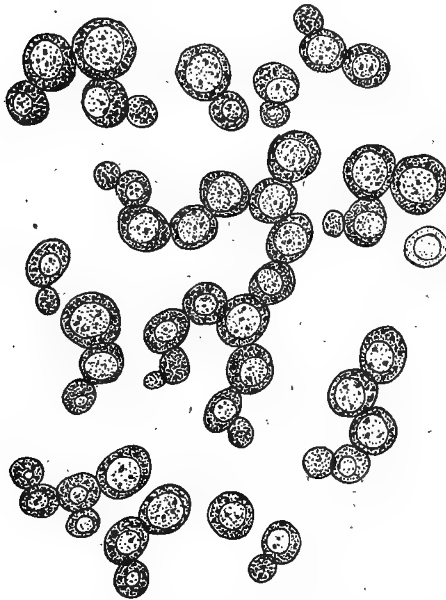


FIG. 70.—*Saccharomyces cereviseæ.* The form or variety known as brewers' top yeast. (*Oberhefe.*)

b. *Saccharomyces anginæ.* Pathogenic.

c. *Saccharomyces ellipsoides.* Common in fermenting fruits, jams, jellies, fruit juices, etc. Other species are active in various vegetable food fermentations.

d. *Saccharomyces Blanchardi.* Pathogenic.

e. *Endomyces albicans.* Pathogenic, causes thrush.

f. *Cryptococcus Gilchristi.* Pathogenic; general infections.

g. *Cryptococcus hominis.* Pathogenic.

2. Gymnoascomycetes.

a. *Trichophyton tonsurans.* Pathogenic, causes scalp disease (ringworm), also attacks other external tissues.

b. *Trichophyton Sabourandi.* Pathogenic. Attacks scalp and beard (ringworm).
c. *Trichophyton violaceum.* Pathogenic. Like (b). Violet color.
d. *Trichophyton mentagrophytes.* Pathogenic. Causes beard and body ringworm.
e. *Trichophyton cruris.* Pathogenic.
f. *Microsporum Audouini.* Pathogenic.

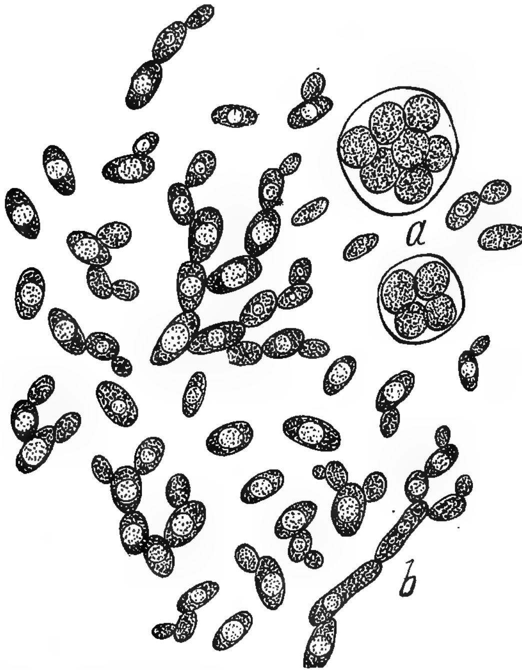


FIG. 71.—*Saccharomyces cerevisea.* The form or variety known as brewers' bottom yeast. (*Unterhefe*). *a*, Spore formation; *b*, elongated cells, which develop under certain conditions of moisture, food supply, etc.

g. *Achorion Schœnleini.* Pathogenic. Is the cause of that very common scalp disease of children known as favus.

3. Carpoascomycetes.
    a. *Penicillium crustaceum.* This is a blue-green mold which is believed to be pathogenic in chronic catarrhal conditions of the Eustachian tubes and of the stomach.
    b. *Penicillium glaucum.* This is the omnipresent blue-green mold so common in the household, infesting all exposed moist organic substances. Supposed to be non-patho-

genic, although some credit it with being the cause of pellagra.

c. *Aspergillus fumigatus.* Said to be the cause of pellagra.

d. *Aspergillus concentricus.* Causes ringworm. Common in the Malay peninsula, China and in the Philippines. Limited to tropical countries.

d. *Aspergillus flavus.* Pathogenic. Found in chronic discharges from ear.

e. *Aspergillus repens.* Much as (d).

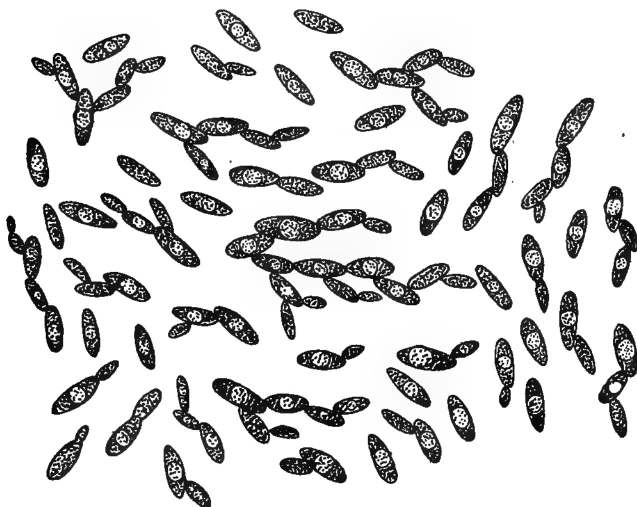


FIG. 72.—*Saccharomyces ellipsoides.* Very common in fruit products as jams, jellies, etc. Living yeast cells show budding of cells and vacuoles. Dead yeast cells usually occur singly, the vacuoles are wanting and the cell walls are more distinct, generally due to the absorption of coloring substances from the medium in which they occur.

f. *Aspergillus pictor.* Pathogenic. Occurs in Central America, where it causes a mange disease.

g. *Aspergillus oryzæ.* Nonpathogenic. Cultures of this fungus are used in the manufacture of saké (Chinese and Japanese rice wine). The fungus growing and feeding upon the steamed rice grains converts the starch into saccharine substances which are then acted upon by the yeast ferment.

III. Hyphomycetes. Systematic position of the pathogenic members not well defined. Life history not yet fully worked out.

1. *Discomyces bovis.* (Actinomyces). The so-called ray fungus which causes the condition in cattle known as actinomycosis, a disease which can be transmitted to man.

2. *Discomyces maduræ.* (Mycetoma). Causes the cattle disease known as madura foot, which can be transmitted to man. Essentially a tropical disease. Two varieties (black and white) of the disease are reported.

3. *Malassezia furfur.* This is the fungus which causes a skin disease (*Tinea versicolor*) which is quite common in tropical as well as in temperate climates.

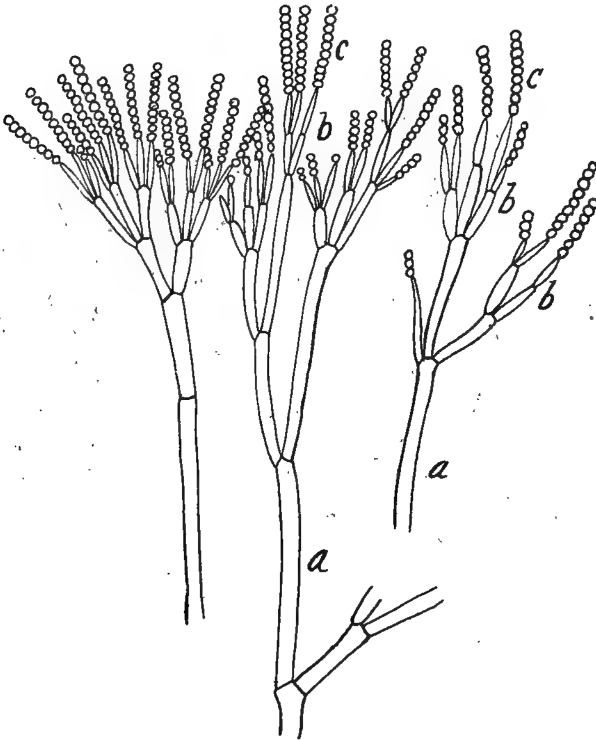


FIG. 73.—Three terminal hypæ showing the characteristic spore formation of *Penicillium glaucum.* This fungus is a true saprophyte and is never found on living fruits or vegetables. Mouldy food substances are quite universally rejected as being unfit for human consumption.

4. *Microsporoides minutissimus.* Causes a skin disease known as Erythrasma or Dhobie's itch. Found in the tropics.

5. *Trichosporum giganteum.* Causes a disease of the hair. The spores of the fungus are arranged about the hair in a peculiar mosaic.

Molds differ from bacteria in that they thrive best in acid media and in that they are not so readily killed by means of the usual chemical disinfectants. Heat (dry as well as moist) kills the hyphal structure quite

readily, but the spores are quite resisting, though less so than the spores of bacteria. They can be cultured on potato, on bread, or on other organic food materials (kept moist in a moist chamber). The following medium is very satisfactory.

Peptone	1 gm.
Maltose	4 gm.
Agar	1.5 gm.
Water	100 cc.

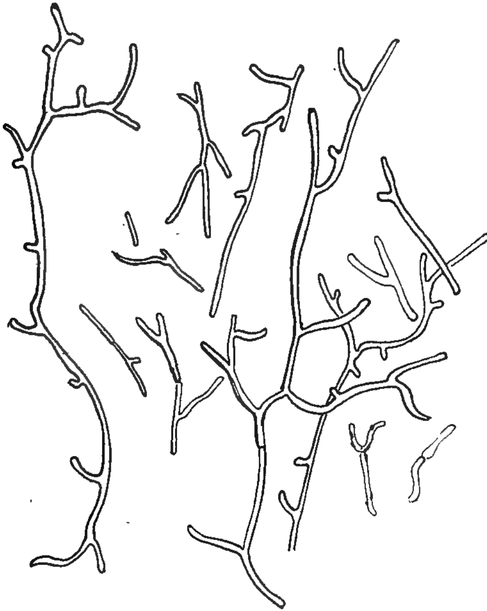


FIG. 74.—*Actinomyces bovis.* Showing the hyphal structure of this pathogenic fungus. There are numerous fungi of the mold group that cause local pathologic conditions of the skin and mucous membranes.

Mix, dissolve, filter, titrate to reaction +2 and sterilize in the usual way. Culturing is usually done in Erlenmeyer flasks (250 or 500 c.c.) with a thin layer of the medium in the bottom. Before placing the mold material in the flask (by means of a platinum loop) allow it to macerate in 60 per cent. alcohol for two hours which will kill the bacteria present without destroying the life of the mold. The acid reaction of the medium (+2) will, however, usually prevent bacterial growth.

Yeast organisms may be studied very conveniently in the hanging drop. The development of mould may be observed between two sterile slides. Since these organisms are much larger than bacteria there is little

difficulty in examining them under the low power of the microscope. Mount in water or in a weak solution (0.10 per cent.) of caustic potash or soda. In looking for yeasts and molds in liquids, centrifugalizing may be desirable. Staining methods will rarely be necessary.

While it is true that not all molds are pathogenic, yet it must be remembered that many are decidedly so, besides most of them are very objectionable on account of the disagreeable moldy odor and taste, if for no other reason. Moldy food substances are not fit for consumption and molds should not occur in any of the pharmaceuticals, syrups, soda fountain preparations and fruit juices. Most of the yeasts are non-pathogenic. The common yeast has even been used as an intestinal disinfectant in typhoid fever, yet no preparations in the drug store should be allowed to undergo yeast fermentation for the reason that the process changes the quality and flavor of the substances thus attacked. Fruit pulp, fruit juices and syrups of all kinds are peculiarly liable to the attacks of the yeast organisms and every precaution should be taken to guard against such infection. This is not a simple matter because the yeast cells and the yeast spores are found everywhere and develop very readily in all saccharine, slightly acid substances. Moist heat sterilization or pasteurization are the most effectual means for preventing yeast fermentations.

The yeast cakes used by the housewife in making bread consist simply of pure cultures of Saccharomyces. The cakes must be kept quite dry and in the cold (ice chest) to prevent decomposition. Even under the most favorable conditions they soon become worthless. As soon as the cake is mixed with the bread dough with adequate warmth, the yeast cells begin to feed upon the various available food substances present and multiply rapidly (by budding), resulting in the formation of alcohol and liberation of CO_2 gas, which latter in an attempt to escape, causes the so-called rising of the bread. If the dough is not thoroughly mixed, the gas liberation is uneven and the bread will be unsatisfactory, because there will be large cavities in some parts of the loaf and in other parts the loaf will be solid. Bread must be baked quickly, after the rising has reached the proper degree, otherwise the loaf will be flat and doughy. The housewife in the country simply prepares sour dough cakes which take the place of the manufactured yeast cakes used in the city. In biscuit making the desired CO_2 gas liberation is brought about by the use of baking soda and sour milk or by means of baking powder alone.

The alcoholic fermentation in the manufacture of beer is caused by the several varieties and forms of *Saccharomyces cereviseæ* (*Torula cerevisεæ*). In beer making, the barley grain is first acted upon by the starch enzyme (diastase) which converts the starch into maltose (malt) and the maltose

is in turn converted into alcohol by the Saccharomyces. If the fermentation product (as grape wine, apple cider, beer, porter, etc.) is exposed to the air for a time, the *Mycoderma aceti* enters and at once begins to convert the alcohol into acetic acid and we finally have vinegar. "Hard cider" is simply apple wine in which the acetic acid fermentation has progressed to an advanced stage.

In the manufacture of the Japanese and Chinese rice wine (saké) the maltose fermentation of the starch (in the rice grain) is brought about by the *Aspergillus oryzæ* as already stated. The process of beer and saké manufacture may be compared as follows:

1. Material Used

BEER: Carefully selected barley is cleaned in running water, then macerated in water to induce germination. Rice, wheat and other cereals may be added. Hops are used.

SAKÉ: A good quality of rice is thoroughly washed in cold water, then softened by a steaming process. No hops used.

2. Diastase Fermentation. Malting

BEER: During the germinating process a ferment or enzyme (diastase) is liberated which converts the starch into saccharine compounds. The ferment is unorganized (non-living) and is soluble in water. The germinating and fermenting grain constitutes the beer wort.

SAKÉ: The steamed rice is spread on mats and inoculated with tha spores and hyphæ of *Aspergillus oryzæ*. This fungus liberates an enzyme (disastase) which converts the starch into saccharine substances. The enzyme produced by the fungus is soluble in water. Fermentation takes place in a warm room.

3. Alcoholic Fermentation

BEER: The beer wort (Bierwürze) is now ready to be acted upon by the yeast organisms (*Saccharomyces cerevisecæ*) which enter from the air or which may be added in pure culture. The yeast organisms convert the saccharine substances into alcohol and carbonic acid gas (CO_2).

The diastase and the yeast ferments are both active during this process.

SAKÉ: The saké wort (*moto*) is prepared by mixing the steamed rice and fungus (*A. oryzæ*) in vats. Yeast cells (*Saccharomyces* of saké) enter from the air and cause alcoholic fermentation, converting the saccharine substances into alcohol and carbonic acid gas (CO_2).

The diastase ferment (produced by *A. oryzæ*) and the alcoholic ferment (Saccharomyces) are active during the entire process.

4. Expressing, Cooling, Clarifying and Pasteurizing

These processes are very closely similar in beer and saké brewing. The differences, if any, are slight and pertain to modifications of methods employed by different manufacturers. Preservatives, as salicylic acid, may be added. Both beverages may be reinforced with alcohol. This is not generally done with seké as the brewers declare that the addition of foreign alcohol destroys the characteristic flavor or bouquet.

5. Kinds or Brands

Many different brands varying in color, taste, alcoholic percentage, ash percentage, etc. The alcoholic percentage ranges from 1.5 to 6. The ash percentage is about 8.

Different brands varying in quality. The alcoholic percentage ranges from 14 to 18. There is a sweet variety (*Mirin*) and a white variety (*Shiro*). Ash percentage about 3, frequently less.

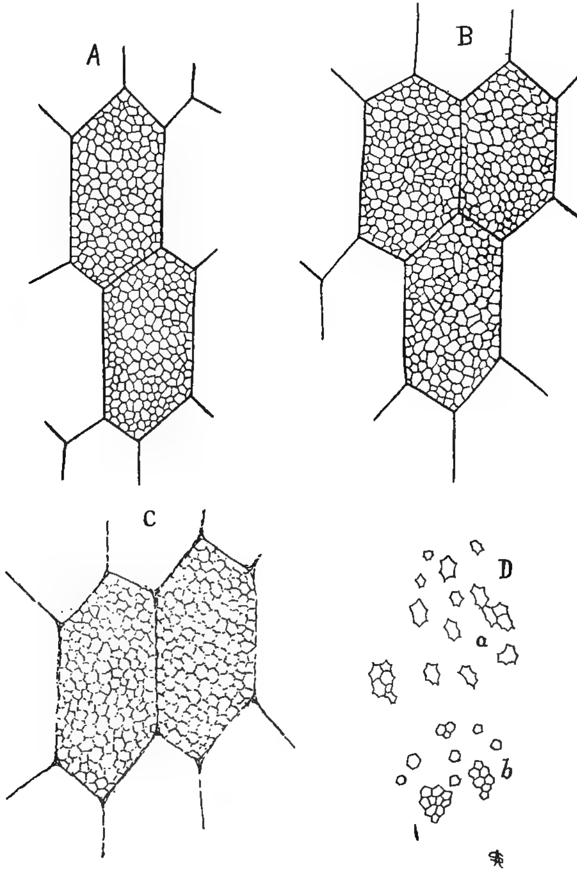


Fig. 75.—Saké making. *A*, *B*, Rice cells entirely filled with starch granules; *C*, rice cells after steaming, the starch granules are broken up; *D*, rice starch granules *a*, dextrinized, *b*, normal.

6. Use and Properties

A beverage, usually taken in comparatively large doses, producing a mild form of intoxication.

Usually taken in small amounts, producing a speedy, though transient, form of intoxication. Taken as a wine. In Japan saké is usually heated before drinking.

There are numerous varieties of *Saccharomyces* concerned in beer brewing. There are several kinds of upper or top yeasts (*Kahmhefe Oberhefe*) and several kinds of bottom or lower yeasts (*Unterhefe*), each kind possessing supposedly special properties. Just what part the more or less incidentally associated organisms (as bacteria, molds, and foreign

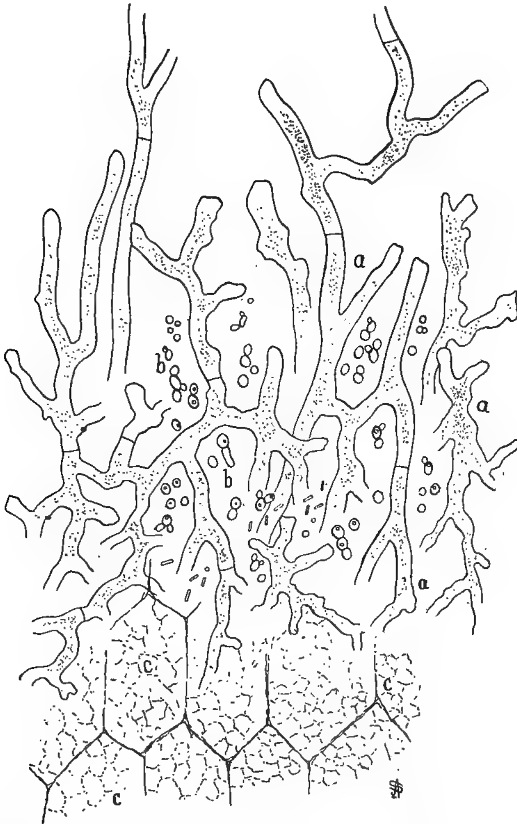


FIG. 76.—Saké making. Steamed rice cells (*c*) attacked by the hyphæ (*a*) of *Aspergillus oryzæ* which feed upon the dex rinized rice starch, converting it into saccharine substances. Yeast cells and bacilli are usually associated with the hyphal fungus, feeding upon the saccharine substances formed.

yeasts) may play in the fermentation processes is not clearly understood. It is known that some of these extraneous organisms may develop to such an extent as to modify the quality of the product completely. Such fermentation diseases are a source of much annoyance to manufacturers, often resulting in great financial loss, but this has also been the great stimulus in compelling the use of pure cultures and in perfecting those methods which are known to improve the keeping qualities of the articles,

whether foods or drink. Saké in particular, does not keep well, even with the greatest care in manufacture and with the use of preservatives. Certain brands of beer, wine, saké, smoking tobacco, cigars, tea, etc., are known to lose their characteristic flavors within short periods, due to the

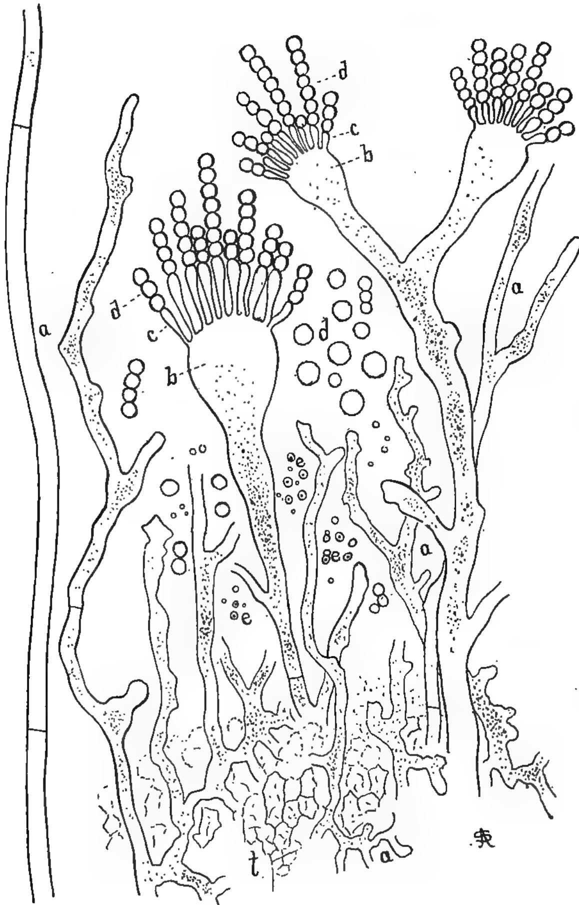


FIG. 77.—Saké making. *Aspergillus oryzæ*, showing vegetative hyphæ (*a*), and spore-forming hyphæ (*b*, *c*, *d*).

invasion of some "disease" producing organism. In many instances manufacturers have been blamed for inferiority in the quality of fermented products when in reality said articles left the establishment in perfect condition as far as quality is concerned, but were subsequently (in shipment, in storage, etc.) attacked by some objectionable organism, resulting in a complete change of flavor or bouquet.

The Japanese soya sauce (fermented soya beans, *Glycine hispidus*) and miso, a soup stock of wheat and soya beans, is prepared through the action of *Aspergillus oryzæ* and *A. wentii.* The Javanese arrak is made from rice which is first acted upon by a fungus (*Ragi*) in many respects similar to *A. oryzæ*, and subsequently, the alcoholic fermentation is carried on by the Saccharomyces, thus the method of arrak manufacture is closely similar to

FIG. 78.—Saké making. *A*, Dead or dying yeast cells; *B*, active yeast cells which convert the saccharine substances formed by the aspergillus into alcohol. *C*, *D*, yeast cells and hyphæ of aspergillus from the fermenting vats.

that of saké. More generally, however, arrak is made from fermented molasses. There are many other species of mold, including the very common *Penicillium glaucum*, which have the power of converting starch into saccharine compounds in the presence of moisture, but thus far these are not used industrially. An alcoholic drink of the East Indies is prepared from a starchy root as follows: A number of people, usually girls, sit about a large vessel masticating the roots which are then expectorated into the vessel. The ferment ptyalin of the saliva converts the starch into saccharine substances which is then acted upon by the Saccharomyces,

resulting in an alcoholic drink which is said to have a very peculiar flavor. Pressed yeast cakes for bread making are prepared as follows:

The filtered saccharine yeast mash in vats, is inoculated with pure cultures of *Saccharomyces cerevisece*. Active fermentation takes place in the presence of pure air which is supplied through pipes leading into the vat.

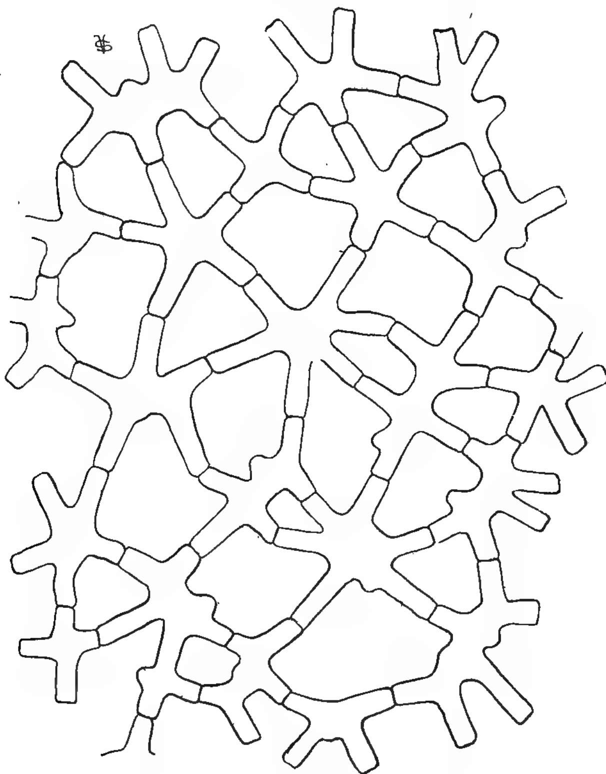


FIG. 79.—Showing the characteristic stellate cells of the pith of some reed used as filtering material in clarifying saké. Bundles of the pith are placed in the bottom of a perforated cask, forming a layer a foot or more in depth; through this the saké percolates. The impurities are caught in the intercellular spaces of the pith.

The white scum or foam which forms is poured on fine sieves, washed with sterile water, and then centrifugalized to remove most of the water. This partially dry material is then pressed into cakes, thoroughly dried at a low temperature, and wrapped in lead foil to exclude air. Starch is sometimes added as a dryer, but this is no longer necessary because of the improved methods of manufacture. Good yeast should be of a yellowish color, easily powdered and should have a pleasant "yeasty" odor.

The so-called Chinese yeast, concerned in various fermentation proc-

esses is a mixture of Mucor species, yeasts and bacteria. The following species of mucor are prevalent—*M. racemosus*, *M. alternans*, *M. spinosus*, *M. circinelloides* and *M. Boidinii*. These have the power of converting starch into saccharine compounds, which are then acted upon by the Saccharomyces. Various alcoholic ferments have been employed in China and Japan since time immemorial.

Nuclein is prepared from yeast and other vegetable cells and is very much used in the treatment of certain diseases due to pathogenic bacteria. It is said to have strong bacteriolytic properties and to increase phagocytosis.

TOXIC YEASTS AND MOLDS

Within recent years numerous outbreaks of cattle poisoning have occurred which have been traced to fodder used. Pammel of the university of Iowa has gathered numerous reports of occurrences of this kind and he is inclined to lay the blame for many cases of poisoning of this kind to fungi. Several species of Fusarium are mentioned as being responsible for equine diseases. *Fusarium equinum* Norgaard, is said to cause epidemic itch among horses. Another species is said to cause fatal meningitis in cattle. Pammel reports that sorghum cane fodder is frequently causative of fatal poisoning among cattle. Many cases of epidemic poisoning among cattle have been laid to silage, especially numerous are the case reports from the southern states. Some have suggested that the trouble is due to botulism (fatal poisoning due to a toxin formed by the *Bacillus botulinus*, the so-called sausage bacillus). The facts are that most of the cases of poisoning among cattle are inadequately investigated, and hasty conclusions are often based upon insufficient data.

A corn silage fungus, the *Monascus purpureus*, is supposed to be responsible for poisoning. Moldy fodder is universally recognized as being poisonous, but it has not yet been definitely determined which of the several symbionts which usually occur on such fodder, are primarily responsible for the ill effects. Numerous parasitic Saccharomyces have been found, in Daphnids, in horses, in guinea pigs, in pigeons, and in humans. Higher fungi are responsible for skin diseases, etc. It is however, only recently that the toxigenic parasitic yeasts have received any considerable attention.

It is but natural to suppose that a fungus which has adapted itself to parasitism upon animals, must have undergone extensive physiological as well as morphological adaptation changes, and it is a fact that most of the fungi of this kind are morphologically unrecognizable or unidentifiable and the tendency is to place them in a separate group, the so-called *fungi imperfecti*. As a rule they show remarkable life habits and peculiar

spore formations. Since the yeasts are sugar feeders and vegetarian we may expect to find them in plants and in fodder containing more or less sugar, and also for these reasons we may expect to find them parasitically associated with herbivorous animals, less commonly with omnivorous animals and least of all with carnivorous animals. One of the many reasons why our knowledge of food poisoning (in cattle as well as in humans) is so incomplete is because most of the methods of investigation of cases are chemical. Rarely is the microscope brought into play and even when this is done, the work is left to amateurs who usually report negatively, not because there is nothing to report, but rather because they fail to observe or fail to recognize the foreign organisms which may be present.

The following descriptions of parasitic Saccharomyces will serve to illustrate the points above set forth. In order that the student may realize the remarkable life habits and morphological characteristics of the two parasitic yeasts, he should familiarize himself with yeasts in general. Such information may be gleaned from any of the more complete texts on general botany and the special treatises on yeasts and on fermentation.

The first case pertains to the poisoning of sheep in a San Francisco stock yard. The toxicological examination (chemical) was negative. The following is the report of the microanalyst who was called into the case.

"I hereby submit a report on the poisoning of sheep which occurred at the San Francisco stockyards, Feb. 12, 1918, and for several days subsequently. I beg to state that certain phases of the observations made by myself are not finally conclusive. However, the following statements, recommendations and conclusions are warranted, based upon the tests and observations made to date.

The sheep (some 257 in number) were evidently killed by a toxin (poison) formed in the intestinal tract of the animals, due to the presence of a parasite which belongs to the yeast group (Saccharomycetes). The spores of this organism, which are apparently derived from the barley as well as from the barley screenings, enter the stomachs of the animals, with the food, where they develop into mature vegetable cells, whereupon these multiply quite rapidly (by a process known as 'budding'). The toxin or poison (evidently an *exotoxin*) is formed by the growing and budding vegetative cells in the stomach of the animals. The parasite next passes into the small intestine along with the food, where further development is completely checked (due to the alkaline reaction and enzymes present). Spore formation takes place in the large intestine and the spores escape with the excreta.

The spores are very small, resembling bacteria, and each spore bearing cell forms from fifty to one hundred and more. Air currents spread the

dried excreta containing the spores about, and those which lodge upon the barley straw and barley grains (and no doubt also upon other cereals and other forage plants) on entering the intestinal tract of susceptible animals which may happen to feed upon such contaminated material, will multiply in the manner already stated. The probabilities are that wild rabbits, rats and mice, as well as sheep, cattle, domestic rabbits and guinea pigs, are the carriers and disseminators of the infection.

It is evident that not all animals, even of the same kind, are equally susceptible to the infection and the poison formed, as not all animals died which were fed approximately equal amounts of the spore contaminated

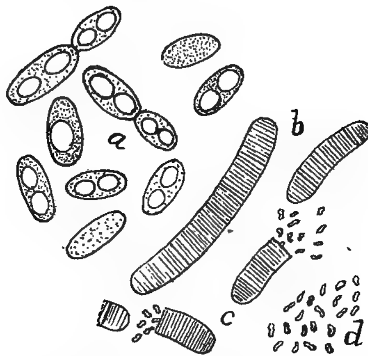


Fig. 80.—A toxigenic saccharomycete responsible for the fatal poisoning of sheep. It is an obligative parasite and will not develop outside of the intestinal tract of susceptible animals (sheep, goats, rabbits, guinea pigs and probably other herbivora). *a*, the vegetative cells which occur in the stomach, where they grow and multiply by budding. *b*, the spore bearing cells, showing numerous transverse markings, some heavy and the rest light. The sporangium breaks across along the heavier lines and the spores are thus allowed to escape as shown at *c*. *d*, a group of the small spores, measuring about four microns in length.

material. It is also evident that a definite number of the spores must be taken in with the food in order to produce fatal intoxication. It is further evident that the organism in question does not grow and multiply outside of the stomachs of living susceptible animals. As soon as the animal dies the vegetative cells also die, only the matured spores surviving, as already explained. The barley and barley screenings themselves contain no toxin as was proven experimentally.

A careful microscopical examination of the ground alfalfa, barley, barley screenings and "black strap" molasses samples submitted to me proved the absence of poisonous weeds. The barley, and especially the barley screenings contained a considerable amount of barley smut (a fungus belonging to the Ustilago genus) but not enough to produce fatal poisoning or even toxic symptoms, and none of the symptoms of the experimental

animals which died resembled those of either acute or chronic smut poisoning (ergotism).

While the "black strap" used in sheep feeding does not carry the infection herein referred to, it no doubt serves as a food for the parasitic organism, and it is advised not to use it with barley screenings or with any other forage material which may contain the spores of the parasite in question. The black strap in all probability hastens the growth and multiplication of the vegetative cells of the toxigenic parasite.

I would advise that the lots of barley and of barley screenings of which samples were submitted for experimentation, be not fed to horses, cattle, sheep or rabbits. It is highly probable that it might be fed to hogs with impunity, as these animals are comparatively immune to most toxic and toxigenic foods. I would also advise the following:

1. That barley screenings be not fed to sheep, young cattle, goats or rabbits. It would, in fact, be inadvisable to feed this material to any animals excepting, perhaps, hogs.

2. A new lot of barley or barley screenings should be tested as follows before feeding it:

(*a*) Feed the barley or barley screenings to several young rabbits (these animals are quite susceptible to the poison). If one or more die within 10 to 48 hours the article in question should be rejected.

(*b*) If none of the experimental rabbits die within 48 hours, feed a reasonable allowance to several heads of sheep, and if no deaths or illness results within 48 hours, the article in question may then be fed to the entire herd, allowing rather scant portion at first.

Among the causes which have been suggested as being responsible for the death of the sheep are:

1. *Acute Gastritis.*—The autopsies showed no evidence of such disease.

2. *Chemical Poisons.*—The findings of the city toxicologist were negative.

3. *Added Poisonous Weeds.*—The microscopical findings were wholly negative.

4. *Botulism.*—Symptoms not those of botulism.

5. *Ergotism.*—Symptoms not those of ergot or smut poisoning.

6. *Bloating.*—No evidence of bloating.

7. *Use of Fermented Barley.*—Barley and screenings in question showed no signs of ever having undergone fermentation.

8. *Overfeeding.*—Denied by Taafe and Co. and autopsies showed no evidence of overfeeding.

9. *Alfalfa.*—Control tests proved that the alfalfa used (dried, shredded or ground alfalfa) was not poisonous.

10. *Green Alfalfa.*—The poisoned animals were not fed green alfalfa.

The details of the experiments and observations upon which this

report is based, including a full description of the toxigenic parasite, will be given in a later report."

A saccharomycetous ascomycete (*Nematospora Lycopersici, n. sp.*)[1] was found on some ripe tomatoes obtained from a Berkeley (California) restaurant. The tomatoes were from a lot in cold storage which, so it was claimed, were imported from the South Sea Islands. The specimens

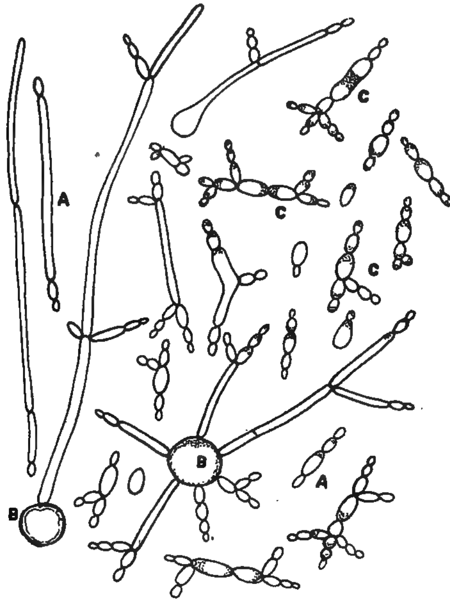


FIG. 81.—Various forms of vegetative cells of *Nematospora Lycopersici*. Extremes in cell formation are not shown. Very frequently some of the hyphal filaments resemble the hyphæ of true molds, but the individual cells do not branch. B, arthrospores; C, the beginning of spore sac formation.

under consideration appeared normal with the exception of an area about ¾ inch in diameter. This area was slightly depressed, of a cancerous raw reddish color. The epidermal tissue appeared markedly indurated and somewhat shrunken, but the hypodermal tissue as well as the parenchymatous tissue underneath appeared to be nearly normal. The microscopical examination showed the presence of a fungus in the seed chamber and in the mucilaginous tissue surrounding the seeds, as well as in the parenchymatous tissue beneath the epidermis.

This fungus proved of special interest because every slide mount examined, showed the complete life cycle of the organism, including the formation and development of the polymorphic vegetative cells and the

[1] Albert Schneider. A Parasitic Saccharomycete of the Tomato. Phytopathology. **6**: 395–399. 1916.

various stages of gametic fusion and of ascospore formation and the formation of Arthrospores. The vegetative cells increased numerically by budding as typified by the saccharomycetes generally. The normal vegetative cells may be described as elliptical to distinctively egg shaped, without vacuoles and without recognizable nuclei. The plasmic substance lines the inner wall of the cell and is more abundant at one end of the cells, usually the distal end in case of cell aggregates. The vegetative

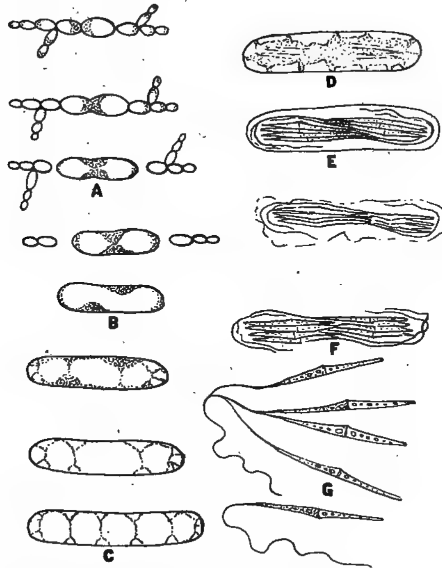


FIG. 82.—Various stages in gametic fusion and spore formation of nematospora. *A*, two somatic or vegetative cells unite end to end with solution of the contact cell walls; *B*, the plasmic contents of the two cells fuse; *C*, the fused plasm becomes grouped into four masses; *D*, plasmic differentiation has proceeded to the formation of the eight spore forming plasmic masses which soon draw away from the wall of the spore sac and occupy a middle position in the spore sac (ascus); *E*, fully formed spores; *F*, the ascus wall soon dissolves setting free the eight spores in two groups of four spores each which remain attached to each other by means of the whip-like appendages; *G*, gradually the spores become separated and are distributed through the medium.

cells may however undergo remarkable changes in form. They may become greatly elongated, narrowed or widened. Occasionally a cell may become bent or elbowed, narrowed at one end and enlarged at the other (gourd form). Daughter cells are always developed apically, never laterally as in many of the true Saccharomycetes. The exceptions are the cell formations at the junctures of two cells. Daughter cell forma tion is bipolar, that is starting with a single vegetative cell, new cells may form from the two apices and this is in fact the rule.

The plasmic contents of all cells inclusive of ascospores and of arthro-

spores, appear to be homogeneous with the exception of a comparatively small number of large spherical 0.5 micron granules. The plasmic granules are especially prominent and numerous in the arthrospores and in the arthrospore sphærocytes. They are highly refractive and stain readily. They are actively motile, especially in the sphærocytes where they also show remarkable Brownian vibration.

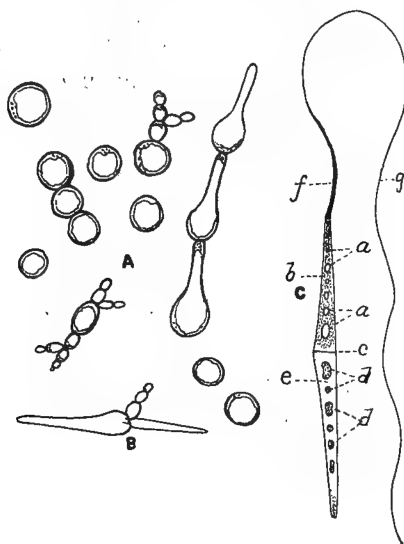


Fig. 83.—Arthrospore formation and ascospore development of *Nematospora*. Ordinary vegetative (somatic) cells are gradually transformed into spores; *A*, arthrospores derived from vegetative cells; *B*, an ascospore entering upon a new vegetative cycle; *C*, detailed structure of a mature ascospore more highly magnified; *a*, vacuolesin the chromatin-bearing cell of the spore; *b*, chromatin substance; *c*, transverse septum; *d*, plasmic masses in the achromatin cell of the spore; *e*, achromatin; *f*, the portion of the ligule which stains very heavily; *g*, the greatly elongated ligule by means of which the spore attaches itself to various substances with which it is brought in contact. The ascospore is distinctly two-celled. The spore wall as well as the septum are thin. There is a distinct widening of the spore at and near the transverse septum.

Ascospore formation is generally the result of the gametic union (isogamous) of two elliptical vegetative cells. The apical cell membranes which are in contact dissolve. The plasmic contents of the two cells fuse. The complete changes are shown in Fig. 81. Eight spores are formed in each spore sac. At an early period in the development of the spore sac (ascus) the associated vegetative cells become separated and the spore sac exists as an independent cell structure. There are indications that a spore sac may be derived from a single vegetative cell, especially when spore formation becomes very active.

As a rule active ascospore formation is accompanied by active arthro-

spore formation. Arthrospores are simply enlarged vegetative cells which as a rule assume the spherical form with thickening of the cell-wall. As a rule the arthrospores also become separated from the vegetative cells. Occasionally two or three vegetative cells in one group may become transformed into arthrospores and remain united for a time. Occasionally arthrospores take on the gourd form as shown in Fig. 83.

The ascospores are two-celled, rather slender and tapering pointed. The two cells differ materially. The end which is directed toward the middle of the spore sac stains very heavily and has a long slender gelatinous ligule or filament which is motionless. This filament serves to attach the

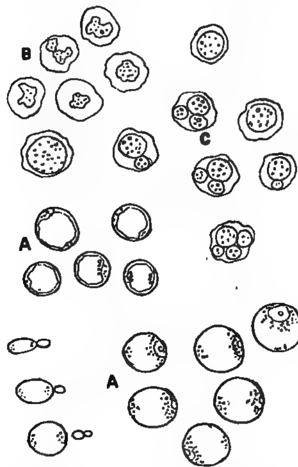


FIG. 84.—Phases in the development of the arthrospores of *Nematospora* (*A*); *B*, dying spores as indicated by plasmolysis; *C*, sphærocyte formation in the spore. The mature arthrospores always take a bipolar position in the liquid medium, so that the plasmic granules always appear in profile relative to the observer.

spore to its fellows and to other objects with which the filament may come in contact. Gradually, when the spore begins a new cycle of cell formation by budding, the ligule disappears. There are indications that the chromatin cell of the spore serves as a source of food supply for the achromatin cell which is chiefly concerned in starting a new cycle of cell formation. The chromatin cell of the spore gradually shrinks and the unused portions of the cell-contents disintegrate and dissolve in the surrounding medium.

The fungus is typically parasitic in habit and dies with the invasion of mold and of rotting bacteria, preceded by very active asco- and arthrospore formation. Spore bearing material injected into a guinea pig was without notable results.

As decay of the tomato advances due to the invasion of rotting bacteria, all vegetative cell multiplication of the parasite ceases. Gradually ascospore formation ceases also and the existing vegetative cells become transformed into unusually large, absolutely spherical arthrospores. These arthrospores resemble sphærocytes very closely. Each cell contains a homogeneous nucleus of very indistinct irregular outline with a more distinct but also homogeneous spherical nucleolus. The plasmic granules are unusually large, spherical in form, highly refractive and stain readily. They are slightly motile and as a rule occur in pairs, resembling diplococci. These plasmic granules occur on the exterior of the homogeneous plasmic supporting substance in this regard differing from the plasmic granules of chlorophyll bearing plants in which they occur within the plasmic substance.

The arthrospores occupy a definite position in the medium in which they live (the liquid of the tomato). The nucleus always occupies such a position as to bring the edge of the nucleus into view. Very rarely the spore is sufficiently tilted to present the view indicated in (A), Fig. 84. In not a single instance was the spore to be seen in exactly vertical view. No explanation is offered as to why the spore should assume this very definite position.

Plasmic granules are sparingly present in the vegetative cells, from one or two to five whereas in the arthrospores there may be as many as one hundred. As the arthrospores die the plasmic granules apparently increase in number somewhat as some show a remarkable increase in size and they become absolutely motionless. All Brownian vibration ceases also. The plasmic supporting substance shrinks, thus bringing the granules closer together until there is finally a closely crowded mass of plasmic granules as shown in (B), Fig. 84.

CHAPTER XIV

PROTOZOA IN DISEASE

Certain low forms of animal life are causative of such diseases as malaria and sleeping sickness. These organisms resemble each other in that they are minute, of simple structure (single celled) and in that they show active motion due to the presence of pseudopodia, of flagellæ or cilia, or due to cell undulations. They are found in stagnant water containing decaying vegetable and animal matter and in decaying organic matter. Most of them are non-pathogenic and all are quite readily killed by means of heat and the common chemical disinfectants. They do not occur in pure, fresh well, spring, or hydrant water.

The following are the more important species of protozoa and the principal activities in which they are concerned:

I. Rhizopoda.—These move by throwing out slender protoplasmic projections. Silicious coverings may be present.

The amebas form the type group of the Rhizopoda. These organisms are very widely distributed in aqueous substances rich in organic matter. Two subdivisions of the group are recognized, the amebas proper which feed upon dead organic matter and also on minute organisms, such as bacteria, and yeasts; and the entamebas which are parasitic upon a variety of organisms, plant as well as animal. Even the parasitic forms feed upon some of the microörganisms found in association with the host. The non-parasitic amebas usually contain one nucleus whereas the parasitic forms are generally multinuclear. The non-parasitic forms are of no special significance from the standpoint of health and preventive medicine. They are scavengers in so far as they feed upon the organisms of decay and the products of decomposition, provided the water supply is adequate, for they are all aquatic in habit. If amebas are abundant in water supply, it is evidence of high organic contamination.

The parasitic forms no doubt began existence as saprophytes, gradually changing to a parasitic mode of living as the opportunities for taking up the food materials elaborated by the host organism developed. The entamebas of the intestinal tract and of the mouth also feed upon the bacteria and other microörganisms found in these localities, but they also set free toxic agents which give rise to the phenomena designated as amebic dysentery, amebic pyorrhea, etc.

The amebas are also classed with the Sporozoa, but it has not been proven that all of the organisms which are classed as amebas form swarm spores. The primary cause of malaria certainly belongs to the sporozoa. Cytologists are gradually recognizing the fact that many of the so-called single-celled organisms, such as ameba, paramecium, etc., are not as simple in structure and in physiological activity as was generally supposed. Many of these organisms contain highly complex cell constituents which enable them to compete quite successfully in the struggle for existence, with the highly complex multicellular organisms.

1. *Entamœba coli.*—Inhabits the large intestine. Probably harmless. Amœba belong here and not in the sporozoa. May be confused with phagocytes.
2. *Entamœba histolytica.*—Causes entero-colitis and dysenteric ulcerations. It is also found in abscesses of the liver. Occurs in tropical countries, less common in temperate zones.
3. *Entamœba buccalis.*—Found in dental caries. Probably not pathogenic.
4. *Entamœba undulans.*—Occurs in the intestinal tract.
5. *Leydenia gemmipara.*—Identity doubtful. Supposed to have a causal relationship to carcinomatosis (cancer).

II. FLAGELLATA.—Motion due to flagellæ. Some possess an undulatory motion. Have been classed as bacteria (Spirillæ).

1. *Spirochœta recurrentis* (*Spirillum obermeieri*).[1]—This is the organism which causes relapsing fever. The disease is so designated because after apparent complete recovery, one or more relapses invariably follow. It is not a very fatal disease (4 per cent. of deaths) and is, so far, rare in the United States. It is and has been very prevalent in parts of Europe. The disease can be transmitted, by inoculation, to man, monkeys, mice and rats. An immunity treatment has been attempted with some success. Most authorities class the organism as a fungus (Spirillum).
2. *Spirochœta Duttoni.*—This organism is the primary cause of the South African tick fever (Tete fever), so-called because the carrier is a species of cattle tick (*Ornithodoras moubata*).
3. *Spirochœta Novyi.*—Said to be the cause of American relapsing fever.

[1] The systematic position of the spirochætes is still in dispute. They probably belong to the animal kingdom. Note the tentative position given them by the committee on the classification of bacteria appointed by the Society of American Bacteriologists. The fully life history of these orgaisms has not yet been worked out. What has been described under the name *Treponema* (*Spirochœta*) *pallidum* is in all probability the male generation of this organism, the female cells being irregular in outline and found in the lymphocytes and in other body cells.

4. *Spirochæta vincenti.*—Pathogenic; causes throat inflammation (Vincent's angina).

5. *Treponema pallidum.*—The specific cause of syphilis. Often other related organisms are found associated with it. This organism stains with difficulty.

6. *Trypanosoma Gambiense.*—This is the cause of the dread sleeping sickness of Africa. The transmitter of the infection is the tsetse fly (*Glossina palpalis*). Investigations have shown that the destruction of the tsetse fly would also eradicate the disease (Koch), which has practically depopulated large districts in Africa. Related organisms cause diseases in horses (surra, dourine and mal de caderas). There are also many trypanosomes of frogs, fish and birds, but these are probably harmless to man.

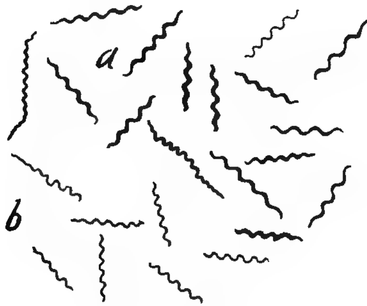


FIG. 85.—*A*, *Spirocheta refringens; b*, *Spirocheta pallida.* The cause of syphilis.

III. FLAGELLATA. (Mastigophora).—Most of the organisms belonging to this group are ameboid. There may be a fairly distinct cell membrane, and some have a distinct mouth part or end, the so-called cytostome, which leads to a blind œsophagus. They contain flagellæ, in addition to possessing an ameboid movement. Some of the representatives of this group appear to have a complex internal structure. The earlier stages may be without flagellæ and may be readily confused with amebæ.

Species of *Leishmania* cause sores and ulcers (in tropical countries). Certain tropical *Lamblia* and *Trichomonas* species may cause intestinal and other disturbances.

IV. INFUSORIA. (Ciliata).—These have numerous very fine cilia and contractile vesicles. The bodies are oval and may be free swimming or attached. They have a complex internal structure and are supposed to be the highest of the entire group of protozoa. There is a distinct body covering or membrane and a distinct mouth part. They are essentially saprophytic scavengers being abundant wherever there is an abundance of organic matter in water. They are wholly aquatic in habit.

To this group belong the widely distributed Paramecia, which are all true scavengers, feeding upon decayed and decaying organic matter, vegetable as well as animal, including the organisms which give rise to the decomposition changes. They show marked preference for decaying vegetable matter. For example, decaying fish food or fish meal infusion, well diluted with water, is likely to contain a pure culture of Paramecia. The accompanying rotting bacteria are actively devoured by the paramecia.

The complexity (physiological as well as morphological) of the paramecial cell has suggested the use of this group of organisms for the purpose of making comparative toxicological tests, in place of the higher animals now almost universally employed for such tests.

The infusoria proper (*Ciliata*), while exceedingly abundant and widely disseminated, are mostly non-pathogenic. The *Balantidium coli* is a common hog parasite which may also cause serious dysentery in man.

V. Sporozoa.—Have no cilia, move by plasmic contraction of the cell and reproduce by spores. Of this group, the most important species is the *Plasmodium malariæ* which is the primary cause of ague or malaria. The carriers of the infection are certain mosquitos (species of *Anopheles*). If the Anopheles group of mosquito could be exterminated throughout the world, malaria would disappear also. The organism is introduced into the blood by the sting of the insect. In the blood it undergoes certain cycles of development. The fever paroxysms are due to the sporulation of the organisms in the circulatory system. During the intervals (non-sporulation) there is no marked febrile disturbance. There are several species of Plasmodium causing the several forms of malaria. The tertian form (*P. vivax*) has a cycle of forty-eight hours; the quartan (*P. malariæ*) has a cycle of seventy-two hours; and the malignant tertian (*P. falciparum*) has a cycle of forty-eight hours. In the latter type the paroxysms are so severe as to give rise to a continued fever. Quinine is fatal to the Plasmodium and this remedy should be given as a prophylactic and as a cure.

The draining of swamps and other breeding places for mosquitos has reduced malaria. The use of mosquito netting, screens, etc., has also checked this disease. Small water areas may be treated with crude petroleum oil which kills the mosquito larvæ.

The primary cause of yellow fever is as yet unknown but it has been definitely determined that the carrier is a mosquito, the *Aëdes* (*Stegomyia*) *calopus*. Yellow fever is essentially a tropical disease, though it may flourish in temperate zones until checked by frost which is so readily fatal to the carrier, the mosquito. It has been ascertained that the Aëdes does not occur far from human habitations, that it breeds generally in barrels and cisterns containing rain water, rather than in ponds or larger bodies of

water, more remote from habitations. Air currents may carry them greater distances. These discoveries have made possible a very effectual campaign against this dread disease. The Federal Government aided by State and Local Boards of Health have insisted on a discontinuance of those breeding places which can be controlled easily. The larger more public breeding places were covered with crude oil. Screening windows and doors and sulphur or Pyrethrum fumigation of mosquito-infected houses and rooms was insisted upon and individuals were instructed in methods of self-protection against the bites of mosquitos. As a result the yellow fever ravages are now reduced to a minimum.

CHAPTER XV

DISINFECTANTS AND DISINFECTION. FOOD PRESERVATIVES. INSECTICIDES[1]

The pharmacist should be well informed regarding disinfectants and their uses in order that he may assist physicians and health officers in carrying out sanitary rules and regulations in which disinfectants play so important a part. The pharmacist should know how to disinfect sick rooms, private homes and public buildings. He should in addition be informed regarding the essentials in the construction of sanitary homes, shops and stores. He should be able to give good advice regarding water supply, sewage disposal and on preventive medicine generally. He should be well informed regarding the preservation of foods, the use and abuse of food preservatives and on food adulteration and should be prepared to test foods as well as drugs as to quality and purity. He should be informed regarding the nature and use of insecticides and pest exterminators generally.

Disinfectant is synonymous with germicide and means any substance, usually in the form of a liquid or gas, capable of destroying bacteria and their spores, more particularly the pathogenic forms. A septic substance is one contaminated or infected with pathogenic or otherwise objectionable bacteria. An aseptic substance is one free from bacterial infection or contamination, but not necessarily possessed of disinfecting or even preserving power. More broadly speaking, disinfectant means any ponderable or imponderable agent or substance, destructive to bacterial life and it is in this sense that the term is here used. Preservatives may be defined as mild disinfectants; that is, when used in larger amounts or stronger concentration, preservatives become disinfectants. Furthermore, the term preservative usually applies to substances added to foods for the purpose of preventing or retarding microbic infection and microbic development. It is, however, also applied to other substances. We speak, for example, of wood preservatives, leather preservatives, fur preservatives, etc., meaning thereby substances which will prevent certain decomposition or other detructive changes in the articles named, due to a variety of organisms as mould, larvæ, insects, mites, etc.

[1] Each student is required to use the following handbook in connection with the study of antiseptics and their practical application. Dakin and Dunham. Handbook of Antiseptics. The MacMillan Company, 1917.

The chief purpose in disinfection is to check and prevent the spread of communicable diseases, by destroying the primary causes thereof, namely, the pathogenic bacteria or other disease producing organisms. The agents or substances which have disinfecting powers or properties are legion. We can only refer to a few of the more important ones, those which are commonly employed, giving the methods of their use and explaining their action.

Disinfectants differ greatly as to germ destroying powers and attempts have been made from time to time to standardize them or, in other words, to determine their comparative germicidal efficiency, but thus far no satisfactory or generally acceptable method has been devised. All methods appear to have some objectionable features. The technic and principles involved in the standardization of antiseptics include the following:

1. Selecting some antiseptic as the unit of comparison, as a solution of phenol.

2. As test objects, definite quantities of bacterial cultures are used; as bouillon cultures of the typhoid bacillus, colon bacillus, hay bacillus, etc. Some experimenters first air dry the bacteria before exposing them to the disinfectants to be tested. There are a number of methods known as the "silk-thread method," the "garnet method," "the glass-rod method," "the platinum-loop method," "the spoon method," and others.

3. Exposing the bacteria from a standard culture, for definite periods (uniform for the series of tests) of time, to varying strengths of the disinfectants to be tested.

4. Plating out (in Petri dishes) the exposed bacteria in order that the death point may be ascertained.

The results are expressed numerically by dividing the strength of the disinfectant tested which will kill a given organism in a given time by the strength of the phenol solution which under the same conditions will kill the same organism in the same time. To illustrate, we will suppose that a 1–40 solution of formaldehyde will kill the typhoid bacillus in ten minutes at 37° C. and that a 1–110 solution of phenol will kill the same organism in the same length of time and at the same temperature, then we get as the phenol coefficient of formaldehyde, 0.36 ($40/110 = 0.36$), which means that formalin is only about one-third as active as phenol as far as the destruction of the typhoid bacillus is concerned. The phenol coefficient[1] is also known as the Rideal-Walker (R-W) coefficient, named after the Eng-

[1] For a full discussion of the methods for determining the phenol coefficient and the albumen coagulation coefficient, the student is referred to the following books: Schneider. Bacteriological Methods in Food and Drug Laboratories. P. Blakiston's Son and Co., 1915; or, Tanner. Bacteriology and Mycology of Foods, John Wiley and Sons, 1919.

lish investigators who worked out the details of the method. In time no doubt an international standard method for testing disinfectants will be adopted. This would be of inestimable value for comparative purposes.

1. Physical and Mechanical Disinfectants

The following is an outline of the physical and mechanical means of disinfecting.

a. Cleanliness.—That is, bacterial cleanliness, or absence of bacteria, brought about in a variety of ways. The liberal use of pure water for washing, bathing and cleansing purposes, is one of the oldest methods for getting rid of pathogenic and otherwise objectionable organisms. It is, at the present time, one of the most effectual means of disinfection, practised by the housewife, the nurse, the physician, in fact by all classes and conditions of peoples. By bacterial cleanliness we bring about a dilution, an attenuation, a dissemination of objectionable organisms to such a degree, that bacterial localization and infection are greatly retarded or are made impossible. Cleanliness prevents filth and dirt accumulation.

b. Pure Air.—Pure air, that is air free from disease organisms, is a prime essential in preventive medicine. Not only should the air we breathe, be free from bacterial infection, but it should also be free from smoke, fumes, noxious gases, soot and dust. The air in many of our large cities is often quite unsuitable for breathing purposes due to fumes, soot and smoke from numerous furnaces and factories, stenches from sewage, from stock yards, from gas factories, etc. This should not be. Stock yards, glue factories, etc., should be sufficiently remote from cities to avoid permeating the city with the horrible stenches emanating therefrom. Smoke, fumes and noxious gases should not be permitted to escape. The recent tests with smoke consumers, with the precipitation of fumes and smoke by means of electricity, etc., would indicate that it is possible to prevent the pollution of the atmosphere by the above agents. Just as soon as there is a smoke consumer on the market that is a practical success, every smoke producing furnace should be supplied with one, irrespective of cost. The same should apply to the use of smelter fume precepitators. Streets should be kept comparatively free from dirt and dust by means of sprinkling cart and street sweepers and cleaners.

The "no spitting" ordinances are largely a failure simply because no provision is made to supply the appurtenances necessary to carry them out. It is not sufficient to simply put up a notice stating that "It is unlawful to spit upon the floor," but cuspidors, or other receptacles must be provided in sufficient numbers, conveniently placed, and furthermore said receptacles must be kept clean and sterilized from time to time, otherwise they may become the breeding places and disseminators of disease. The

spitting habit of the adult male is largely due to the use of tobacco, especially chewing tobacco. While it is not possible to discontinue spitting altogether, it can certainly be greatly reduced. Women rarely spit in public and men can, if they will, also discontinue the nasty habit.

A most serious defect in places of habitation is the lack of pure air, as in small bed-rooms, in the Pullman sleepers, in sweat shops, in factories, in school-rooms. Next to the crowded sweat shops in our large cities, the lower berth in the American Pullman car, is most unsuitable for human habitation. Rooms for living purposes, sleeping purposes, for factory use, office use, etc., etc., should not only be large enough, but there should be adequate provision to renew the air constantly, no matter how warm or how cold it may be. We need a thorough sanitary supervision of all building construction, whether private home, school, factory, sleeping car, office, or street car. There is plenty of pure air and every individual should have an ample supply, for pure air is one of the most potent factors in preventive medicine.

c. Heat.—Heat is one of the best disinfectants known. Dry and moist heat are used, both of which have been sufficiently treated in the preceding chapters. Mere dryness is in itself a germ destroyer. Microbes require moisture for their growth. Most bacteria (vegetative cells, not spores) succumb in a dry atmosphere in a comparatively short time, several hours to several days. The spores may, however, survive dryness for many months.

The dry-air temperature usually employed for germicidal purposes, ranges from 140° C. to 170° C., acting for one hour or longer. A dry heat of 145° C. acting for one hour is sufficient to kill all bacteria, including the spores. Temperatures used for purposes of disinfection and sterilization range from 55° C. to 120° C. 55° to 75° C. is usually employed in the pasteurization of milk and in sterilizing sera, vaccines, certain culture media (as egg albumen, blood serum), etc. Moist heat of 100° C. in the form of circulating steam vapor is much used. To obtain a moist temperature above 100° C., an autoclave is necessary, or liquids may be employed which boil at a temperature higher than 100° C. as cumene, oils, etc.

d. Cold.—Cold, 10° C. and lower, has decided antiseptic properties, that is, it checks bacterial growth and activity very effectually, as has already been explained. Prolonged freezing is, however, necessary to kill bacteria. Cold may therefore be considered a most excellent check upon bacterial activity, but it is a very poor germicide. Cold is a universally recognized and an extensively used food preservative, due to its checking influence upon bacterial growth.

e. Agitation.—The agitation of gases and liquids reduces the bacterial activity therein. Still waters become stagnant but running waters do not, in the comparative sense, due in part to the difference in the oxygen con-

tent. Agitating and churning contaminated liquids checks bacterial development somewhat. The active circulation of contaminated air reduces the number of bacteria present. Agitation is, however, not a satisfactory means of sterilization and disinfection.

f. Sedimentation and Filtration.—Sedimentation in sewage waters and other contaminated liquids, combined with filtration, is a very effective means of purification. Precipitation and filtration, aided by chemicals as alum, iron sulphate, and other coagulants, are much employed in the purification of water supplies.

g. Free Circulation.—Free circulation of air and water are most favorable to sanitation because of the checking influence upon bacterial activity and also because of the disseminating and diluting effects upon the organisms which may be present. Circulation is strictly speaking a form of cleansing.

Purification of flowing water, as rivers and small streams, is effected very largely by oxidation and dilution. The agitated water takes up oxygen by absorption which combines with the organic particles suspended in the water rendering it unsuitable as food for bacteria. As the water flows along, the bacteria are scattered more and more. Sedimentation is also an important factor in the destruction of bacteria. Gradually the bacteria settle to the bottom of the stream where they are brought in competition with other bacteria, protozoa, algæ, perhaps hyphal fungi, etc., which tend to check and even entirely inhibit their further development.

h. Light.—Sunlight has most marked germicidal powers, due in part, to the drying effects produced and in part to the actinic or chemically active rays of the sun's light. Numerous investigators have demonstrated the germ-destroying effects of the blue and violet-rays and the ultra violet end of the solar spectrum. Bacteria cannot survive in sunlight. Electric light is said to have the same effect upon bacterial life as sunlight. The *X*-rays destroy bacteria, likewise does radium, and these agents have been extensively tested in the treatment of skin diseases and superficial tuberculosis as lupus, and in cancer, but without satisfactory or conclusive results.

i. Electricity.—The electrical current in itself appears to be without germicidal powers, but electricity is used to precipitate smelter fumes, and organic impurities in water, as already stated. Electricity is used to stimulate seed germination and it may be possible to utilize electrical discharges or currents in the treatment of communicable diseases.

2. Chemical Disinfectants

Chemical disinfectants may be divided into gaseous (or vaporous) and liquid (solutions). The liquid disinfectants are superior to the gaseous disinfectants because direct contact with the articles to be disinfected can

be brought about, as in washing, immersing or mixing. Gaseous disinfectants are effective for surface sterilization, especially useful for inaccessible rooms, buildings, ships, paintings, books, fabric, etc. Both have their special advantages, however.

The number of chemical disinfectants, variously classed as gaseous, liquid, patent, proprietary, efficient, useful, useless, etc., is very great. We shall mention only a few of the more powerful kinds. No reliance should be placed in any patented or proprietary disinfectant until its value has been demonstrated by tests made by reliable bacteriologists, giving its phenol coefficient. Nor is this all, not only must the disinfectant have actual germ-destroying powers, but it must also be practically usable and it must not be misrepresented as to its value and its application and use in practice.

The resistance of pathogenic germs to disinfectants is extremely variable. Furthermore, the various disinfectants produce changes in the tissues and substances in and upon which they act, which changes tend to modify, check or inhibit the disinfecting powers. Thus a number of disinfectants may have the same laboratory phenol coefficient and yet their value as disinfectants in actual practice is widely different because of the difference in the effects produced in and upon the substances with which they are brought in contact.

As a rule, the action and use of disinfectants is variable according to the following conditions:

1. Disinfectants are more active when warm or hot. In all disinfections hot solutions should be used, if possible and if practicable.

2. Gaseous disinfectants act only in the presence of moisture, as will be explained under formalin and sulphur disinfection.

3. The thoroughness of disinfection is directly proportional to the time that the disinfectants are allowed to act.

4. The activity of disinfectants is directly proportional to the degree of concentration, though there are noteworthy exceptions. Absolute alcohol, for example, is of very little value as a disinfectant, whereas the weaker solutions (40 to 70 per cent.) are very active germ destroyers. The same is true of ether, chloroform, glycerin and a number of other substances. Most disinfectants have a concentration of optimum or maximum efficiency which is the degree of concentration generally employed in practice.

5. In actual practice the cost of disinfectants is a factor of some importance, as is indicated by the table giving the comparative phenol coefficient and the relative cost:

6. It is known that the disinfecting power of metallic salts is proportionate to their electric dissociation, that is, the more strongly a salt is

dissociated by electrolysis the stronger is its disinfecting power. It follows that anything which interferes with the electrolytical dissociation of germicides weakens the germicidal power. For example, the addition of sodium chloride lowers the germ destroying powers of corrosive sublimate through such interference. This is a matter of great importance in determining the efficiency value of antiseptics.

7. The chemical composition of the material associated with the germs to be destroyed, has a marked influence upon the action of the germicides. Thus germicides give different results when acting upon the same organism in water, in beef broth, in salt solutions, in and upon tissues, etc. For this reason the value of germicides in actual practice cannot be based exactly upon uniform laboratory results.

8. Not only do different species of disease germs differ in resistance to germicides, but the different strains of the same species react differently with the same germicide. Certain substances appear to have an elective affinity for certain organisms, as for example, quinine for the malaria germ, and mercury salts for the syphilis germ.

Disinfectants destroy or kill germs in different ways. In some cases the death of the organism is due to oxidation as when ozone, hydrogen peroxide and sulphites are used, or death may be due to interference with nutrition, but more generally it is due to the coagulation of albumen and abstraction of water from the cell-plasm, as in the use of dry heat, phenol, alcohol, tannic acid and metallic salts. As already explained in another chapter, lysins act by actually disintegrating the bacterial cells.

As a rule germicides are most active when dissolved in water, though some authorities declare that bichoride of mercury, phenol, thymol and lysol are more active when dissolved in 50 per cent. alcohol. The activity of phenol as a germicide is greatly increased by the addition of hydrochloric acid, whereas lime reduces its potency. Solutions of germicides in oils are inert because oil does not penetrate the bacterial cell; however, the oil itself may be fatal to bacterial life, in which case the added germicide is unnecessary. Chemical germicides do, however, increase the potency of the volatile coal-tar products as gasoline, benzine and xylol, provided the germicides are soluble in or readily miscible with these substances.

The following are the more important disinfectants given approximately in the order of their usefulness and potency.

a. Carbolic Acid (*Phenol*).—Very widely used, in strengths of from 1 to 5 per cent. As a disinfecting wash for all manner of septic things, a 5 per cent. solution is commonly employed. A 2.5 per cent. (also the 5 per cent.) solution is much used as a disinfectant for hands and the skin generally and for septic wound irrigation. A 0.5 to 1 per cent. solution is used as a mouth wash and gargle. Phenol does not kill spores hence

should not be used after anthrax, tetanus, malignant edema, and other diseases due to spore bearing bacteria. Phenol coagulates albumen, but not as actively as does corrosive sublimate.

Carbolic acid (5 per cent.) is much used for disinfecting liquid discharges in dysentery, typhoid, cholera, and for the disinfection of sputa and expectorations in tuberculosis, in pneumonia, etc., using about two times as much of the disinfectant as material to be disinfected, allowing the mixture to stand for several hours at least.

A 5 per cent. solution may be prepared as follows:

Carbolic acid (95 per cent.)	6½ oz.
Water	1 gal.

Shake thoroughly until all of the acid is dissolved.

Carbolic acid does not destroy, bleach or discolor cloth fabric, does not corrode metal, has a marked characteristic odor, is a powerful escharotic poison, and the crystals are readily liquefied by heat, by alcohol and by water.

b. Liquor Cresolis Compositus U. S. P.—This most efficient germicide is a liquid soap with 50 per cent. cresol, miscible in all proportions with water. The cresols used should have a high boiling-point (187° to 189°C.). The germicidal power of this substance is nearly double that of carbolic acid. It does not coagulate albuminous matter and kills spores.

There are a number of germicides similar to carbolic acid having marked germicidal properties including creolin, cresol and lysol. These are somewhat superior to carbolic acid. Lysol is a cresol mixed with soap which greatly facilitates the solution of the cresol, being therefore similar to liq. cres. comp. U. S. P. They all kill spores.

c. Tricresol.—Tricresol is a mixture of orthocresol, metacresol and paracresol. It dissolves in water in the proportion of 2.5 per cent. and is about three times as active as carbolic acid. It is less irritating than carbolic acid for which reason it is preferred in sterilizing sera (about 0.25 per cent.) and other solutions intended for hypodermic use. Tricresol kills spores and albuminous matter does not interfere with its action.

Tricresol, cresol, lysol, solveol, solutol and creolin are usually employed (as germicides) in 1 per cent. solutions and are generally conceded to be equal to about 2.5 per cent. solutions of phenol. They, however, have no superiority over the liq. cres. comp. U. S. P.

d. Formalin.—The 40 per cent. commercial article is used. It has many advantages as a disinfectant. It does not injure, fade or decolorize cloth or other colored fabric and does not corrode metal (excepting hot steel and iron). It kills spores and is an efficient deodorant. Albuminous matter does not interfere with its action and hence it is an efficient sickroom disinfectant. It disinfects and deodorizes all discharges from

patients very quickly and completely, when used in 4 to 5 per cent. solutions.

As a gaseous disinfectant it is active in a moist, warm atmosphere. It does, however, not kill insects and other higher organisms and in this regard it is inferior to sulphur dioxide, but has the advantages of not decolorizing fabric and being a better deodorant. There are several proprietary disinfectants composed of soap and formalin, as lysoform.

e. Sulphur.—Sulphur in itself is odorless, tasteless and wholly inert as a germicide, but when undergoing oxidation into sulphur dioxide (combustion), in the presence of moisture, it is a very active disinfectant and is at the same time fatal to insects and in fact to all forms of animal life, including rats, mice, etc. But it cannot be used to disinfect fine fabrics, paintings, books, etc., because of the destructive effects upon pigments.

Under ordinary conditions the gaseous substances, as formaldehyde (formalin) and sulphur dioxide, are surface disinfectants only and are used where surface disinfection is all that is required, as in the sterilization of clothing, wood work, walls, ceilings, pictures, furniture, etc.

f. Bichloride of Mercury.—This is the most potent and most extensively used of all antiseptics. A 1–1000 aqueous solution (used hot whenever and wherever possible) makes a most satisfactory germicidal wash for floors, walls, wood work of all kinds, in fact anything requiring disinfection, excepting metals which would be corroded (excepting of course platinum, gold, silver) and substances rich in albuminous matter as pus, sputum, and other sick room discharges, which are coagulated by this germicide, checking further action.

The 1–1000 solution is sufficiently powerful to kill all non-sporogenous bacteria at the ordinary room temperature in one-half hour. For spores a stronger solution (1–500) and longer exposure are desirable (one hour).

The chief disadvantages to the use of corrosive sublimate are its highly toxic nature, its corroding effect upon metals and its coagulating effects upon albumen which hinders penetration. It should also be borne in mind that soap interferes with the action of corrosive sublimate.

A 1–1000 solution is made as follows:

Bichloride of mercury,	61½ grs.
Citric acid or salt,	61½ grs.
Water,	1 gal.

The sodium chloride or citric acid is added to retard the decomposition of the bichloride. Tablets are now on the market made from mercury cyanide. They are held to be more decidedly antiseptic than either the iodide or the bichlorid of mercury, and are so prepared that one tablet added to a pint of water will make a strength of 1–1000.

g. Chlorinated Lime.—Also known as chloride of lime. This is an oxidizing disinfectant and deodorant, most extensively employed for the disinfection of stools, urine, sputa and other excreta. Eight ounces of the chlorinated lime are added to one gallon of water. This solution is placed in the vessel which is to receive the discharges, using at least double the amount to be disinfected and allowing the mixture to stand for one-half hour or longer. Chlorinated lime destroys color and corrodes all textile fabrics and most metals. It must be kept in an air tight receptacle as it loses in strength on exposure to air. The solutions should be made as required.

h. Lime.—Lime (unslaked lime, quicklime) is very useful for the destructive disinfection of cadavers dead of infectious diseases, using twice the amount of lime, by weight, to the substance to be disinfected. The lime is powdered or crushed and packed about the cadaver in a box or coffin. Neither water nor moisture need be added.

i Milk of Lime.—Lime is slaked in the usual way. From the slaked lime the milk of lime is prepared by adding eight parts of water. The preparation should always be made from freshly slaked lime. It is much used for the disinfection of stools and sputum, using an amount equal to the amount of material to be disinfected. Whitewash is much used to disinfect and preserve fences, stables, sheds, walls, ceilings, etc.

j. Copper Sulphate.—Blue vitriol is a very useful disinfectant for sick room excreta of all kinds, using a 5 or 10 per cent. solution, bulk equal to bulk of material to be disinfected, stirring and mixing and allowing to stand for 3 to 4 hours. Iron sulphate (copperas) is similarly used, though it is somewhat weaker in action.

k. Permanganate of Potassium.—This is another of the oxidizing antiseptics, having a rather limited use. It is furthermore comparatively expensive. Freshly prepared solutions are used, ranging in strength from 1–1000 up to 5 per cent. Quite extensively used as a disinfectant for hands. Has been administered internally to oxidize alkaloidal poisons in the stomach and in the intestinal tract.

The following antiseptics are used more or less in surgery and as skin and other tissue disinfectants. Some of them are used as general disinfectants, but as a rule they are not sufficiently powerful to be of much practical value.

a. Iodoform.—Formerly much used as a dressing for syphilitic ulcers. It is not germicidal but has decided aseptic and sedative properties, hence also used in scalds and burns. It may, however, cause dermatitis. It is insoluble in water but freely soluble in ether and alcohol. The ointment (containing 10 per cent. iodoform) is still much used. Aristol, europhen, iodol, losophen and nosophen are iodoform derivatives, have similar

properties, less odorous, less irritating and less poisonous. The persistent disagreeable odor of iodoform is a great objection to its use.

b. Boric Acid and Borax.—Boric acid is a very mild antiseptic and hence is of little practical value as an active germicide but it is a good mild antiseptic. It can be applied to comparatively aseptic cuts, bruises, wounds, etc., in saturated solution (aqueous) or in powder. It can be applied as a dusting powder to many conditions where a mild antiseptic is indicated. In saturated solution it makes a good gargle, mouth wash, eye wash, etc.

Borax is similarly used and has similar properties. The choice between the two is decided by the difference in reaction. Boric acid is slightly acid in reaction, whereas borax is slightly alkaline. The preparation boroglycerin is much used as a dressing for inflamed and infected mucous membranes.

Sixteen grains of salicylic acid and 96 grains of boric acid dissolved in a pint of sterile water makes Thiersh's fluid. This is useful in cleansing mucous membranes, such as those of the mouth, nose and eye, and it may be used in the form of irrigations for cleansing purposes.

c. Creosote.—This excellent germicide is rarely used for general external disinfection though it is more active than phenol and does not coagulate albumen and is less toxic and less irritating. In doses of from 1 to 10 minims (given internally) it is much used as an antiseptic and stimulant in tuberculosis and to correct intestinal fermentation. The carbonate of creosote is said to be especially efficacious in lung troubles (tuberculosis). Creosote is essentially an intestinal antiseptic.

d. Hydrogen Dioxide.—This is the most active of the oxidizing disinfectants, used in solutions of from 10 to 15 per cent. It is a very active bleaching and deodorizing agent. It is not used for general disinfection but is one of the best known local germicides, applied to abscesses, ulcers, used as a spray, as a gargle, etc. Much employed in dental work. Used by bacteriologists to determine the amount of bacteria in milk (indicated by gas liberation when added to the milk in fermentation tubes).

e. Naphthalene Derivatives.—These are used as intestinal antiseptics but are of doubtful value in the treatment of intestinal diseases. They are not acted upon in the stomach secretions but on reaching the intestinal tract they undergo a chemical change and act as antiseptics. Their prolonged use produces irritation of intestines, bladder and kidneys. To this group belong betanaphthol, betol, naphthol, naphthalin, and others.

To the group of so-called intestinal antiseptics belong antipyrin, acetanilid, phenacetin, phenecol, quinine, salicylic acid, salol, salophen, guaiacol, resorcin and many other substances. Their value as intestinal antiseptics is very problematical and doubtful.

f. So-called Respiratory Antiseptics.—There are a great variety of

volatile or gaseous substances which are said to act as antiseptics to the respiratory tract when inhaled, as oil of thyme, eucalyptol, oil of eucalyptus, menthol, camphor, euthymol, campho-phenique, mint oil, etc., but their value in this regard is *nil*. They may have some stimulating effect upon the tissues of the respiratory tract but they do not destroy any germs which may be present upon or within the cells of the respiratory passages.

The following table taken from the work by Ellis gives the minimum proportion of germicidal activity of well-known disinfectants. The figures indicate the strength of solution necessary to prevent bacterial development when added to substances capable of giving rise to bacterial growth. The figures are not absolute for reasons which have been fully set forth in the beginning of this chapter. The table is merely a guide to the relative activity of the germicides named.

1. *Very active antiseptics.*	
Mercuric iodide,	1–40000
Silver iodide,	1–33000
Mercuric chloride,	1–14300
Silver nitrate,	1–12500
2. *Active antiseptics.*	
Osmic acid,	1–6666
Chromic acid,	1–5000
Chlorine,	1–4000
Iodine,	1–4000
Chloride of gold,	1–4000
Bichloride of platinum,	1–3333
Hydrocyanic acid,	1–2500
Bromine,	1–1666
Copper chloride,	1–1428
Thymol,	1–1340
Copper sulphate,	1–1111
Salicylic acid,	1–1000
3. *Fair antiseptics.*	
Potassium bichromate,	1–909
Potassium cyanide,	1–909
Ammonia,	1–714
Zinc chloride,	1–526
Mineral acids,	1–500
Lead chloride,	1–500
Nitrate of cobalt,	1–500
Carbolic acid,	1–333
Potassium permanganate,	1–285
Lead nitrate,	1–277
Alum,	1–222
Tannin,	1–207

4. *Indifferent antiseptics.*

Arsenious acid,	1–166
Boric acid,	1–143
Arsenite of soda,	1–111
Hydrate of chloral,	1–107
Salicylate of soda,	1–100
Iron sulphate,	1–90
Caustic acid,	1–56

5. *Feeble antiseptics.*

Calcium chloride,	1–25
Sodium borate,	1–14
Alcohol,	1–10

6. *Very feeble antiseptics.*

Ammonium chloride,	1–9
Potassium iodide,	1–7
Sodium chloride,	1–6
Glycerin,	1–4
Ammonium sulphate,	1–4

The following table gives the efficiency value of some disinfectants. It will be seen that this value is of necessity variable, depending upon the variation in the market price of the disinfectants. In will also be seen that in the proposed rating the remarkably high coagulation coefficient of some of the more important chemical disinfectants lowers the efficiency value greatly.

The efficiency value of any disinfectant is found by dividing the phenol coefficient by the other coefficients as follows:

$$\frac{\text{Phenol coefficient}}{\text{Tox. coefficient} + \text{Coag. coefficient} + \text{Comp. cost}} = \text{Efficiency value}$$

In the table the first figure in the comparative cost column (4th column) is the market price per pound of the disinfectant and the second figure is the comparative cost (compared with phenol at 15 cents per pound).[1]

[1] The comparative cost is a variable quantity. The present cost of phenol is much higher than 15 cents per pound. The above figures would have to be revised to make them applicable to the prevailing market price for the several disinfectants.

EFFICIENCY VALUE OF A FEW DISINFECTANTS

Name of disinfectant	Phenol coeff.	Tox. coeff.	Coag. coeff.	Comp. cost	Eff. value	Special properties
Phenol	1.00	1.00	1.00	0.15 1.00	1.00	Odor.
Boracic acid	0.23	0.05	0.00	0.15 1.00	0.20	Odorless.
Chloronaphtholeum	6.06	0.16	0.00	0.15 1.00	5.22	Odor.
Copper sulphate	0.50	0.30	750	0.20 1.20	0.007	Odorless. Slight color.
Lysol	2.12	0.45	0.00	0.65 4.33	0.44	Odorless.
Mercuric chloride	43.00	50	650	1.27 8.46	0.06	Odorless.
Neko	20.00	0.30	0.00	0.50 3.33	5.66	Odor.
Potassium permanganate	0.85	0.50	0.00	0.25 1.66	0.04	Odorless. Deodorant. Color. Stains
Silver nitrate	38.00	3.00	475	5.64 37.60	0.075	Odorless. Stains.
Trikresol	2.62	0.90	0.00	0.40 2.66	0.73	Odor.

THE WORTH HALE TOXICITY COEFFICIENT

Worth Hale of the U. S. Public Health Service Hygienic Laboratory has worked out a method for determining the comparative toxicity of disinfectants of which the following is a brief summarized outline.

Test Animals.—The animals upon which the substance in question is to be tested shall be white mice of not less than 15 nor more than 30 grams weight.

Dilutions and Dosage.—The dose is to be calculated per gram of body weight and should when diluted equal between 0.03 and 0.04 c.c. per gram weight; that is, 0.6 to 0.8 c.c. for a 20-gram mouse. The diluent is to be distilled water and primary dilutions are to be made of such strength that the dose is easily measured with a 1 c.c. pipette graduated into hun-

dredths. This is most easily accomplished by the use of the substance in greater concentration than is required to kill in the above volume doses.

Administration of the Test Solutions.—After the required dose of the diluted disinfectant has been estimated it is measured into a suitable dish and is then diluted further to the required volume by adding sterile distilled water in sufficient quantity. A series of mice are then injected subcutaneously with varying amounts of the substance until the least fatal dose (L.F.D.) is determined, the mice being kept under observation.

Time Limit of the Observation.—After the animals have been inoculated they are kept under observation for a period of 24 hours unless death results in a shorter period of time.

Phenol Comparative Test.—Mice of the same lot are similarly injected with pure phenol properly diluted to make the measurements of the dose easy and then further diluted in a small dish to equal a volume dose of 0.03 to 0.04 c.c. per gram of body weight and the fatal dose determined as above. This least fatal dose (L.F.D.) of phenol is unity and the least fatal dose of the substance in question is estimated in per cent. of this.

Determining the Comparative Toxicity.—The phenol toxicity of the disinfectant tested is to the toxicity of phenol as x is to 100. The example given below would be represented in the following proportion—$4.5 : 18 :: x : 100 = 25$ per cent., that is disinfectant "A" is one-fourth as toxic as is pure phenol.

Name of disinfectant	Mouse weight	Dose per gram body weight	Result	Time, H. m.
Disinfectant "A"	21.15	0.0012	Survived	
	20.64	0.0016	Survived	
	18.32	0.0018	Died	10:30
	19.05	0.0020	Died	2:15
Pure phenol	18.46	0.0035	Survived	
	20.10	0.0040	Survived	
	19.23	0.0045	Died	1:15
	18.90	0.0050	Died	0:25

Valuable information regarding the comparative toxicity of many of the substances used as disinfectants may be obtained from a study of the comparative medicinal doses. For example, the doses of phenol, betol, resorcinol and corrosive sublimate are 1 grain, 3 grains, 4 grains and 1/30 grain respectively. These doses are practically in proportion to the toxicity of the substances named and stating the dosage in the terms of

the phenol toxicity coefficient as proposed by Hale, we would get the following results.

Phenol	100
Betol	33.3
Resorcinol	25
Corrosive sublimate	3000

As a rule, however, the exact composition of disinfectants is either not known or is not disclosed by the manufacturers and in such cases the only thing to be done in order to ascertain whether or not the claims of the manufacturers are correct is to make tests as above outlined. However, in cases where the composition of the disinfectant is definitely known, whether a simple or compound substance, its comparative toxicity can be determined by ascertaining the toxicity of the ingredients and rating with the standard, namely pure phenol.

The Toxicity and Germ Destroying Power of Some Disinfectants

The values given are obtained from various sources and in some instances require further verification. The table will serve as a guide to a valuation of the disinfectants for purposes of general disinfection.

GERM DESTROYING POWER AND TOXICITY OF DISINFECTANTS

Name	Destroying Phenol 1	Toxicity Phenol 100
Alcohol	0.03	0.05
Alum	0.64	10.00
Ammonia	2.40	15.00
Ammonium chloride	0.03	10.50
Ammonium sulphate	0.015	5.00
Antozone	0.00	——
Arsenious acid	0.50	5000.00
Arsenite of soda	0.33	3000.00
Bacterol	1.58	45.00
Benetol	1.23	33.00
Bichloride of platinum	10.00	——
Boracic acid	0.23	5.00
Bromine	5.00	——
Cabot's sulpho-naphthol	3.87	11.00
Calcium chloride	0.08	3.50
Camphor	——	33.30
Carbolene	1.36	11.00
Carbolozone	1.48	6.40
Car-sul	2.00	16.00
Caustic acid	0.17	120.00
Chinosol	0.95	25.00
Chloride of gold	12.50	——
Chlorine	12.50	——

GERM DESTROYING POWER AND TOXICITY OF DISISNFECTANTS (*Continued*).

Name	Destroying Phenol 1	Toxicity Phenol 100
Chloro-naphtholeum	6.06	16.00
Chromic acid	15.00	100.00
Copper sulphate	0.50	30.00
Corrosive sublimate	43.00	3000.00
Cre-bol-you	——	9.00
Cremolene	1.26	——
Creo-carboline disinfectant	4.00	30.00
Creola	0.52	12.80
Creolin (Pearson)	3.25	18.00
Creoleum (Dusenberry)	1.00	9.00
Creolol (Rudish's)	1.24	13.00
Creo-Sul	——	15.00
Creosol (Saponified)	1.03	6.40
Cresoleum	2.90	11.00
Cresylone	——	56.00
Crude carbolic acid	2.75	90.00
Cupric chloride	4.20	80.00
Cyllin	11.00	40.00
Dioxygen	0.02	——
Electrozone	0.90	——
Ether	——	0.50
Ferrous sulphate	0.27	40.00
Formacone liquid	0.04	——
Formaldehyde	0.30	75.00
Germol	2.12	16.00
Glycerin (sp. g. 1.25)	0.015	0.50
Hycol	12.30	32.00
Hydrate of chloral	0.32	10.00
Hydrocyanic acid	7.50	10000.00
Hydrogen peroxide	6.30	2.00
Hygeno A	3.56	17.00
Iodine	12.50	400.00
Iron sulphate	0.27	40.00
Izal	8.00	40.00
Killitol	0.02	——
Kreosota	1.26	5.60
Kreotas	1.10	5.60
Kreso	3.92	22.50
Kresolig	2.18	56.00
Kretol	0.92	14.00
Lead chloride	1.50	200.00
Lead nitrate	0.83	300.00
Liquid creoleum	——	2.00
Liq. cres. comp. U. S. P.	3.00	56.00
Lincoln disinfectant	1.48	17.00
Lisapol	——	50.00
Listerine	0.01	0.20
Lysol	2.12	45.00

GERM DESTROYING POWER AND TOXICITY OF DISINFECTANTS
(*Continued*)

Name	Destroying Phenol 1	Toxicity Phenol 100
Mercuric chloride	43.00	5000.00
Mercuric iodide	120.00	1000.00
Milkol	——	11.00
Mineral acids	1–1.50	120.00
Naphthalene	2.50	7.50
Naphthol phenoline	6.40	6.40
Neko	20.00	30.00
Nitrate of cobalt	1.50	——
Noncarbolic disinfectant	——	7.50
Osmic acid	20.00	1000.00
Phenaco	15.00	32.00
Phenol (pure)	1.00	100.00
Phenol disinfectant	0.61	——
Phen. disin. & cleans	——	7.50
Phenol liquid U. S. P	1.77	80.00
Phenol sodique	0.01	4.50
Phenosote	3.43	19.00
Phenotas disinfectant	1.37	9.00
Pi-ne-ex	——	10.00
Pino-lyptol	0.27	3.20
Platt's chlorides	0.01	——
Potassium bichromate	3.00	500.00
Potassium cyanide	3.00	1500.00
Potassium iodide	0.02	5.00
Potassium permanganate	0.85	25.00
Public health dis	0.48	——
R. R. Roger's disinfec	3.03	56.00
Salicylate of soda	0.30	5.00
Salicylic acid	3.20	6.50
Sanax	0.22	22.00
Sanitas	0.30	——
Saponified cresol	1.03	6.00
Silver nitrate	38.00	300.00
Sodium borate	0.04	5.00
Sodium chloride	0.02	0.05
Sulpho-naphthol	3.87	15.00
Tarola	3.12	16.00
Trikresol	2.62	90.00
20th Cent. disinfectant	0.13	10.00
Veriform germicide	0.43	15.00
Victor sanitary fluid	——	13.00
Wescol disinfectant	——	22.00
Worrel's disinfectant	0.01	——
Zenoleum	2.25	19.00
Zinc chloride	1.56	100.00
Zodane	0.04	——
Zodone (4)	1.62	8.60
Zonol	2.37	10.00

3. Procedures for Disinfection

A. *Surgical Disinfection.*—a. The operating room must be clean and free from pathogenic and other objectionable organisms. The room must therefore be disinfected from time to time, after the method of procedure for any room which may be assumed to be infected, as will be explained under room and house disinfection. As to when, how often or how completely the operating room is to be disinfected that must be left to the judgment of the surgeon in charge.

b. Surgeons should be especially careful regarding personal cleanliness, irrespective of the routine personal disinfection and sterilization performed preparatory to an operation. They should always be smooth-shaven as the beard is a carrier of germs.

c. On preparing for an operation the surgeon removes coat, cuffs and collar in an ante-room; rolls up shirt sleeves and proceeds to wash and scrub hands with tincture of green soap, then in 1–1.5 per cent. tinct. cres. comp. U. S. P. or lysol, rinse in sterile water, dry with a clean sterile towel and dip in 50 to 60 per cent. alcohol. Formalin and carbolic acid should not be used as hand disinfectants (by the surgeon) because of the benumbing effects of these chemicals, causing a lessening in the delicacy of touch. A 1 per cent. solution of potassium permanganate is recommended as a disinfectant for hands. In many hospitals nothing more than a thorough scrubbing with green soap is employed for the hands of surgeons, with wholly satisfactory results.

Before entering the operating room the surgeon and attendants don sterilized gowns with hoods covering head, hair, and face (beard), leaving only the mouth, nose and eyes free. The hands of the attendants are covered with sterilized rubber gloves.

d. The surgical instruments are washed and wiped dry; boiled for ten minutes, in water with 1 per cent. soda, and laid in a tray containing 5 per cent. carbolic acid solution. Before using, they are rinsed in boiled distilled water. Never sterilize metallic instruments in corrosive sublimate, or in any corrosive disinfectants of any kind. Only a short exposure would suffice to dull the keen edge of knives, scalpels, and other cutting instruments. Do not sterilize steel instruments in hot air as high temperatures reduce the temper, and do not sterilize them placed with rubber goods.

B. *Sick Room Disinfection.*—Disinfection in the sick room of a patient afflicted with some communicable disease, may be divided into disinfection of dejecta, urine and sputum; disinfection of the patient; disinfection of clothing and bedding; disinfection of the sick room itself; and precautionary disinfection of the attending physicians, nurses and attendants. In case of fatal termination of the malady there is included

disinfection after postmortem and sterilization of the dead body. In all cases, whether the patient dies or recovers, the entire sick room, including bed, chairs, bedding, etc., must be thoroughly disinfected. The methods of procedure may be outlined as follows:

a. *Disinfection of Excreta.*—To disinfect dejecta, urine and sputum, a 4 per cent. solution of chloride of lime or a 20 per cent. solution of milk of lime will be found very efficient, using amounts of the disinfectants equal to the bulk of the excreta to be disinfected, mixing well and allowing to stand for one hour. The disinfectants are first placed in the veesels intended to receive the excreta, more being added afterward if it is thought desirable. If sputa and other excreta are received upon napkins or other cloth, these should be burnt at once, or if that is not convenient they may be placed (entirely immersed) in the disinfectant. For tuberculous sputum the chloride of lime is best. Spit cups should be kept two-thirds full of the 4 per cent. soluton. Paper spit cups are to be destroyed by burning as soon as possible.

Sulphate of copper (5 to 10 per cent. solution), or carbo-hydrochloric acid solution (5 per cent. each of phenol and hydrochloric acid) may be used in place of the chloride of lime and milk of lime. Bichloride of mercury and phenol (without the hydrochloric acid) are not very satisfactory for disinfecting excreta because of the coagulating effects upon albuminous matter. Liquor cres. comp. U. S. P., lysol and tricresol (2–2.5 per cent. solutions) may be used. Weak disinfectants or untried patent or proprietary disinfectants should never be used for above purposes. For example, permanganate of potassium, boric acid, borax, glycothymoline, borol, etc., would be valueless as disinfectants for excreta.

b. *Disinfection of Patient.*—This includes cleaning the body surface with soap and water, with 50 to 70 per cent. alcohol, washing with 1 to 2.5 per cent. solutions of phenol, cres. comp., lysol or creolin, when so ordered by the attending physician. Bichloride of mercury (1–2000 to 1–1000) may be used for skin disinfection. A saturated solution of boric acid, normal salt solution or a 1–1000 solution of permanganate of potassium may be used as a wash or irrigation for non-infected wounds and cuts, etc., but not for ulcers, abscesses, etc.

Irritating disinfectants should not, for very obvious reasons, be used. In every case the mode of procedure in the disinfection of the patient will be outlined by the attending physician.

Nurses, attendants and physicians must observe the necessary precautions against becoming disseminators of the infection and must resort to certain methods of self disinfection after each visit to the patient, as in small-pox, plague, diphtheria and other communicable diseases.

c. *Disinfection of the Clothing Worn by the Patient and of the Bedding.*—All clothing worn by the patient and all bedding, as soon as ordered changed, should at once be immersed in a hot, 5 per cent. solution of carbolic acid or a 2.5 per cent. solution of cres. comp. or lysol. After soaking for several hours the clothing should be boiled in water for 30 minutes at least. After thorough drying, perferably in the sun, the clothing should be well ironed. The ironing process in itself has very marked germicidal powers. Clothing may also be disinfected in formalin (4 per cent.). Sulphate of copper and sulphate of iron discolor and corrode the cloth. All cloth fabrics and clothing which has been in close contact with a patient suffering from diphtheria, cholera, plague or smallpox, should be destroyed by burning whenever feasible.

d. *Disinfection of the Sick Room.*—The bed frame, the chairs and other wooden furniture, the floor and the wood work of the room, may be washed or wiped with corrosive sublimate (1–1000), formalin (3–4 per cent.) or phenol (5 per cent.), if contamination is suspected or if so ordered by the physician, even while the room is still occupied by the patient.

Just as soon as the patient is taken from the room, a thorough disinfection should be carried out at once, the disinfection including furniture, clothing of the patient, bedding, mattresses, pillows, etc., excepting such articles as are ordered destroyed by burning.

Every pharmacist should fully inform himself regarding the state laws and city ordinances governing health and quarantine regulations. State and city boards of health usually issue free bulletins on methods of disinfection in communicable diseases. Copies of these should be on hand for ready reference.

For room disinfection, formalin or sulphur are used. With formalin the procedure is as follows: For every 1000 cubic feet of space there is required one pint of formaldehyde (the 40 per cent. commercial formalin) and 8 ounces of commercial potassium permanganate. Place the permanganate in an agate lined or iron pail of about ten times the capacity of the disinfectant to be used, spreading the permanganate evenly over the bottom. Set pail containing the crystals upon a brick, iron stand or other support, in a tub, pan or dish partially filled with water. See that windows and doors are closed and sealed (excepting the exit). The room should be warm and moist, a condition which may be effected by suspending sheets wrung out of hot water about the room. In a steam heated flat, steam may be allowed to escape from the air vent of a radiator; or steam may be generated outside of the room and conducted into it by means of rubber tubing. Do not have an open fire or flame in the room to be disinfected as the gas to be liberated is somewhat inflammable. Having ascertained that all is in readiness, pour the formalin

solution from a dipper or wide mouthed vessel over the permanganate; leave the room at once, close and seal exit, plugging key hole and crevices in door. Eighty per cent. of the gas is liberated within ten minutes or less. Leave the room sealed for at least six hours, preferably twelve hours. At the end of this time disinfection is complete. Open doors and windows. Traces of formalin may be destroyed by sprinkling or spraying ammonia in the room.

It is advised to use a separate container for every pint of formalin used. A large piece of matting or other absorptive material may be placed under each container to guard against the possibility of staining the floor, in case the floor requires such protection.

In case sulphur is used, prepare the room (as to sealing, air moisture and warmth) as for formalin disinfection, taking the precaution to remove (and disinfect separately, by means of formalin and bichloride of mercury) paintings, clothing and other fabric which must not be bleached by the sulphurous acid fumes. For every 1000 cubic feet of space, use 3.5 pounds of flower of sulphur. Place the sulphur on a bed of sand or on ashes in an iron pot or pan which is supported on a brick or iron stand in a dish of water. Pour a little alcohol over the sulphur and ignite.

Sulphur candles are now found upon the market and are more convenient than sulphur. Place a sufficient number of the candles upon bricks in pans of water and light them. Liquefied sulphur dioxide put up in convenient containers may be employed, using 15 ounces to each 1000 cubic feet. Open the can by means of a can opener, set it in a pan or dish and allow the gas to evaporate.

Remember that the sulphur dioxide corrodes metal, bleaches clothing, hangings and draperies and, like formalin, is without disinfecting power in the absence of moisture.

After the disinfection with formalin or sulphur dioxide is completed, it is often desirable to go over the floors, furniture, bed frames, etc., with a 1–1000 bichloride of mercury solution.

Mattresses, heavy quilts, pillows and furniture cushions are difficult to disinfect with formalin or sulphur dioxide. These should be disinfected by steam under pressure. In such diseases as plague, diphtheria and cholera, such articles should be destroyed by burning. Anyway, a sick room should have simple furniture and merely such articles as are absolutely necessary and such as can be disinfected readily.

The so-called carbo-gasoline method of book disinfection is highly recommended. Immerse books, papers, clothing and other articles to be disinfected for twenty minutes in the carbolized gasoline. Take from the disinfecting solution and allow to dry in the open. The carbolized gasoline consists of Baume 88° gasoline or gas machine gasoline to which

2 per cent. of carbolic acid is added. No injury is done to the books or clothing, provided they are carefully handled until dry. Gasoline will, however, injure oil paint lettering, etc.

D. *Postmortem Disinfection and Sterilization of Cadaver.*—After autopsies on bodies after infectious disease, thorough disinfection must be resorted to. A liberal use of a 4 per cent. solution of calcic hypochlorite, allowing this to act for at least one hour, will serve the purpose.

In cases of death from contagious diseases all orifices of the body should be packed with cotton well soaked in a 1–500 bichloride solution. The entire body should be washed with 1–1000 bichloride solution. Cremation is desirable and the funeral should be private.

The so-called embalming fluid of funeral directors are aqueous solutions of various chemical disinfectants, having corrosive sublimate and formalin as the chief ingredients. The following formula is said to have the approval of the National Funeral Directors Association of the United States:

Formalin (40 per cent.)	11	lb.
Glycerin	4	lb.
Borax	2.5	lb.
Boric acid	1	lb.
Potassium nitrate	2.5	lb.
Solution of eosin (1 per cent.)	1	oz.
Water, to make	10	gal.

The salts are dissolved in six gallons of water; the glycerin, formalin and eosin added and enough water to make up the ten gallons.

E. *Disinfection of Public Buildings and Public Conveyances.*—Only rarely will it become necessary to disinfect an entire large building, whether private or public, and then the method of procedure is much the same as for the sick room disinfection, already described, treating each room as though it were independent of other rooms, excepting that inner connecting rooms need not be closed and sealed.

In disinfection, one important fact should never be lost sight of, namely, that it is just as important to destroy the carriers of disease (flies, fleas, rats, mice, and other animals), as the disease germs themselves. This is particularly important in public disinfection, so much so that it is a general rule to always use a disinfectant which destroys the disease carriers, as sulphur dioxide. In the yellow fever district, for example, the chief fumigating agent is burning Pyrethrum which is a sure death to the Aedes mosquito as well as to other insects.

Wherever and whenever practical therefore, sulphur dioxide should be used for public disinfection. In many European cities, the health department is provided with portable generators which are run alongside the

building to be disinfected, the sulphur dioxide generated and conducted into the room, hall, cellar, or area way to be disinfected, by means of tubing. This is the safest and most satisfactory way. If such apparatus is not available, the flower of sulphur, sulphur candles, or liquefied sulphur dioxide may be used (15 ounces to each 1000 cubic feet of space). Street cars, railway cars, large public conveyances generally, may be disinfected much like rooms, after being well sealed. A safe rule is to use double quantities of the disinfectant for public conveyances, as compared with a sick room, because of the fact that it is difficult to seal such public conveyances well. After the disinfectant has acted for a sufficient length of time (twelve to twenty-four hours), the place is opened, aired and then all of the wood work (of furnishings as well as the floor, walls and ceiling) is either washed or sprayed with a 1–1000 bichloride of mercury solution or a 3–5 per cent. formalin solution.

In such communicable diseases as have no animal carriers (other than the patient himself) or where for obvious reasons such carriers are not present, formalin will always be the preferred disinfectant, whether for private or public disinfection, bearing in mind that heat and moisture are necessary adjuncts to its use. *Formaldehyde is not effective in a dry, cold atmosphere* because under those conditions the formalin is converted into solid polymerized paraformaldehyde, which as such, is inert.

Public or private disinfection by means of formalin may be carried out as follows, the method selected depending upon time, place and opportunity.

a. *Wet Blanket Method.*—Immerse blankets or sheets in the formalin solution and suspend them about the room to be disinfected. The room may first be sprayed with a hot 4 per cent. solution of formalin which furnishes warmth and moisture. The operator must work rapidly as formalin is very irritating to eyes and respiratory tract.

b. *Methyl Alcohol Lamps.*—Formalin may be generated in the space to be disinfected by oxidizing the methyl alcohol and converting it into formaldehyde. Lamps of special construction are necessary. The vapor of methyl alcohol is passed over a highly heated plate whereupon it is oxidized into formaldehyde ($CH_3OH+O=HCHO+H_2O$) with liberation of water. This method of disinfection is now rarely employed.

c. *Sanitary Construction Company's Lamp.*—The mechanism consists of a tank to hold the formalin, connected with a spiral tube through which the solution is slowly passed through a flame. The heat vaporizes the formalin which is then conducted into the room (through the key hole) by means of suitable tubing. This apparatus is much used by health officers.

d. *The Shering Lamp.*—These small compact and most convenient

lamps can be secured from any wholesale drug supply house. With this apparatus the solid tablets of paraform or paraformaldehyde are used. The heat from the lamp decomposes the tablets, producing formaldehyde. The lamps are placed in position, in sufficient numbers, lighted and the small tray of each lamp is supplied with a sufficient number of tablets. As a precautionary measure each lamp should be placed on a brick in a pan or dish of water. The air in the room must be warm and moist.

e. *Formaldehyde Candles.*—These consist of a mixture of paraformaldehyde and paraffin, wax, tallow or other combustible, which may be moulded into candles. The candles are placed in a fireproof dish or pan and ignited. For room disinfection these candles are most convenient as well as satisfactory.

F. *Disinfection at Quarantine Stations.*—All civilized nations maintain a system of vigilance as a protection against the introduction, from foreign countries, of certain communicable diseases designated as quarantinable. The first disease against which a quarantine was established was the plague. In the fourteenth century certain Italian cities established a quarantine against this dread disease and the word "Quarantine" came into general use because of the fact that the period of detention was about forty days (Ital. *quarantina*). The actual period of detention as now enforced varies somewhat depending upon the nature of the disease against which the detention is maintained, as determined by the period of incubation. The quarantinable diseases recognized by the United States are plague (bubonic), small-pox, yellow fever, Asiatic cholera, leprosy and typhus.[1] The enforcement of the quarantine regulations is under the direction of the U. S. Public Health Service. The most important quarantine stations in the United States are at San Francisco, New Orleans, New York and Boston, ranking in importance in the order named. The Station at San Francisco is of special importance because upon its efficiency depends very largely the exclusion of plague, cholera and small-pox, the three highly communicable diseases so prevalent in the Orient. Of course a national quarantine to be effective must be complete, covering every port of entry, whether large or small, maritime or inland. This is very often not the case and as a result an epidemic may enter *via* a minor port where the service is inadequate due to incompetent or insufficient inspection.

The quarantine officers are kept informed as to the occurrence of epidemics or sporadic cases of quarantinable diseases in foreign countries

[1] National quarantine against foreign disease is entirely distinct from state or city quarantine. The following diseases are recognized as quarantinable by most state boards of health: Scarlet fever (including scarlatina and scarlet rash), diphtheria (including membranous croup), small-pox, epidemic cerebro-spinal meningitis, anterior poliomyelitis, leprosy, and bubonic plague.

and port cities thus putting them on their guard as to the need of special vigilance regarding imports and immigration from such places or cities. However, every ship from a foreign port on arriving within the quarantine zone of the station is visited by the boarding officer who immediately proceeds to get data regarding the sanitary conditions on board, as to deaths, sickness of any kind, etc. All passengers, including the ship's crew, are lined up and inspected by the boarding officer. If nothing untoward is reported or detected the captain of the ship is given a clean bill of health and the vessel is permitted to dock and discharge passengers and cargo.

If however the boarding officer finds a case of small-pox or other quarantinable disease on board, the ship is anchored near the station; the passengers and crew are landed at the quarantine station and, with the aid of the ship's officers, the quarantine officer proceeds to disinfect all persons and their personal effects, the same class distinction (first cabin, second cabin, steerage, ship's crew) being maintained as on ship. Each day, as long as the quarantine lasts, all persons are examined by the chief officer of the station, to note, if possible the first manifestations of new cases. Just as soon as a new case is found the patient is at once taken care of in an isolated hospital. Suspects are kept under observation in an isolation camp.

All personal effects, including every bit of clothing worn, is disinfected in enormous double walled cylinders, by means of hot formalin laden steam under pressure. Sterilization is made absolutely complete without any injury to the clothing.

The ship with its cargo is next disinfected with sulphur dioxide gas generated in iron pots or pails placed upon sheets of tin. A little alcohol is poured over the sulphur, ignited, the exits closed down and kept closed for twelve hours. If the cargo contains combustible material as alcohol, oil, benzine, etc., the sulphur dioxide is generated upon a special boat or float which is run alongside and the fumes conducted into the hold of the ship to be disinfected. The sulphur fumes kill all organisms present, including fleas, rats and mice. In fact sulphuring of ships must be resorted to quite frequently for the sole purpose of killing rats and mice, even though there may have been no disease on board.

4. Purification and Sterilization of Water Supplies

Every city, town, hamlet and home should have an ample supply of pure water for drinking, cooking and cleansing purposes. Impure waters, that is waters which require sterilization in order to render them potable, are always dangerous. It is therefore of prime importance to secure a pure supply of water, sufficiently pure to make the work of sterilization and

purification wholly unnecessary; if that is not possible, and it generally is not, under our peculiar communal condition, then said questionable water supply should be thoroughly sterilized and purified, according to the most approved modern methods. We cannot condemn too strongly the generally prevalent methods of emptying the sewage of our cities and towns into rivers and lakes and then again supplying this sewage contaminated water to towns and cities for drinking and cooking purposes. There should be an efficient state board of health coöperating with a federal department, and there should be efficient and competent sanitary inspectors to look after the water supplies of private homes, of towns and in the country.

The suitability of water for drinking purposes is inversely proportional to the number of bacteria present. Pure spring or well water contains very few bacteria, rarely exceeding 50 per cc. Sewage contaminated water, which is still used for drinking and cooking purposes, may contain several million bacteria per cc. It has been proven time and again (statistically) that the mortality rate (due to disease) of cities is inversely proportional to the purity of the drinking water supply. It is self evident that water purification should be considered a subject of the utmost importance. It should receive more attention than it does.

The sedimentation and filtration method for removing dirt, sand and other coarser particles from the water supplies of large cities, is practised and has been practised for years in many of the European cities. This is satisfactory as far as it goes, but it does not go far enough. The filtering material used (sand, charcoal, etc.) does not remove bacteria and other small organisms, excepting those which are attached to the coarser particles remaining upon the filtering material. Furthermore, unless the filter is frequently changed or sterilized, the filtering material will become the breeding place of germs and thus contaminate the water still more.

Various chemical disinfectants have been tried, but most of them have proven unsatisfactory for various reasons. The use of high attenuations (1–5,000,000 to 1–50,000) of copper sulphate has been highly recommended, especially by the U. S. Dept. of Agriculture, and has in many instances given excellent results, especially in the destruction of low forms of algæ and protozoa. As a means of destroying bacterial life the method is, however, not a success. Dr. Kraemer and others recommend the use of copper foil or plates immersed in the water as a means of destroying pathogenic and other bacteria, but this method does not appear to have met with any general approval. Kraemer sums up the copper foil treatment of water as follows:

1. The intestinal bacteria, like colon and typhoid, are completely destroyed by placing clean copper foil in the water containing them.

2. The effects of colloidal copper and copper sulphate in the purification of drinking water are in a quantitative sense much like those of filtration, only the organisms are completely destroyed.

3. Pending the introduction of the copper treatment of water on a large scale the householder may avail himself of a method for the purifications of drinking water by the use of strips of copper foil about 3½ inches square to each quart of water, this being allowed to stand over night, or from six to eight hours, at the ordinary temperature, and then the water drawn off or the copper foil removed.

The alum method of purifying water has met with considerable success, but more recently the alum-sodium hypochlorite combination has proven more satisfactory. The alum coagulates and precipitates the organic impurities and the sodium hypochlorite, through its electric dissociation, acts as a germ destroyer. The coagulated and precipitated organic material holding most of the bacteria is then removed by filtration. The amount of chemicals used depends somewhat upon the degree of contamination. With highly contaminated waters it is customary to use 3.3 per cent. of alum as the coagulant, subsequently introducing 1.2 per cent. of the hypochlorite. The water is then filtered, whereupon it is ready for use.

Small quantities of drinking water may be purified as follows: Dissolve a level teaspoonful of powdered chloride of lime in a teacup of water. This solution is diluted with three cupfuls of water, and a teaspoonful of this mixture may be added to each two-gallon pail of drinking water. This will give 0.4 or 0.5 part of free chlorine to a million parts of water and will, in ten minutes, destroy all typhoid and colon bacilli or other dysentery-producing organisms in the water. Moreover, all traces of chlorine will disappear rapidly.

There are in use a number of methods for dissociating sodium hypochlorite by electricity. Some of them are patented and modifications thereof are in use by city water purification works, giving excellent results. Dr. C. P. Hoover, assistant chemist of the Columbus Board of Health, has the following to say regarding the process:

"There are two general types of electrolyzers for dissociating sodium chloride. In one the cathodic and anodic products are allowed to recombine in the main body of the electrolyte and in the other, known as the diaphragm process, the products are removed separately from the cell as produced.

"For the production of sodium hypochlorite the non-diaphragm process has been considered best because it dispenses with the destructible diaphragms and the loss of energy that all such diaphragms occasion.

"When a direct current of electricity is passed through a solution of sodium chloride, sodium is liberated at one pole and chlorine at the other.

The liberated sodium reacts on the water breaking it up into hydrogen and hydroxyl ions to form sodium hydrate. The sodium hydrate in turn combines with the chlorine to form sodium hypochlorite, (Na O Cl) which becomes active in the sterilization of the water."

Pharmacists find considerable demand for distilled water for drinking purposes as well as for use in dispensing. However, some of the leading authorities declare that drinking distilled water is objectionable, because of the disturbance of the osmotic pressure in the cells of the digestive tract. That is, the distilled water acts as a mechanical poison. There is an excessive endosmosis inducing an abnormal distention of the cells, causing physiological disturbances. This action is due to the fact that the mineral salts present in natural drinking water are absent in distilled water.

The pharmacist can prepare cheaply and simply a marketable drinking water which does not have the objectionable qualities above referred to. Instead of distilling the water, filter it, using a Pasteur-Chamberland filter. Whether a large or small filter is used will depend upon the number of customers to be supplied. In all probability a two- or three-tube filter is large enough for the average retail store. "Rapid safety filters" are of no value whatever, and should not be used, as they are in no sense germ-proof. They merely remove the coarse filth. It is true that the Pasteur-Chamberland filters are not absolutely germ-proof, but they remove most of the microbes present, as may be determined bacteriologically by the pharmacist himself. The few germs which may pass through the filter are killed by heating the water to the boiling-point or 30 minutes. Such filtered and heat sterilized water should be sold in large sterile glass or earthenware containers. It is more palatable than distilled water and does not interfere with the osmotic balance of cells.

5. Food Preservatives

The use of food preservatives is as old as the history of man. Since remotest antiquity man has found it necessary to accumulate a supply of food during the seasonal periods of plenty in order to tide over the periods of scarcity. The very first observation made was that the accumulated and stored food soon showed a tendency to undergo decomposition. The next observation no doubt was that under certain conditions some organic food kept better than under other conditions, thus, for example, primitive man gradually learned that sun-dried meats did not decompose nearly as quickly as undried meats. No doubt the value of smoking meats was soon ascertained, in all probability purely accidentally, from meats, etc., which had been exposed to the smoke of the camp fire. The preservative value of heat, as in cooking and roasting, was noted. Next, no doubt the preservative properties of certain chemicals used with foods, as ashes

from the camp fire, salt, brine, vinegar, wine (alcoholic beverages) and sugar was noted. Thus primitive man made use of the germicidal powers of sunlight, drying, dry heat, moist heat, wood ash, smoke, creosote (in smoking meats), salt solutions, acids (in vinegar) and alcohol, without having any idea as to why these agents retarded or prevented the decomposition of organic food substances.

In modern times the use of food preservatives is based upon the germ theory of decomposition. The time-honored preservatives above referred to have continued in use and many new ones have been added, as benzoic acid, sodium benzoate, boracic acid, borax, salicylic acid, sodium sulphite, sulphurous acid, formalin and many others. A somewhat generalized theoretical assumption is that the chemical preservatives in foods are more or less injurious to health. It cannot be denied that some of the preservatives used are irritating to the kidneys and skin and some perhaps interfere more or less with food digestion and assimilation. It has long been known, for example, that the prolonged consumption of salted meats produces serious skin affections designated as scurvy. The sulphites are irritating to the kidneys; formalin interferes with digestion of foods, etc. However, there can be little doubt that in the comparative sense it is far more conducive to health and longevity to eat preserved foods than foods which are more or less decomposed. We are daily making use of foods which contain small quantities of natural preservatives. Cranberries, for example, contain benzoic acid; formalin and phloroglucin are present in minute quantities in certain plants; a multitudinous variety of salts, acids, sugars, aromatic oils, etc., are present in food plants. Food chemists do not appear to be seriously worried about these natural preserving agents nor about the old-time artificial preservatives as smoke creosote, salt, brine, sugar, and vinegar, and it is reasonable to suppose that careful investigation will disclose new chemical preservatives which are superior to those mentioned. The whole discussion regarding artificially added chemical food preservatives will no doubt simmer down to the following: *What is the smallest amount of the least objectionable chemical food preservatives which must be added to certain food substances in order to preserve them until they are to be consumed?* Also the following correlative rule should hold good: *No chemical food preservatives whatsoever should be used as such excepting in cases where modern methods of heat and cold sterilization and preservation fail or are inapplicable.*

The use of sugar and of salt in moderation are, of course, always permissible, since these substances are essentials in many foods. The objection and danger in the use of food preservatives lie in the fact that careless manufacturers are too prone to use them in order to avoid employing harmless, though perhaps less simple, and more expensive means of food

preservation. Chemical preservatives make it possible for the unscrupulous to use decomposed and otherwise objectionable food material. Furthermore, there is a strong tendency to use chemical preservatives in excess, in spite of the strictest legal quantitative limitations.

The following is a brief summary of the more common food preservatives and their use.

The physical and mechanical means of food preservation have been referred to, likewise the use of heat, cold, smoke, etc. One of the most satisfactory methods of preserving foods, now employed in all up to date canneries, is a combination of heat sterilization with air exclusion (air pump and by displacement). The food products as meat, corn, beans, asparagus, peas, jams, jellies, preserves, etc., etc., are heated (100° C. to 120° C.) to destroy all germ life, the containers (tins, glass) are also heated and then nearly filled to exclude as much air (oxygen) as possible. Air (oxygen) is necessary for the growth of bacteria, yeasts and molds, hence a well filled container, with a minimum of oxygen is less likely to show decomposition effects ("swells," "leaks") than containers which are not well filled. It is claimed that wholesome fruit, meat, etc., (free from decomposition), which is well sterilized by steam heat and put up in well sterilized containers requires no chemical preservative whatever. It is, however, customary, in the case of fruits, to add sugar as a preservative and also for the purpose of rendering the article more palatable. The sugar from sugar cane is quite universally used in preference to the sugar from the sugar beet. This is no doubt due to the fact that sugar beet sugar contains slightly more organic impurities and is, hence, under similar methods of use as to quantity, degree of heat sterilization, etc., slightly more likely to undergo decomposition.

Preservation of food substances by drying is coming into use more and more. By this method it is possible to keep, for variable periods of time, a great variety of foods as apples, peaches, pears, bananas, potatoes and many other vegetables, besides bread, meats, eggs, milk and other substances, which were formerly more generally preserved by the canning method. Eggs may also be preserved entire by giving them a coating of tallow, wax, paraffin or soluble silicate, which exclude the air, or they may be preserved in brine, salt or other so-called harmless chemical preservative.

Herring, cod and other fish are often preserved in a brine of salt or of equal parts of salt and borax or boric acid. Of meats, fish is particularly liable to decomposition and it is declared that certain kinds cannot be preserved in salt alone, that it is necessary to add boric acid, rubbing the preservative well into incisions made along the spinal column where the decomposition develops earliest. Salt is used with meats generally and with butter. Two per cent. of salt in butter is sufficient, though as much as

15 per cent. and more is sometimes added to increase the weight. A combination of salt and saltpeter is added to meat (brine). The saltpeter gives a red tint to meat besides serving as a preservative. Saltpeter is considered more or less injurious to health, when taken with food to the amount of 0.5 of 1 per cent. or more.

Borax and boric acid is often added to milk. 4.4 grains to the pint (0.05 per cent.) keeps milk sweet for a time (10 to 14 hours and longer). Small doses of borax and boric acid (up to 1 gram per day) is considered harmless. Certain preservatives of a proprietary nature as "Preserving Salts," "Preservative," consist of borax and salt in the proportion of three to one.

Formalin (the 40 per cent. commercial solution) added to milk, to the amount of 1–50,000, retards souring for several hours; 1–10,000 prevents souring for twelve hours and longer, and in this amount it does perhaps very little harm, though it is believed, due to its coagulating effects, to interfere with the digestibility of milk, particularly in children. Several marketed milk preservatives have formalin for their principal ingredient ("milk-sweet," "iceline," "freezine").

Sulphurous acid and sulphites are added to vinegar, pickles, catsups, etc., anchovy pastes, canned and dried fruits, etc., to the amounts of 0.2 to 1.15 per cent. The part active as a preservative is the available SO_2 which is gradually oxidized into sulphates. These agents are deodorant, as well as preservative, because of the high oxidizing power.

Butchers use sulphite preservatives to dust over sausage meats for the double purpose of giving the meat a red color (due to the O combining with the hemaglobin of the blood) and to destroy possible odors of decomposition. 0.05 per cent. of sulphites is sufficient to check decomposition in fresh meats, though the best results follow the use of 0.5 per cent. 0.2 per cent. has germicidal powers when combined with cold. Sometimes aniline color is added to the sausage meat preservatives.

Sodium benzoate is perhaps the most extensively employed preservative and at the same time the least harmful. 0.1 per cent. added to food articles, as meats, fruits, catsups, vinegar, cider, etc., checks decomposition. Generally, however, more than 0.1 per cent. is added, from 0.2 to 0.5 per cent. The percentage of benzoate preservative is likely to vary because of its volatile nature; canners quite generally add an excess knowing that much of it will be carried off with the vapors escaping during the heating process. As a result it follows that products declared to contain 0.1 per cent. of benzoate may upon chemical examination show the actual amounts to range from a mere trace (0.05 per cent. to 0.5 per cent.).

Next to benzoate, salicylic acid is perhaps the most common food preservative, used much like benzoate, in strengths varying from .01 to 0.25

per cent. It is frequently added to beers, cordials, wines and foods (2 to 4 grains to the pint) containing sugars. It is also used as a surgical dressing, but other less irritating wound disinfectants are given the preference.

Crude pyroligneous acid is used as a meat preservative. This acid is obtained by the destructive distillation of wood and contains creosote and other tarry matter and imparts the odor and taste of smoked products. Meats, fish, etc., are immersed in a solution of this acid, dried and sold as smoked. This constitutes the "quick" or "dip" method of smoking meats as compared with the usual slower method of exposing the meats to the smoke of slowly burning wood.

The following are a few of the less commonly employed preservatives: Fluorine compounds are used in strengths of from 0.03 to 0.02 per cent. Alum is sometimes used in pickling vegetables and meats (brine) because of the hardening effects produced. Copper sulphate is much used in pickling cucumbers, peas, string beans and other green vegetables for the purpose of deepening the green color. Sodium and calcium carbonate are sometimes added to cider and wine to check the souring process (by combining with the fruit acids). Formic acid is a powerful preservative. 0.014 to 0.08 per cent. retards fermentation. Saccharin, sucrol and dulcin are sweetening as well as preserving agents. Peroxide of hydrogen is used as a preservative. It is also a deodorant. The use of saccharin in food is no longer permissible in the United States.

6. Insecticides and Other Pest Exterminators

The farmer, fruitgrower and florist have many enemies belonging to the insecta and to other divisions of the animal kingdom, which interfere with the productiveness of crops. The remedies employed against these pests are numerous. We shall mention only a few of the more useful ones, explaining their action very briefly. They may be grouped into powders, gases, sprays and washes.

A. *Powders.*—These may be applied by the "pepper box" method, the material being placed in a box, usually of tin, with perforations, through which the powder sifts on shaking. Or a blowing device may be used, like the ordinary bellows box for blowing insect powder, or modifications of this simple device. A third method known as the sifting method is much in vogue in the cotton fields. The powder is placed in a porous bag or cloth, fastened to a stick and shaken over the plants to be treated. Only three powders are used to any considerable extent, as follows:

a. *Slaked Lime.*—Dry air slaked lime is reduced to a uniformly fine powder which is then ready for use. It is very efficacious with all slimy animals, as slugs and snails. It is applied to plants when the pests are

active, that is, in the early morning or in the evening. Lime is used where paris green is not permissible, as with fruit plants and edible herbs.

b. *Sulphur.*—The flower of sulphur or ground sulphur is a very widely used remedy for fungous pests, as mildew; also for the red spider and thrips. Sulphur is active only in the sunlight, particularly on a hot day.

The flower of sulphur gives better results than the ground sulphur because it "sticks" better. It should be applied evenly and not too thickly. Remember that sulphur dioxide is very injurious to plants, therefore fumigation by burning sulphur is out of the question.

c. *Paris Green and Other Arsenicals.*—These are generally not used in the dry powdered form. When so used they are diluted with flour, dust or other inert powdered material. Must be sparingly applied and evenly distributed, otherwise serious damage may be done to the foliage.

B. *Gases.*—Gases diffuse with great rapidity and when applied within an enclosed space will, in a short time, be uniformly distributed throughout the enclosed space. The rapid diffusion of gases is a great hindrance to their practical utilization in the open as in orchards, fields and gardens. Their use is quite limited.

a. *Carbon Bisulphide.*—This is not used with growing plants though it is applied to stored seeds, and dry plants and grains, for the purpose of killing insects and other destructive animals. It is also used to kill pests which live in the soil, as the grape Phylloxera. For this purpose a machine is used which injects the bisulphide into the soil. To destroy pests in drug plants, seeds and grain, enclose them in a space, place a dish containing the bisulphide on top of the material. The vapor being heavier than the air, gravitates downward and soon fills the entire enclosed area. The amount necessary to do the work will depend upon the nature of the material to be treated and the tightness of the enclosure. Roughly estimated a dram of the carbon bisulphide to five pounds of the material is sufficient. Grainmen usually apply one pound to the ton of grain, if the bin is tight.

Carbon bisulphide is one of the most effective remedies against the gopher and the ground squirrel. Use the remedy after a rain as the soil is then less porous. Pour an ounce over a rag or other porous substance (horse droppings are much used), stuff this into the hole and plug with a ball of dirt. The bisulphide is also used to kill the yellow-jacket, which is very injurious to fruit, also the root crown borer of the peach, and to disinfect grapevine cuttings, etc., etc.

b. *Hydrocyanic Acid Gas.*—This is about the only gas which is powerful enough to kill insects and yet not injure the foliage. It is used by covering the tree, shrub or bush with a tent cloth or canvas which should be oiled to keep in the vapor. The vessel containing the chemicals is placed underneath. Exposure of from thirty to fifty minutes is usually sufficient.

About one ounce of potassium cyanide to 150 cubic feet of space is required.

The gas is extremely poisonous and is often destructive to foliage. It is preferably applied at night as it is then less injurious to the foliage.

C. *Sprays and Washes.*—Plant pests are most generally destroyed by spraying agents or washes. A wash is really a more liberal application of the spray, the two being alike as to the results to be attained from their use.

For low plants the remedy can be applied by means of a sprinkling can but the better method is to use some form of force pump with spray nozzle. A good spray pump should maintain a uniformly constant as well as adequate pressure, should be simple of construction, with all parts readily replaceable. The nozzle should break up the stream into a fine mist.

It is, of course, desirable to get as much as possible of the spray to remain on leaf or stem and to have it evenly distributed. If put on too abundantly the fine droplets or gobules on the leaf will run together and roll off to the ground. The nozzle must not be held near the plant to be sprayed in order to get the desirable dew-like deposit on the leaf.

For scale insects a thorough moistening is necessary, wetting the bark, the scale and eggs. In order to accomplish this the nozzle must be held close.

The following table by Woodworth will indicate the method of preparing and using the more important spraying solutions:

The well known Bordeaux mixture, so extensively used as a spray and wash is prepared as follows:

Water,	50 gal.
Copper sulphate,	6 lb.
Unslaked lime,	4 lb.

The adhesive properties can be increased by adding soft soap in quantity equal to that of the copper sulphate. It is also advisable to dilute the mixture for spring spraying. It is the most effective and perhaps the cheapest fungicide that can be used.

Aphides (plant lice) and similar plant parasites may also be destroyed with weak solutions of alum (1.5 to 2 per cent.). Beetles may be killed by sprinkling a mixture of equal parts of red lead, sugar and flour, near their hiding places, or a mixture of borax 20 parts and precipitated carbonate of baryta (native witherite will not answer the purpose). A great variety of substances are recommended for the extermination of ants, as borax, camphor, balsam of Peru, spraying with benzine, etc. In lawns and in the open generally (in ant hills) they are most quickly destroyed by means of carbon bisulphide. This kills the ants as well as the larvæ.

The exterminators for pests of all sorts is legion and those especially interested must consult some standard work on formulas such, as the Scientific American Cylcopedia of Formulas (Hopkins).

TABLE OF FORMULÆ FOR WASHES AND SPRAYS

Name	Strength desired	Ingredients	Per cent.	For 5 gallons (oil can)	For 40 gallons (barrel)	Directions for mixing
Lime, salt and sulphur. For peach worm, San Jose scale, etc.	For winter use only	Lime	9.00	3 lbs.	24 lbs.	Boil sulphur and one half the lime 1½ hours; mix and add other ingredients and boil ½ hour longer.
		Salt	3.00	1 lb.	8 lbs.	
		Sulphur	4.50	1½ lbs.	12 lbs.	
Sulphide of potash. For red spider.	For summer use	Potash	.80	4⅓ oz.	2 lbs.	Mix these with a very little salt and water and dilute after reaction is complete.
		Sulphur	.92	5 oz.	2½ lbs.	
Resin soap. For scale and other insects.	Usual strength	Resin	2.40	1 lb.	8 lbs.	Boil two hours; dilute with warm water.
		Caustic soda	.60	¼ lb.	2 lbs.	
		Fish oil	.30	2 oz.	1 pt.	
	Extra strong for winter use.	Resin	4.00	1½ lbs.	12 lbs.	
		Caustic soda	1.00	6 oz.	3 lbs.	
		Fish oil	.50	3 oz.	1½ lbs.	
Kerosene emulsion. For scale and other insects.	Weak for plant lice	Soap or milk sour.	.15	1 oz.	½ lbs.	Mix hot, with spray pump, 15 minutes; use either sour milk or a soap solution.
			2.50	1 pt.	1 gal.	
		Kerosene	5.00	2 pts.	2 gals.	
	Usual strength	Soap or milk sour.	.25	1½ oz.	¾ lb.	
			4.00	1½ pts.	1½ gals.	
		Kerosene	8.00	3 pts.	3 gals.	
	Usual strength	Soap	2.00	¾ lb.	6 lbs.	Mix 5 minutes.
		Kerosene	8.00	3 pts.	3 gals.	
Poison. For sleaf- and fruit-eating insect.	Usual strength	Paris green or other arsenical.	.12	¾ oz.	6 oz.	

Sufficient water is to be used in each case to make up the amount indicated in the next columns.

CHAPTER XVI

STERILIZATION AND DISINFECTION IN THE PHARMACY

It is only within very recent years that sterilization in the pharmacy has received any serious attention. Certain pharmacopœias, notably those of Austria and Belgium, give specific directions regarding the sterilization of certain medicamenta, particularly those intended for hypodermic use. The German, English, Italian, Swiss and other pharmacopœias give directions regarding certain sterilizing processes which may be applied to a few articles. Fischer, Stich, Deniges, Mario, Schoofs and other European investigators have given the subject much attention and have perfected many of the details of procedure.

Some of the non-official methods of sterilization are of very doubtful practicability. Particularly the methods recommended for the sterilization of pharmaceutical solutions by means of the ultra-violet rays and by means of chemical disinfectants. Lesure sums up the use of the ultra-violet rays as follows: "A series of experiments shows that, at present, the ultra-violet rays can scarcely be regarded as a practical means of sterilizing pharmaceutical solutions, such as hypodermic injections. It is not yet possible to sterilize liquids in small closed glass vessels, since the glass absorbs the rays of shortest wave length, which are precisely those of most active sterilizing power. Possibly on a large scale solutions could be sterilized in bulk and then filled, *in vacuo*, into sterilized small receivers. The rays might be useful for substances which are decomposed by treatment in the autoclave. Some substances are, however, so readily decomposed by ultra-violet rays, that their solutions can never be sterilized therewith. Such are solutions of quinine salts, of mercuric iodide, of atoxyl, of eserine, of apomorphine and some glucosides, as for example gentiopicrin. Opaque solutions and suspensions of solids cannot be thus sterilized. The permeability of the different solutions to the rays also varies very greatly. Apart from the question of decomposition, it is found that, in the case of gentiopicrin, completely sterile solutions were not obtained even after an exposure of half an hour; on the other hand ancubin solutions were completely sterilized in thirty seconds." The decomposition changes due to the ultra-violet rays are not clearly understood. The indications are that there are no very marked chemical changes in such substances as cocaine and pilocarpin hydrochloride after three hours' exposure. Arbutin shows a change in a few minutes. There is so much

uncertainty as to the results that the method cannot as yet be recommended for practical use.

The addition of disinfectants to medicines for purposes of sterilization has recently received some attention. The use of formaldehyde, ether, chloroform and alcohol, have been recommended, each having its special use in practice. The general criticisms made regarding the use of the ultraviolet rays also apply here. Currie recommends a formalin method as follows: applicable to infusions of calumba, gentian, quassia and senega. "The infusions of calumba and quassia are simply evaporated to one-eighth of their bulk, filtered, and 4 minims of the ordinary 40 per cent. solution of formaldehyde added to each fluid ounce of the concentrated infusion. On dispensing, the requisite amount is put in a shallow basin and brought sharply to the boil, thus dissipating the formaldehyde. The infusion is then diluted to the normal strength with sterilized distilled water. Infusion of gentian is made from gentian root alone, and concentrated. To this is added essence of lemon (1 in 10), and the official tincture of orange in the proportion of 2 fluid drams of the former and 1 fluid ounce of the latter to each pint of the infusion. There is also added 4 minims of 40 per cent. solution of formaldehyde to each fluid ounce of infusion. Infusion of senega is concentrated by evaporation and to prevent precipitation, 5 grains of potassium bicarbonate are added to each fluid ounce of the concentrated solution, and 4 minims of 40 per cent. solution of formaldehyde. In case of both gentian and senega infusion, the formaldehyde is dissipated at the time of dispensing, in the manner already described. The advantages of this process are ease of manipulation, cheapness, and the certainty of the antiseptic condition of the infusion while being kept in stock and until dispensed. The quantity of formaldehyde remaining in the diluted infusion is infinitesimal, and may be ignored for all practical purposes."

It is known that weak solutions of hypodermic and intravenous solutions, unless sterilized, will show numerous bacteria upon standing for a time. One per cent. solutions of pilocarpin, atropin, cocaine, morphine, and fluid-extract of ergot have been found to contain millions of bacteria per cc. However, 10 per cent. iodoform glycerin, camphorated oil (1 in 10, solutions of apomorphin (0.2 in 20), quinine (1 in 10), antipyrin (5 in 10), cocaine (10 per cent.) are usually quite free from bacteria. In a general way the bacterial content of medicinal solutions decreases directly with the degree of concentration. Pus microbes die at once in ether and in a saturated solution of quinine, whereas they remain active in a 10 per cent. solution of cocaine. A 2 per cent. solution of morphine kills pus microbes in twenty-four hours, while pure glycerin kills them only after an exposure of six to eight days.

A perfectly safe rule for the pharmacist is to consider all medicamenta which he handles and which he may be called upon to dispense, as being possibly contaminated and to sterilize and disinfect all articles which in his judgment as a qualified pharmacist may require such treatment, in so far as it is practically possible. The retail pharmacist must not place too much confidence in the assertions of comparatively little known manufacturers and wholesale houses, regarding the sterile conditions of the articles which they may supply.

The medicines found in a drug store and dispensed by the pharmacist may be grouped as follows:

A. Medicines which do not Generally Require Sterilization

a. For internal administration per mouth. They may be contaminated or may become contaminated on standing for a time. Such medicines should be rejected. Do not attempt to render them usable by sterilization.

b. Mouth washes and gargles.

c. Enemas. Enemas for young children and such enemas as are to be applied to inflamed or otherwise pathologic conditions of the intestinal mucous membrane, should be sterilized.

d. Medicamenta which are to be applied to the intact skin, or to the scalp.

B. Medicines Which Require Sterilization

a. Those intended for intravenous and hypodermic use. Not only must these be absolutely sterile but they must be in perfect solution, before using.

b. Those to be applied to cuts, bruises, abrasions, wounds, ulcers, sores, and to the broken skin generally.

c. Those to be applied to inflamed mucous membranes, as enemas, douches, etc.

d. Solutions for the irrigation of the bladder.

e. Eye medicines, as washes and other solutions, intended for direct application to the eye.

1. Methods of Sterilization

The following methods of sterilization are applicable in the pharmacy and should be consistently practised:

A. *Sterilization of Containers.*—The glassware and other containers used in the pharmacy should be cleaned and sterilized as follows:

a. *Bottles and Glassware Generally.*—Wash and rinse in warm water to remove dust, dirt, sand, straw, etc., then wash and rinse in hot water with 2 to 5 per cent. sodic hydrate. Neutralize the sodic hydrate by washing

and rinsing in 2 to 5 per cent. hydrochloric acid. Finally wash and rinse in hot sterile water and allow to drain. Wipe dry and plug lightly with cotton. Place the plugged bottles in a hot-air sterilizer and heat for one hour at 130° C. to 140° C. Keep these cleaned, sterilized, and cotton plugged bottles in clean container in a dry clean store-room, until wanted for use.

b. *Porcelain and Similar Containers.*—May be cleaned and sterilized like glassware. Plugging with cotton is as a rule inadmissible.

c. *Large Flasks, Jugs, Etc.*—Large containers are as a rule difficult to sterilize and for this very reason are often subject to special neglect. Proceed much as for bottles, observing greater caution as to changes in temperature. Large bottles, carboys and similar containers cannot be sterilized by means of boiling hot water as they are very apt to crack. They may be sterilized by means of carbolic acid (5 per cent.), lysol (1.5 per cent.) or formaldehyde (4 per cent.), then thoroughly rinsed in sterile water, allowed to drain, plugged with cotton, carefully heated in hot-air sterilizer for one hour or more at 115° to 120° C. Cool gradually.

d. *Tin Containers.*—Wash and rinse thoroughly in water; boil for thirty minutes, drain and dry and sterilize in dry-air sterilizer for one hour at 100° C.

B. *Sterilization of Apparatus and Tools.*—It is of the highest importance that mortar and pestle, spatulas, percolators, pill and suppository machines, mixing plates, etc., etc., should be clean and sterile. This means a liberal use of hot water, green or soft soap, and clean towels. The sink, the floor of the dispensing room, the tables, chairs, desks, in fact everything in and about the dispensing room should be scrupulously clean.

C. *Sterilization of Corks and Other Stoppers for Containers.*—It would be energy wasted to clean and sterilize the containers if the stoppers are not also clean and sterile. Sterilize corks by washing in hot 60 to 75 per cent. alcohol, drain and heat in hot-air sterilizer for one hour at 130° C. Keep these corks in sterilized wide-mouthed ground-glass capped bottles. Take out corks as wanted by means of a sterile pair of pincers, not by means of fingers. Other stoppers, as of glass, of wood, of rubber, must also be cleaned and sterilized. Rubber caps, rubber stoppers, and other rubber goods may be sterilized by boiling in water for thirty minutes.

D. *Sterilization of Surgical Supplies.*—a. Bandaging materials, cotton, absorbent gauze, etc., may be sterilized by wrapping in cheese cloth or filter paper, first placing a grain of fuchsin or other aniline dye in the center of the package (wrapped in paper or cloth), and sterilizing in steam for one hour. The dye particle is introduced as a test object to ascertain if the steam has penetrated the entire package. If it has penetrated the entire package it will be indicated by a spreading of the color. Afterward, dry

for one hour at 100° C. in the hot-air sterilizer. For this purpose the form of Arnold steam sterilizer shown in Fig. 18 will be found very useful.

b. Sewing materials, such as needles, forceps, catgut, etc., require careful sterilization before using. All metal instruments and appliances, including silver wire, can be sterilized in 5 per cent. carbolic acid if necessary or they may be boiled for 30 to 50 minutes. Wipe perfectly dry with sterile towels and place in hot-air sterilizer for one hour at 100° C. In order to keep them in sterile condition for immediate use they must be kept wrapped in sterilized cloth or cotton.

c. Catgut requires thorough sterilization as not infrequently spores of disease germs (as anthrax) are present. The so-called cumol (cumene) method of catgut sterilization is quite generally adopted in the hospitals of Germany and of other European countries. Wind the catgut in the usual ring form, dry in hot-air sterilizer for two hours at 70° C., place rings in a vessel (beaker, etc.) with cumol on sand-bath and heat to 155° C. or 165° C. (the boiling-point of cumol), turn off the gas and allow to remain in the hot cumol for one hour. The cumol dish should be covered with a fine mesh wire screen to guard against catching fire. Take the catgut rings out of the cumol by means of sterile pincers and place in benzine for three hours, then allow the benzine to evaporate in sterile Petri dishes.

d. Silver catgut is preferably sterilized in 1 per cent. silver citrate (itrol) or 1 per cent. silver lactate (actol), allowing it to remain for six hours which destroys even the anthrax spores. Next expose the catgut to light (in sterile dishes) for a day or two, then wind or fasten on glass and preserve in 95 per cent. alcohol with 10 per cent. glycerin. Actol and itrol ionize silver far less actively than silver nitrate, hence their preference.

e. Catheters, drainage tubing and other rubber materials are sterilized by boiling in water with 5 per cent. sodic hydrate. Rubber goods will not stand prolonged and frequent boiling. Do not sterilize metal ware with rubber goods.

e. *Sterilization of Medicines.*—As a rule, medicines which are prepared under aseptic surroundings and conditions do not require sterilization. However, the ideal conditions rarely exist and subsequent sterilizations become desirable and even necessary.

Tooth powders, dusting powders and similar substances may be sterilized at a dry temperature of 70° C., for three to four hours. Salves and pastes are difficult to sterilize. Low temperatures (from 60° C. to 70° C.) for several hours may be employed.

Solutions for subcutaneous injection, for wound irrigation, for bladder irrigation, solutions of boric acid, of tannic acid, aquæ, normal salt solutions and all weaker solutions of chemicals, intended for washes and irrigation in surgery, should be sterilized by boiling for five minutes. Strong

solutions of chemicals (as acids, alkalies, etc.) do not require sterilization as they are themselves strongly germicidal.

Alkaloidal and glucosidal solutions, and solutions of alkaloidal salts, tinctures and fluidextracts, should be carefully filtered and sterilized in sealed containers at a temperature of 60° C., one hour each day for six days. Concentrated alkaloidal solutions may be similarly sterilized. It is not advised to employ a higher temperature for these substances inasmuch as the decomposition changes, if any, which may take place at 100° C. are not clearly understood. To be on the safe side, the lower temperature (60° C.) should be employed.

In the case of solutions or emulsions for hypodermic use, prepared with oil, the oil is first to be treated with alcohol (95 per cent.) to remove the oleic acid. Oily solutions of calomel, yellow oxide of mercury, lecithin, and of camphor are to be prepared with sterile materials, then placed in a boiling water-bath for ten minutes or in an air-bath at 100° C. An interesting requirement is exacted by the Italian Pharmacopeia as regards the glass of the containers for hypodermic injections: Ten to twelve ampuls or five or six bottles are filled with a clear solution of 1 per cent. mercuric chloride, then sealed. They are then left in an autoclave at 112° C. for half an hour, at the expiration of which time no brownish turbidity should be perceptible.

Some of the points pertaining to the sterilization of alkaloidal, glucosidal and other substances which are quite readily decomposed or altered by light and heat, will be treated under ampuls.

2. Preparation of Ampuls

Ampuls (Lat. ampulla; Fr. ampoule;—a flask) are small glass containers filled with medicinal substances usually in solution. These have come into great prominence within recent years, due to the methods of sterilization now required and practised in well regulated pharmacies. Ampules are really nothing more than very small flasks, the size being suited to single doses of the medicine, as a rule. They were introduced into France about thirty years ago by Limousin and have now come into general use in France, Italy, Spain, Holland and England. It is only recently that they have come into use in the United States. C. A. Mayo was among the first American writers to publish the first more complete information regarding their origin, manufacture and use. (See Proc. A. Ph. A., vol. 57, 1909.) They are generally adopted by the navies and armies of all civilized countries, because of the advantage which they offer for the preservation, storage and transportation of all manner of medicines, particularly those which require sterilization and which are generally wanted for immediate administration. From the standpoint of the physician they are wonderfully convenient and are great time savers.

Ampuls may have any desired capacity, from 1 cc. up to 100 cc., and more, but the more usual capacities are 1 cc., 2 cc., 5 cc., and 10 cc. They are made of alkali-free glass, white or colored (amber). Those supplied by French, German and Italian makers are of different forms, as flask-like, bulb-like, spindling, globose, etc.

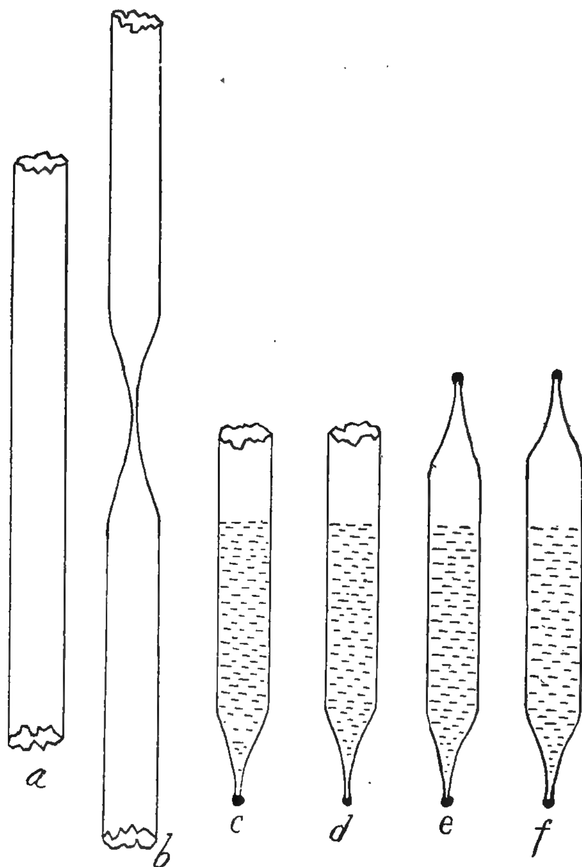


FIG. 86.—Making ampuls. *a*, Piece of glass rod to make two ampuls; *b*, rod *a* heated in the middle and nearly drawn apart; *c*, *d*, two half ampuls filled; *e*, *f*, the completed ampuls.

The following are some of the reasons why ampuls have come into use:

a. Most of the liquid medicamenta and those which are to be dissolved before using, have little or no antiseptic power and under the usual conditions readily become highly contaminated with different organisms. The use of such contaminated medicines has led to serious infections.

b. The necessity of direct administration of medicinal solutions, by

hypodermic, intramuscular and intravenous injection, is due to the desirability of getting prompt therapeutic effects.

c. The direct (hypodermic, intramuscular and intravenous) administration of medicamenta is very frequently necessary because administration per mouth is impossible.

As a rule the pharmacist will purchase ampuls, ready for immediate use by the physician, from some reliable wholesale manufacturing house. In certain districts and under certain conditions this may not always be possible, in which case the pharmacist must prepare the ampuls. The pharmacist should be prepared to make all ampuls which may be desired by the Mhysicians in his community. The following suggestions can be carried out readily:

A. *Glass Tubing.*—Ampuls can readily be made from ordinary alkali-free glass tubing, selecting rods of a diameter to make ampuls of 1 cc., 2 cc., 5 cc., and 10 cc. capacity. This tubing can be secured from any chemical or pharmaceutical supply house. Select rods which are quite free from bubbles and of fairly uniform diameter and thickness.

B. *Breaking the Tubing into Suitable Lengths.*—Break the tubing in lengths of from five to six inches, by filing a scratch with a small file and breaking, with the hands protected by gloves to avoid injury by small bits of glass.

C. *Sterilizing and Neutralizing the Glass Tubing.*—Place the lengths of class rods into water with 5 per cent. of soda and boil for thirty minutes. Neutralize in 5 per cent. hydrochloric acid, rinse thoroughly and again boil in distilled water. Let drain until dry. May be placed in hot-air sterilizer at 140° C.

D. *Making the half Ampul.*—Take one glass tube and heat the middle part in a bunsen burner with rotation until red hot and soft, and pull apart with a fairly quick strong pull. Break off the thin hairlike ends and hold the tips in the fame to seal them securely. A small bead should form as shown in Fig. 86, c,d,e,f. A little practice with a steady hand is necessary to do this neatly. The half ampuls (one end open, the other sealed as explained— are now laid aside in a sterile box or other container, until ready to be filled. Or the two ends of the ampul can be reduced to a capillary tube as follows. Heat the glass tubing in the blow-pipe flame, beginning at one end, until soft and draw out a short distance with a firm pull. Heat at a point about 1 to 3 inches from the narrowing portion of the glass tube and repeat as before. Repeat this until there are a series of tubes of normal diameter with capillary connections. Breaking these apart with the aid of a file, yields empty ampuls open at the two capillary ends.

E. *Filling the Half Ampuls.*—This can be done by means of a burette,

a pipette or a medicine dropper. The burette has many advantages. Many ampuls can be filled from one burette, the exact amounts can easily be measured. The pipette is far less convenient than the burette and is more easily contaminated. A well graduated medicine dropper is very convenient, but all things considered the burette is recommended. The points to be kept in mind are.

a. The finished ampul should not be more than three-quarters full. The length (of untapered portion of tube) of a neat ooking ampul is about three or four times the diameter of the tubing used.

b. In filling, introduce at least 10 per cent. more than the actual dose required, that is, the 1 cc. tube should contain 1.10 cc.; the 5 cc. tube should contain 5.50 cc. of the medicinal substance, etc. This is to make sure that the physician may get a full 1 cc., 5 cc., etc., dose after allowing for unavoidable loss (portion clinging to inside of ampul, remaining in narrowed ends, etc.).

c. In filling do not allow any of the liquid to come in contact with the upper end (open end) of the tube as that might interfere with sealing.

There are many different methods for filling ampuls which may be classed under three heads; filling by gravity, by pressure, and by vacuum; the latter two being but modifications of the same principle involved. There are on the market (France, Holland, Germany) several devices made expressly for filling and sealing ampuls.

F. *Sealing the Filled Half Ampuls.*—This is done by means of suitable side-flame blow-pipe burner, pinching together and drawing our the soft end of the glass by means of pincers and sealing in same manner as the other end. Do not upend the ampul until it is cool, to avoid cracking the glass.

G. *Sterilizing the Ampuls.*—The hypodermic and other solutions usually put up in ampuls can be divided into three classes or groups according to the degree of heat which may or must be used in sterilizing, namely, those which cannot withstand a temperature above 60° C., those which can be sterilized at 100° C., and those which may be sterilized in an autoclave at 120° C. Inasmuch as the autoclave is rarely usable and also because the ordinary steam temperature (100° C.) will meet all of the requirements of the autoclave, the latter piece of apparatus may be left out of consideration by the practising pharmacist.

To bring about a complete sterilization of the ampuls, the discontinued or fractional method should in all cases be carried out. Place the ampuls in a container (beaker, tumbler, etc.) with water to which enough methyl blue or fuchsin has been added to give it a very marked color and sterilize as follows: If a temperature of 60° C. is to be used, apply this temperature (in incubator with Reichert thermo regulator) for one hour each day for four to eight days. Some manufacturers recommend a period of ten days. If the 100° C. is to be used, apply this temperature (in an ordnary Arnold

steam sterilizer) for from 20 to 30 minutes once each day for three days. Should the autoclave be used, one exposure for a period of 20 minutes at 120°C. is sufficient to kill all organisms, including spore.

It is of vital importance in preparing liquids for hypodermic and intravenous injection to have absolutely perfect solutions. There must be no insoluble particles as these might cause serious harm. After the solutions are made they should be forced through a Berkefeld or Pasteur-Chamberland filter. All operations should be done under aseptic conditions, using only chemically pure materials and boiled distilled water. If the contents of the ampuls become cloudy after sterilization, or if the inside of the glass tubes show opacities, something is wrong and such ampuls should be rejected. Also reject all "leaks," indicated by the aniline color which will appear on the inside of the tube.

The finished ampuls are now ready for use. The physician simply breaks off one end of the ampul, inserts the hypodermic needle (sterilized) upends the ampul and aspirates the contents of the ampul into the syringe by simply drawing down the piston. A second method is to remove the piston from the syringe tube, break off one end of the ampul, insert this end into the open of the piston tube, break off the other end of the ampul, whereupon the contents will flow into the piston tube; afterward replace the piston rod. In this latter method great care must be observed so as not to get small particles of broken glass into the hypodermic syringe.

Use white glass for making ampuls. Those filled with solutions which are affected by light may be kept in an amber-colored bottle or other container which is impervious to light.

The following substances are commonly put up in ampuls. Many others can be so put up. Each ampul should contain enough material for one dose or for one application, as the case may be. In the columns to the right are given the sterilization temperatures; the preferred or only usable temperatures being given in degrees, the permissible method being indicated by "Yes" and the inadmissible method being indicated by "No." In case of doubt it is always advisable to use the lower temperature (60° C., hourly for from four to eight days).

Name of Article	Sterilizingg Temperature		
	Incubator 60° C.	Steam 100° C.	Steam (autoclave) 120° C.
Adrenalin	Yes	100°C.	No
Alkaloids salts generally	60°C.	Yes?	No
Antitoxins	60°C.	No	No
Argyrol	60°C.	Yes?	No
Arsacetin	Yes	100°C.	No
Arsenate of iron	Yes	100°C.	No
Arsenic	Yes	100°C.	No

Name of Article	Sterilizing Temperatures		
	Incubator 60° C.	Steam 100° C.	Steam (autoclave) 120° C.
Atoxyl	60° C.	No	No
Atropin	60° C.	Yes?	No
Bacterins	60° C.	No	No
Cacodylates	60° C.	No	No
Caffeine	Yes	100°C.	No
Caffeine benzoate	60° C.	No	No
Calomel cream	Yes	100° C.	No
Camphorated oil	Yes	100° C.	Yes
Chemicals in solution	Yes	100° C.	Yes?
Cocaine	60° C.	No	No
Duboisine	60° C.	No	No
Ergot	60° C.	No	No
Eserine sulphate	60° C.	No	No
Eucaine	60° C.	No	No
Gelatin	Yes	100° C.	No
Glucosides	60° C.	No	No
Glycerophosphates	60° C.	No	No
Grey oil	Yes	100° C.	No?
Gums	Yes	100° C.	No
Hysocine	60° C.	No	No
Iron cacodylate	60° C.	No	No
Mercury benzoate	Yes	100° C.	No
Mercury cacodylate	Yes	100° C.	No
Mercury salicylate	Yes	100° C.	Yes
Mercury sozo-iodolate	Yes	100° C.	Yes
Mercurial salts generally	Yes	100° C.	No
Morphine	Yes	100° C.	No
Mucilaginous substances	Yes	100° C.	No
Normal salt solution	Yes	100° C.	Yes
Oils	Yes	100° C.	Yes
Paraffins	Yes	100° C.	No
Quinine	60° C.	No	No
Physostigmine	60° C.	No	No
Salvarsan	Yes	100° C.	No
Scopalamine	60° C.	No	No
Sera	60° C.	No	No
Sodium cacodylate	Yes	100° C.	No
Stovaine	Yes	100° C.	No
Strophantin	Yes	100° C.	No
Strychnine	60° C.	No	No
Toxins	60° C.	No	No
Trypsin	60° C.	No	No
Vaccines	60° C.	No	No

Empty ampuls of German and French make can be secured from dealers in glass ware and chemical supplies, likewise the appliances for filling and sealing. These ready-made empty and filled ampuls vary in form as already indicated. Those with a flat bottom and which will remain standing when placed on a flat surface are preferred by some physicians.

The ready-made empty ampuls (still sealed) may be sterilized by boiling for fifteen minutes in a 5 per cent. solution of phenol, rinsing thoroughly in boiling hot sterile water, draining and drying. With the aid of a small sharp file, break off the tips of the ampuls to be filled. Place them in distilled water, bring to a boil, take vessel from the fire for a few moments, pour cold distilled water upon the empty floating ampuls, a partial vacuum is produced in the interior of the ampuls and they quickly fill with water. Now boil for thirty minutes. When water is sufficiently cool take out the ampuls, shake out the water and dry in the hot-air sterilizer at 100° C. They are then ready to be filled, sealed and finally sterilized in the manner already described. An ordinary sterilized hypodermic syringe will be found very satisfactory for filling the ampuls. The suggestions regarding the amount of material to be placed in the ampul, sealing, sterilization, use of the aniline solution, etc., already given, also apply here.

CHAPTER XVII

COMMUNICABLE DISEASES WITH SUGGESTIONS ON PREVENTIVE MEDICINE

The pharmacist should be prepared to assist the physician and the health authorities in the enforcement of the sanitary rules and regulations. To this end he should be informed as regards the source of the more important contagious and infectious diseases and the causes of epidemics and the means available to prevent or to combat such conditions. This does not mean that the pharmacist must have a full knowledge of the pathology

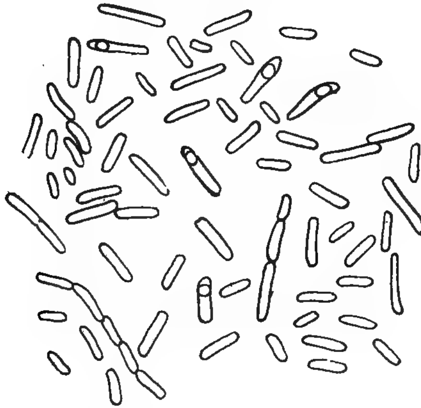


FIG. 87.—*Bacillus botulinus.* This bacillus causes botulism, a form of meat poisoning. There are numerous cases of poisoning resulting from eating infected meats. It should be kept in mind, however, that meat may not be decomposed and may be without bacilli and yet ptomaines may be present. Therefore absence of bacilli and of bad odor does not prove that the meat is wholesome. Meat from animals recently killed, which has been well cared for and which is without bad odor and shows no bacilli, is in all probability wholesome. Ham, canned meats, cold storage meats, etc., may have taken up toxins from contaminated meats, thus being made unfit for consumption even though no bacteria are found.

and therapeutics of disease. He should have at least a general knowledge of the causes of disease in order that he may assist in applying the means for preventing disease. It is not within the province of the pharmacist to cure disease, but he should be a potent factor in preventive medicine.

In many instances, protection against one kind of infection also protects against other infections. About 1893, two health officers (H. F. Mills of the Massachusetts State Board of Health and Dr. J. J. Reinke

of the Hamburg city Board of Health) noted that a reduction in deaths from typhoid fever, due to improved water sanitation, coincided with a reduction in mortality from causes other than typhoid. This is known as the Mills-Reinke phenomenon and has received much attention on the part of health officers everywhere. This observation is however not so recent as is indicated by the date given. For many years, it has been general knowledge among observing health officers and practicing physicians, that an improvement of those conditions which reduce the mortality rate due to any one of the more or less serious intestinal infections, reduces the general mortality rate likewise, and it has been surmised for many years that some kind of genetic relationship exists between all filth borne pathogens and toxigens. It makes little difference against which of the many filth germs the sanitary activities of a city or community are primarily directed, the end result will be a reduction in all filth borne diseases. A thorough and complete sanitary cleanup of a city means a reduction in the following diseases, naming them in the order of their more usual pathogenetic filth relationship. The entire series of intestinal infections, as the diarrheas, dysenteries, enterites, colitis, cholera infantum, cholera morbus, etc.; typhoid fever, tuberculosis (including both the bovine and human types), diptheria, and incidentally also syphilis and gonorrhea. The coincident reduction in the so-called social diseases is no doubt due to the fact that a purely physical cleaning up, encourages or stimulates moral cleanliness. To the list of essentially filth borne diseases must be added Asiatic cholera, amebic dysentery, bubonic plague, and other diseases endemic in certain countries. The exact causality of some of the supposedly water borne diseases, such as goiter, has as yet not been fully worked out.

As early as 1886-1887, the writer was stronly impressed by the high rate of intestinal diseases in the city of Chicago and surmised so some causal relationship between the dysenteries and typhoid. Since the completion of the Chicago canal, the general health of that city has improved wonderfully. Up to that time, the sewage of the city was dumped into lake Michigan (*via* the Chicago river) and again pumped into the city and the inhabitants were obliged to swallow the mixed human and animal excreta. The accumulation of the sewage in the sluggish stream gave rise to an undescribable stench, still fresh in the memories of the older members of the present generation of the city. The digging of the sanitary canal and the proper diverting of the city sewage, is the grandest and best thing ever done by the city of Chicago.

A contagious disease is one which is readily communicable, from one person or animal to another, either through direct contact or very close proximity. An infectious disease is communicable through a considerable

interval of space. Itch, for example, is contagious, but not in the least infectious, whereas whooping-cough is infectious, but not contagious. Some diseases are both contagious and infectious, as small-pox and diphtheria. Malaria and yellow fever are infectious, but not in the least contagious. Since the distinction between contagious and infectious diseases cannot be clearly drawn, these term are discontinued and the term communicable diseases is substituted therefor. By communicable disease we mean any disease which may be transferred from the sick to the well, either directly through close contact or indirectly through more or less

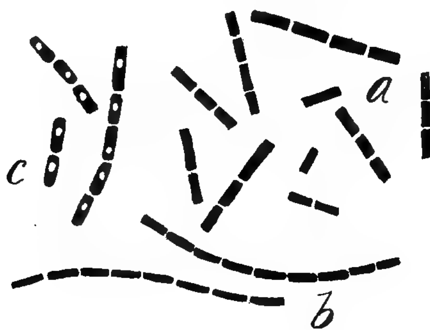


FIG. 88.—*Bacillus anthracis*. This bacillus is spore-forming and causes the cattle disease known as anthrax. This disease is especially common among sheep and cattle and may be transmitted to man, especially those working with the wool, hides and meat of infected animals. The two chief forms of anthrax in man are malignant pustule and woolsorter's disease. The dried spores of this bacillus will live for years and will withstand the boiling temperature for hours. Vaccinating animals against anthrax is commonly practised now. Anthrax is frequently confused with glanders, an equine disease caused by the *Bacillus mallei*. *a*, Non-spore-bearing bacilli; *b*, chains of cells; *c*, spore-bearing bacilli. Cell-walls and plasmic contents are stained, the spores are unstained.

distance in space. A pandemical disease is one which spreads over or pervades the entire earth. La Grippe or influenza is such a disease. The last pandemic destroyed more lives than were killed during the entire period of the World War, and this despite the fact that the mortality rate in this disease is not high. The case rate was very high, about equal in numbers to the entire armies engaged in the World War. In the past Asiatic cholera and plague have been pandemical in scope. In modern times pandemics are prevented by the national quarantine services, and any extensive epidemic in any country or state is prevented by the state and community quarantine. That is, this is certainly true as far as the disease are concerned concerning which we know the cause. The only reason why influenza became pandemical is because we are not as yet aware of the primary cause. A disease which is more or less wide spread over a country is spoken off as an epidemic. For example, cerebrospinal meningitis and pneumonia may be epidemical. Diphtheria is often epidemic in a community, and as above stated, it is likewise infectious and contagious.

The term epidemic is, however, also applied to any communicable disease which has become general in a given community. A more or less common or spreading disease which is limited to and recurs in a given district or country is said to be endemic in that district or country. Endemics are usually due to climatic conditions which encourage certain microbic and other disease-producing invasions.

The causes of disease are of two kinds, primary or inciting, and secondary or predisposing. The primary cause of a disease is that factor or influence which must invariably be active before the disease can possibly develop. For example, the primary cause of diphtheria is the diphtheria bacillus; the predisposing causes are exposure to wet and cold, impoverished condition of body, etc. No matter how numerous or how active the predisposing causes may be, the disease cannot develop until the primary cause acts. There are numerous abnormal or pathological states or conditions without recognizable primary causes, as gout, rheumatism and the senile changes in the body; and again there are certain diseases which evidently have primary causes, as whooping-cough, small-pox and yellow fever, but in which said primary causes are not yet discovered. The following tabulation outlines the primary and secondary causes of disease:

- Communicable diseases.
  - Primary causes (inciting).
    - Bacteria, as in typhoid and Asiatic cholera.
    - Protozoa, as in malaria.
    - Parasitic higher animals, as tape-worm and itch.
    - Fungi, as in ring-worm and pellagra.
    - Undetermined, as in small-pox.
  - Secondary causes (predisposing).
    - Heredity..
      - Race. } (Phylogenetic).
      - Family. } (Phylogenetic).
      - Individual (ontogenetic).
    - Age.........
      - Infancy.
      - Childhood.
      - Adolesence.
      - Adult.
      - Old age.
    - Sex.
    - Environment..
      - Climate.
      - Altitude.
      - Seasons.
      - Unsuitable food.
      - Unsuitable clothing.
      - Poisons.
      - Occupation.
      - Injuries.
    - Habits........
      - Alcoholic.
      - Tobacco.
      - Drugs.
      - Coffee and tea.
      - Gourmandage.

In a general way it may be stated that any cause, factor or influence, which tends to lower the vitality, predisposes to disease. Individuals with a well-balanced physical and mental development are less liable to disease, and when attacked are more apt to recover, than those individuals who have a poor physical development. Undue abstinence is as harmful as over-indulgence. The ascetic is as pathologic as the gouty gourmand.

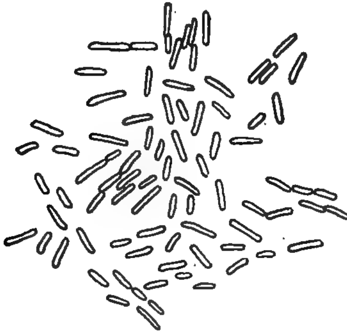


FIG. 89.

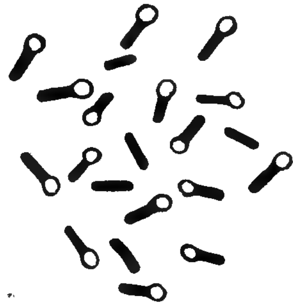


FIG. 90.

FIG. 89.—*Bacillus mallei*, the cause of glanders in horses. This disease can be transmitted to man where it causes symptoms of a suppurative infection of the lymphatic glands. Mallein, which is used in testing horses for glanders, consists of the filtrate (Berkefeld filter) of dead cultures (glycerin bouillon) of the bacillus. A positive malleiu reaction consists in a rise in temperature and local swelling. The dose is 1 c.c.

FIG. 90.—*Bacillus tetani*, an anaerobic spore-bearing bacillus, the cause of tetanus or lockjaw. This bacillus is found in soils and may infect abrasions, cuts and wounds. Treatment with tetanic antitoxin is successful if begun before the symptoms develop. The best time to administer the antitoxin is at the time the injury is received.

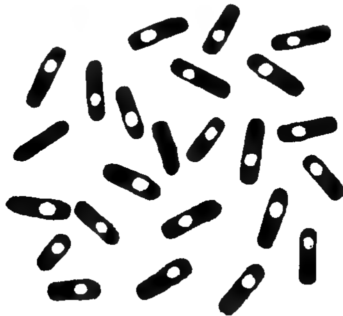


FIG. 91.—A spore-bearing bacillus stained with methyl blue leaving the spores unstained. Fortunately most of the bacilli pathogenic to man do not bear spores.

Irrational diet, drink and food fads, sooner or later leave their pernicious effects upon the system and predispose to certain diseases. Overeating is as objectionable as starvation. Lack of adequate physical exercise has its evil effects as does also over-exertion. Trained or professional athletes are not long lived, many are hopelessly afflicted with enlarged and weakened heart and arteries (aneurism). Pernicious habits of all kinds indicate

weakness and further develop the weakness, which in turn predisposes to certain diseases and render the individual less resistant to the ravages of disease. A good ancestry and inheritance, good wholesome food, comfortable clothing, the right sort of exercise for body and mind, the simple life rather than the strenuous life, avoiding bad habits of all kinds, abundant fresh air, etc., all tend toward longevity. To argue that we should go unclothed is as absurd and unreasonable as to teach that sheep should be shaved. To adhere to a wholly vegetable diet is irrational simply because we are organically adapted to a mixed diet. An excessive meat diet is also very pernicious.

Occupation is a potent factor in predisposing to disease, and in longevity. The following table adapted from a report by Ogle will serve to make this clear. The high mortality rate among street-hawkers is due to several causes chief of which are low-living, exposure to inclement weather, and the greater exposure, in the squalid districts of large cities, to the primary causes of disease. The low mortality rate among clergymen is due to a comparatively simple though comfortable mode of living; while in the case of the farmer and gardener, the out-of-door life is the favorable influence. The list represents ages ranging from twenty-five to sixty years, therefore adults.

Occupation	Comparative Mortality
Clergymen, priests and ministers	100
Gardeners	108
Farmers	114
Carpenters	147
Lawyers	152
Coal miners	160
Bakers	172
Builders, masons, bricklayers	174
Blacksmiths	175
Commercial clerks	179
Tailors	189
Cotton manufacturers	196
Medical men	202
Stone, slate quarries	202
Book-binders	210
Butchers	211
Glass workers	214
Plumbers, painters, glaziers	216
Cutler, scissors makers	229
Brewers	245
Innkeeper, liquor dealers	274
File makers	300
Earthenware workers	314
Street hawkers	338
Inn, hotel service	396

The following are the more important communicable diseases with suggestions on prevention. The information is given for the sole purpose to better qualify the pharmacist to coöperate with the health officers in safeguarding the public health.

A. **Tuberculosis.**—Commonly known as consumption and the "white plague." A universal disease, essentially infectious, especially peculiar to crowded habitations and to lack of pure fresh air. The primary cause is the *Bacillus tuberculosis* (bacillus of Koch), a non-spore-bearing microbe, which is somewhat more resistant to disinfectants and other destructive agencies than most other pathogenic bacteria. The chief predisposing causes are living in crowded habitations and inherited low vitality, especially weak lungs. The disease may be general (general tubercular infection) or it may be localized in any one or in several organs or tissues. Commonly localized in the lungs (phthisis, consumption) and in lymph glands. Lupus and many so-called scrofulous conditions are tuberculosis of the skin; the disease often attacks bones and joints (hip-joint disease of children). It attacks young and old and may occur in all walks of life. The disease enters *via* the air passages and per mouth with food and drink. It is contracted by inhalation through close association with consumptives, and the bovine form or type of tuberculosis is acquired from the milk of tubercular cows. Bovine tuberculosis is especially liable to affect the lymph glands and the joints in children, rarely in adults.

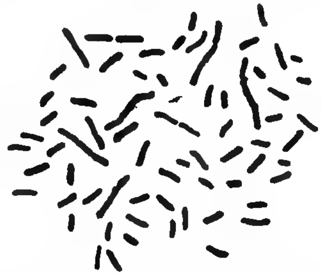


FIG. 92.—*Bacillus tuberculosis.* Although this organism does not form spores it is quite resistant to the action of germicides. The bacillus causing the bovine type of tuberculosis differs slightly in several characteristics from the bacillus of human tuberculosis.

The disease sometimes runs a quick course (quick consumption), but more generally it makes an insidious start and runs a chronic course. Many people have limited local infections which are only discovered at an autopsy. There are many spontaneous recoveries from tuberculosis. Since it is very important to begin early treatment, the physician resorts to several tests for the purpose of determining the possible existence of masked or incipient forms of the disease. These tests are as follows and depend upon the reactions produced by tuberculins when applied to or introduced into the system:

a. *The Calmette or Ophthalmo Test.*—Old tuberculin, precipitated by alcohol is used. The precipitate is dried and made into a 1 per cent. solution in sterilized distilled water or sterile physiologic salt solution. This substance is put up in sterile capillary pipettes, ready for use. A drop of

the solution is placed in one eye, using the other eye as a control. Any abnormality in the eye is regarded as a contraindication. If tuberculosis exists in the system it is indicated by an inflammation in the eye tested. Also known as the Wolff-Eisner test or reaction. It may be necessary to repeat the test several times before satisfactory results are obtained.

b. *The von Pirquet or Cutaneous Test.*—A 25 per cent. solution of tuberculin (O. T.) is applied to the skin with scarification, as in vaccination. The skin is first cleansed with alcohol and control scarifications are made near the test area. This test is also known as the "skin reaction." It is not very reliable. The inflammatory reaction may be simulated by other substances in persons that are known to be entirely free from tuberculosis.

c. *The Moro, Percutaneous or Ointment Test.*—Fifty per cent. tuberculin (O.T.) in lanolin is rubbed into the skin, without scarification. The preparation is put up in collapsible tubes, one tube containing enough material for several tests. If tuberculosis exists, small reddened vesicles appear at the point of inunction, usually on the second day.

d. *The Thermal Test.*—A solution of tuberculin (O.T.) put up in, 8 cc. bottles, representing one milligram per cc. (1–1000) is injected hypodermically. If tuberculosis is present there is a rise in temperature, usually within ten to twenty-four hours after injection.

e. *The Detre Differential Test.*—This test is intended to differentiate between tuberculosis of human origin and that of bovine origin. Three tuberculins are required. Tuberculin O. T., tuberculin B. F., made from tubercle bacilli of human origin and tuberculin B. F., made from tubercle bacilli of bovine origin. Three small skin areas are scarified. Into one tuberculin O. T. is rubbed, into the second humanized tuberculin; and into the third bovinized tuberculin. The resulting reactions indicate whether tuberculosis is of human or of bovine origin.

We cannot go into the details of the reactions. They are not always reliable, neither the positive nor the negative reactions. In the advanced stages of tuberculosis and in moribund cases, the reaction is usually negative. Indeed, in such cases the test is unnecessary as the existence of the disease is evident without special tests.

Tuberculosis is not as infectious as is generally supposed. Those who are in good condition physically may live for years with those afflicted with the disease without becoming infected. Yet, tubercular patients should be isolated from well people as much as possible. The sputum is the principal source of infection, also other secretions; and the breath as in sneezing, laughing and coughing. Plenty of fresh pure dry air should be supplied to patients, large airy sleeping rooms and easily digested wholesome food is essential. Consumptives should not marry, should not kiss healthy individuals, especially children. Expectorated material

should be disinfected at once. Treatment should be begun early. The propaganda favoring well constructed, well ventilated, comfortably warmed homes and less close segregation in cities and a general improvement in sanitation will do much toward eradicating tuberculosis. Tenement houses and large or small crowded houses of all kinds should not be tolerated for moral as well as for sanitary reasons. The milk used must be free from tubercular infection. Dry warm climates and the higher clear atmospheres favor recovery from tuberculosis (California, Colorado).

The following test has been found very reliable in the hands of clinicians.

The Jefimoff-Klein Urinary Test for Tuberculosis

1. Mix 4 cc. of the fresh urine with 2 cc. of a 20 per cent. lead acetate solution, warm and filter several times while hot.

2. The filtrate, while still hot, is mixed with ten or more drops of an alcoholic solution of silver nitrate. A precipitate forms which varies in color from brick red to cherry red, according to the progress of the disease. Normal urine remains uncolored (white precipitate).

The silver nitrate solution is made by dissolving 10 grams of silver nitrate in a minimum of water and adding strong alcohol up to 100 cc.

Most tuberculous urine is amphoteric (amphochromatic), when warmed, that is it will turn red litmus blue, and blue litmus red. This behavior may be utilized as a corroborative test with the above. In advanced stages of the disease the urine becomes markedly acid, and is no longer amphoteric.

B. Typhoid Fever.—This is a filth disease. If the environment were made clean and sanitary, typhoid fever could not exist. The primary cause is the non-sporulating *Bacillus typhosus* which is found in filthy water, in milk and in food materials. Typhoid contaminated *slops*, sewage, wash water, etc., poured on the soil may seep into the well water and finally enter the system in drinking. The bacillus develops readily in the intestinal tract where the reaction is alkaline. It is quite susceptible to the action of weak acids and is easily killed by boiling and by disinfectants. Typhoid is a widely disseminated dangerous infectious as well as contagious disease. In large cities the mortality rate from this disease is directly proportional to the filthiness of the drinking-water supply. In country districts epidemics are very frequently due to contaminated well-water (contaminated from kitchen refuse, barns, cow-sheds, etc.). Epidemics often follow in the wake of the dairyman, who supplies cow's milk in cans washed with or which contain milk, contaminated with polluted water. Typhoid fever is carried in vegetables from truck gardens where human and other excrement are used for fertilizing purposes. The Chinese

truck gardeners are particularly culpable in this regard. Again, the vegetables may be irrigated with stagnant sewage-polluted water. House flies are carriers of typhoid.

The mortality rate in typhoid is high and the disease runs its course in about five weeks. There are some mild cases, the so-called walking or ambulatory cases. All of the excreta from the patient should be disinfected. Among the disinfectants which have been used for this purpose are, corrosive sublimate (1–1000), copper sulphate (5 to 15 per cent.), copperas solution (10 to 20 per cent). These are effective when properly used. Their albumen coagulating coefficient is very high (see table in the chapter on disinfectants) and it is absolutely necessary to stir the mixture of material and disinfecting solution very thoroughly. The noncoagulating disinfectants are to be preferred, such as milk of lime and the coal tar disinfectants. All bed linen, clothing, etc., used by the patient should be disinfected in 5 per cent. carbolic acid before washing. Everything used by the patient should be sterilized, disinfected and kept away from the rest of the family. Those who nurse typhoid patients must be extremely careful not to carry the infection to others. Pillows, mattresses and other large articles used by the patient should be steam sterilized. In simple words, everything about the patient must be scrupulously sterilized in order to avoid spreading the infection.

A national department of health should see to it that the water supply of large cities is free from sewage contamination. Our streams, lakes and reservoirs which supply drinking water, require careful guarding against typhoid infection.

There should be compulsory regulation regarding the position and depth of wells in farm yards and as regards the position of the well relative to barns, cow sheds, privy vaults, etc. Typhoid fever will continue its ravages as long as filth contamination of water supplies and food supplies is permitted.

The Gruber-Widal test for typhoid is an agglutination phenomenon. The agglutinating power of the blood of a typhoid patient is usually noticeable as early as the fifth day of the disease. Preventive inoculation with typhoid bacterin has been used with considerable success, particularly in the British and German armies, ane is now quite extensively used in general practice. Chantamesse and Wright use agar or broth cultures of the typhoid bacillus, killed by heat.

The de Silvestri urinary test for typhoid is made as follows:

In a small test tube overlay 2 mils of ferric chloride to which four drops of concentrated sulphuric acid has been added, with three mils of the filtered urine. If typhoid is present, a more or less maroon colored ring will develop at the zone of contact, and at the

top of the upper layer a turbid ring exhibiting a green fluorescence develops. The reaction is said to be most distinct with urine from persons suffering with paratyphoid A, but also with that of persons suffering with typhoid and paratyphoid B.

The decade just passed has proved to the entire world that typhoid fever is one of the readily preventable diseases. This is done by means of the bacterins (ordinary and sensitized). As the result of the use of these agents typhoid fever has been driven from the army. The bacterins establish immunization which is an efficient as the vaccination against mallpox. It now lies within the means of everyone to protect himself against this disease. (See also the army statistics mentioned under serobacterins).

For some time the human typhoid carriers (that is, persons who harbored the typhoid organism without showing signs of the disease) have received much attention and numerous cases have been traced to such carriers. The attempts to free such carriers from the infecting organisms have as a rule, not met with general success.

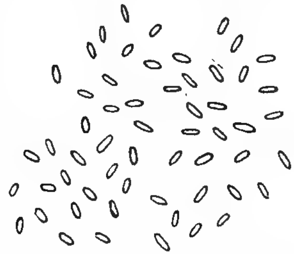


Fig. 93.—*Bacillus pneumoniæ* of Friedländer, also known as *Bacillus mucosus*. This organism is non-sporogeneous and is easily killed.

C. Pneumonia.—Pneumonia with its modifications, as broncho-pneumonia, capillary bronchitis, pleuro-pneumonia, pneumonic pericarditis, etc., is estremely common. The primary cause of pneumonia is the *Diplococcus pneumoniae* of which three types (type I, type II and type III) and one group (group IV) are recognized. Types I and II cause about 33 per cent. of all cases. Type II occurs in about 10 to 15 per cent. of cases which are very severe with a morality rate of 50 per cent. The group IV form the causative organisms in about 45 to 50 per cent. of cases, having about the same mortality rate as for groups I and II, namely 10 to 15 per cent . The important predisposing causes are exposure to wet and cold, weak lungs, infancy, old age, general debility and alcoholism. It is generally limited to the respiratory tract and the contiguous tissues, as the pericardium and the pleuræ. Among infants and young children and those well past middle life, the disease shows a high mortality rate. In youth and early middle life recovery is the rule, provided the physical inheritance and development is good. The mortality rate among those addicted to the use of alcoholic drinks, and those affected with "tobacco heart," is very high.

One attack of pneumonia is supposed to increase the resisting power

to subsequent attacks but such acquired immunity is not by any means permanent. The anti-pneumococcic serum is used with some apparent success though the results are far from satisfying to the majority of those who have tried it. Dr. Shafer has recently recommended a mixed bacterin (composed of disease exudate and pure pneumococcic bacterin) which has been used with some success. At the present time the use of the specific bacterin (ordinary and sensitized) is much used as a cure, employing the polyvalent bacterin in those cases where diagnosis is uncertain, and in cases where it is not convenient or possible to make the differential tests. Three differential tests are recommended, the urine test, the sputum test and the exudate test. Confirmatory cultural tests and microscopical examinations are also made. The details of the differential as well as the confirmatory tests cannot be given here. They may be found in the larger more comprehensive manuals of medical bacteriology.

It is important to guard against exposure to wet and cold, particularly when the vitality of the body is lowered, as through lack of sleep, lack of food, over-exertion, etc. The sputa of patients should be disinfected at once. Well persons having good resisting power may carry the germs and convey the disease to those who have a lower vitality. The room occupied by the patient should be thoroughly fumigated as soon as possible.

D. Small-pox.—Also known as variola and pest. This is a well-known disease which has occurred epidemically from time to time throughout all ages and in all lands. It is most highly infectious and contagious. In spite of all investigations, the primary cause has not yet been discovered. The contagion is wafted from the skin eruptions and is carried in clothing and by everything used or touched by the patient. The contagion may lie dormant in clothing for months. The contagion is known to be filterable, will remain active in glycerin for months, will resist drying for weeks but is quickly rendered inert by bile and by sodium oleate. Heating for 15 minutes at a temperature of 58° C. will also destroy it. Certain cell inclusions (infected epithelial cells) have been considered the primary causative organisms, and have been named *Cytoryctes vaccinae*. These are very minute but may readily be seen in the epithelial debris of the vaccine.

All excreta from the patient should be disinfected with a 5 per cent. solution of carbolic acid or other convenient disinfecting agents as lime, formalin, etc. Bedding, mattresses and other material used in the sick-room should be burned as soon as the patient does not need them any longer.

As the result of the general practice of vaccination (with the modified cow virus) small-pox is no longer the dread disease that it once was. In Germany, where smallpox vaccination has been religiously enforced

from the first, this disease is practically non-existent. In fact so rare, is the disease that the medical society of Berlin some years ago, elected one member each year who shall give a lecture on this disease in order that the fraternity might retain some idea as to the nature of this disease. In Austria, in France and in the United States where anti-smallpox vaccination is less rigidly enforced the case rate is occasionally high, and lesser epidemics appear from time to time, with varying mortality rates. One minor epidemical outbreak in Berkeley (California) of the confluent hemorrhagic type of the disease gave a mortality rate of 100 per cent. Since vaccination is almost an absolute safeguard, there is no need of fearing this disease, even when brought in direct contact with it. One vaccination does not always establish life immunity, as is popularly believed. The rule is to vaccinate in infancy, again about the time of adolescence and again in early adult life. This will usually insure immunity for life. However, vaccination should be carried out after every exposure or whenever smallpox exists in the vicinity, no matter how many good "take" scars there may be. Nurses and physicians in pest hospitals are vaccinated once a year, or oftener, to insure immunity. In the navy it is customary to vaccinate every man every time a port is entered where small-pox is suspected. Small-pox is a quarantinable disease.

There is absolutely no danger or ill effects from vaccination, in spite of the popular newspaper and popular verbal reports to the contrary. In perhaps one case in a million, tetanus or severe septicæmia may be traceable to the use of an impure virus. Septic infection of the scarified area may take place, due to carelessness on the part of the patient, and not due to the virus used, but even this is an extremely rare occurrence. Since the incubation period of small-pox is about twelve days and that of vaccinia (cow-pox) is only five or six days, it is evident that the vaccination will establish immunity even in those who were actually exposed, provided vaccination is done within a few days after exposure.

Primitive (savage) races are very susceptible to small-pox, with a very high mortality rate. This is in part due to the total ignorance of sanitary measures, resulting in the more ready spread of the contagion. Entire savage tribes have been exterminated by this disease. Negroes are far more susceptible than Caucasians. Indians have spread the infection in blankets after having been exposed.

E. Malaria.—This familiar disease, commonly known as ague, the shakes, chills and fever, and intermittent fever, prevails in many areas in the United States and is limited to swampy wet countries. It gradually disappears with the tilling and the draining of soil which remove the breeding places of the only carriers of the disease, namely the mosquitos (Anopheles). The primary causes is the *Plasmodium malariæ* (*Hæmatozoa*

malariæ) which is introduced into the circulation by the sting of the mosquito.

The prophylactic measures consist in the destruction of the mosquitos in rooms. To this end burn two pounds of Pyrethrum to every thousand cubic feet of space. Sulphur (one pound per thousand cubic feet) may be used though it offers no advantage over the Pyrethrum and has the disadvantage of corroding metal and fading colored fabrics. Also destroy the breeding places of the mosquito and keep mosquitos out of houses by means of screens and netting. Protect the person against mosquito stings when travelling in countries known to be infested by the Anopheles group of mosquito. Also take quinine as a prophylactic (3 to 5 grains twice daily), and as a cure. Quinine is, however, more satisfactory as a preventive than as a cure.

The life history of the malarial plasmodium is complex but it has been worked out very definitely. Two life cycles are recognized, the asexual (also known as the human cycle, the cycle of Golgi and the schizogonic cycle), the sexual (also known as the mosquito cycle, cycle of Ross and sporogonic cycle), and occasionally a third cycle known as the parthenogenetic or virgin cycle, which is said to explain the latent occurrences of the organism in the human body. The spindle shaped sporozoites resulting from the sexual generation in the mosquito are introduced into the human body by the sting of the female member of the Anopheline group of mosquitoes (male mosquitoes do not bite). The leucocytes of the blood devour as many of these sporozoites as they can. Those not so destroyed, enter the red blood corpuscles where they undergo the signet ring stage (socalled, because the stained specimens show a resemblance to a signet ring) of development, finally greatly enlarging and partially disintegrating the blood corpuscle by their increase in size and numbers, forming the merocyte. The matured merocytes divide into a number of small bodies which occur free in the blood plasm, constituting the merozoites. This cycle in the human blood is completed in from twenty-four to seventy-two hours, depending upon the species of malarial organism. Each merozoite now enters a red blood corpuscle and a similar cycle repeats itself. At each sporulation a paroxysm of fever manifests itself. It was found that most of the merozoites were asexual, but that some were sexual. The sexual forms require longer time to mature (from eight to ten days) and are known as the gametocytes. From the sporozoite introduced by the sting of the mosquito to fully matured gametocytes (male and female) constitutes the complete asexual or human cycle. The gametes are now ready to enter upon the sexual cycle which can take place only in the salivary glands of the female member of the Anopheline group of mosquitos. The mosquito inoculates itself with gametocytes

upon filling itself with blood of a malarial patient in whom the asexual cycle has been completed. Should the mosquito bite the human earlier, that is, before the gametes are matured, it would not become a transmitter of malaria for obvious reasons. In the sexual or mosquito cycle, the gametocytes derived from the human undergo the preliminary change in the stomach of the insect (flagellation and exflagellation of the male gametocyte and the macrogamete formation of the female gametocyte). The products of exflagellation constitute the male gametes or microgametes which now fertilize the macrogametes, giving rise to the oökinetes. The oögonites now pass through the wall of the stomach and attach themselves to or lie adjacent to the outer lining of the stomach, where they grow to large size forming cysts in which are developed hundreds of tiny spindle shaped nucleated bodies known as the sporozoites, which enter the body cavity of the mosquito from which they gradually are gathered into the salivary glands, where they remain until some of them may be injected with the saliva into the human body, where the asexual cycle again repeats itself.

Malaria could be made to disappear from the face of the earth by doing the following.

1. Destroy all malaria bearing mosquitos, or,

2. Destroy all humans, or,

3. Do away with the breeding places of malaria bearing mosquitos occurring within the zones of dissemination for humans, comprising what is commonly known as malarial control.

Since the malaria lorganism cannot survive unless it is provided with the two hosts, namely man and mosquito, it is evident that the discontinuance of one or the other of the two hosts, would cause the malaria to disappear. Proposition (1) is practically impossible and proposition (2) is not to be thought of. Proposition (3) is practicable as has been demonstrated on numerous occasions. It is reasonable to suppose that if all breeding places of the malaria spreading mosquitos occurring within the reach of humans should be rendered uninhabitable for the mosquito larvæ, for a period sufficiently long to make sure that all latent carriers were also dead, then the disease would be eradicated from the face of the earth.

Malarial control is a very definite phase of sanitary science and those who are interested should consult the following special treatise. Herms, William, B.—Malaria: Cause and Control. The Mac Millian Company.

F. Diphtheria.—This dread disease is both infectious and contagious. The primary cause is the *Bacillus diphtheriæ*, also known as the Klebs-Loeffler bacillus. The chief predisposing causes are exposure to wet and cold. The disease may be localized in the larynx (membranous croup)-

in the pharynx, in the nares, on any of the mucous membranes, and in cuts and wounds. Animals such as cats and dogs may carry the infection. The sick must be isolated and all discharges from nose, mouth and throat as well as the bed linen, etc., must be sterilized and disinfected. Upon recovery, the sick-room must be thoroughly fumigated by means of formaldehyde. Bedding, mattress and pillows must be disinfected. The anti-diphtheric serum should be used early and in large doses. The best authorities look upon this remedy as a specific always effecting a cure, provided it is given in time and given in adequate doses. All those who have been exposed should receive a proplylactic dose of the remedy (about 1,000 units). The other remedial agents as gargles, sprays, etc., should not be neglected. The diphtheria toxin acts on the heart and all patients should be warned against any sudden or severe exertion until complete recovery is assured by the attending physician as death has resulted from a single undue action, as jumping or suddenly rising from bed.

G. Cancer.—The primary cause, the secondary causes and the treatment of cancer are all in the dark as yet. We know that this disease rarely develops earlier than middle life. It usually runs a comparatively short course (several months to two years), producing some rather marked symptoms (the cancerous cachexia), with constant pain, and a very characteristic waxy pallor of the skin. It is to be hoped that the primary cause and the cure will be discovered in a short time. There are some indications that a tendency to cancer is inherited and that the primary cause is an organism resembling the protozoa group. There is a popular belief that eating raw tomatoes causes cancer, and it may be that the plasmodium of cancer resides in some vegetable. Cancer may attack any tissue or organ, although the internal viscera, as liver and stomach, are more commonly affected. Cancer should be treated as a contagious disease though the proof of its contagious nature is not conclusive.

All advertised cancer cures are fakes. There is no known cure for cancer. Surgical removal of cancerous growths has been the means of prolonging life, but the trouble is very apt to recur. Many cases are inoperable.

Among the agencies which have been tried as cures for cancer are mixed streptococcic bacterins; radium emanations from various sources, as radium and other radioactive minerals, water rendered radioactive, and radioactive clay; and plant extracts. None of them have proved satisfactory. Radium has undoubtedly effected some cures and improvements but it is far from reliable. The principle upon which the use of radium emanations are based are as follows. The emanations are destructive to living tissues, but even more destructive to pathological tissues. A carefully adjusted dose of emanations will kill pathological tissue, such as

cancer, without seriously injuring normal tissues. In skin cancer the properly adjusted dosage and time exposure of the radium emanations has resulted in the complete killing of the cancerous epithelium, so that it could be lifted off, showing the normal granulation tissue underneath. Many volumes have been written about cancer and many scientific bodies have given much time and attention to the cancer problem, which is a very serious one, but the solution is not yet found.

H. Plague.—This disease, which is also known as black plague, the pest, bubonic plague, black death, etc., is essentially a filth disease. The primary cause is the non-sporogenous *Bacillus pestis*. The plague has

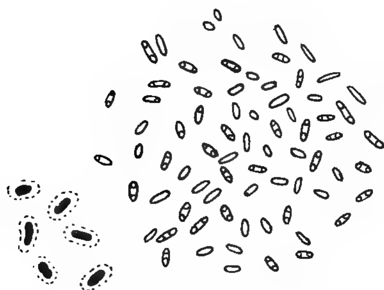


FIG. 94.

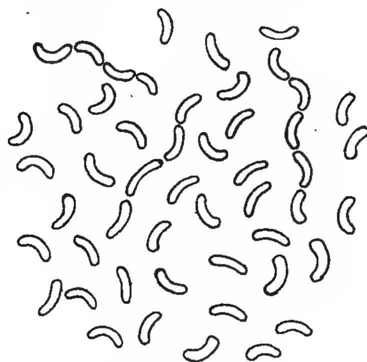


FIG. 95.

FIG. 94.—*Bacillus pestis*. Does not form spores and is very easily killed. The ends stain more heavily than the middle. Involution forms may occur. Sometimes the cells become encapsuled as shown in the figure.

FIG. 95.—*Bacillus choleræ* also known as *Spirillum choleræ*, the case of Asiatic cholera.

occurred epidemically from time to time throughout all ages. It is most virulent and most prevalent (endemic) in the crowded cities of the warmer countries (Oriental cities), where the sanitary conditions are often very bad. The disease is highly contagious and infectious and is communicable not only to man but also to rats, mice, dogs, squirrels and cattle. Rats, and the fleas upon them, are the principal carriers of the disease, although other animals, as ants and flies, may also act as carriers.

There are several forms of the plague of which the pneumonic is the most dangerous and most infectious because the bacilli are spread by coughing and sneezing.

In this disease thorough disinfection is of the greatest importance. The entire body of the patient should be washed with a disinfecting solution (1–1200 bichloride of mercury). Disinfect everything used about the patient. After death or recovery everything used by the patient should be destroyed by burning.

Rats (bearing the infected fleas) are the principal carriers of this disease, and the experience in San Francisco (1906–1909) has demonstrated that plague disappears as soon as the plague infested rats disappear. Destroy rats and mice and see to it that the home is free from fleas. Plague is a quarantinable disease and the federal authorities are constantly on the lookout to prevent the importation of this disease. The Oriental ports are the chief sources of infection.

Yersin's anti-plague serum and Haffkine's bacterin have been used with considerable success as prophylactics and also with some success as cures.

I. Asiatic Cholera.—This is another filth disease essentially of Oriental origin, particulaly prevalent in the crowded unsanitary cities of India and Asia. It is a quarantinable disease. The primary cause is the non-sporogenous *Bacillus choleræ* (*Spirillum choleræ*) also known as the comma bacillus of Koch. The principal sources of the infection are polluted water and food, particularly the former. In fact the sources of infection and modes of entry into the digestive tract are not unlike those of typhoid. Cholera is highly infectious and usually occurs epidemically, often spreading over wide areas. Human excrement carries the infection and when this material is used as fertilizer, which is done in China and other Oriental countries, it becomes the means of initiating and continuing the spread of the disease. The importing by the Chinese of human excrement and animal dung for medicinal purpose should be prohibited as it may be the means of starting an epidemic of cholera in the United States, even though it is unlikely that the infection will survive the trip.

Fortunately the cholera bacillus is easily killed by heat, disinfectants, and by drying. The temperature of boiling water kills it in five minutes. In water it may retain its vitality for a long time. Furthermore, it is not a strict (obligative) parasite and may multiply outside of the body under favorable conditions. Flies carry the infection from cholera stools to articles of food.

Haffkine's attenuated cholera bacterin has been employed successfully as a prophylactic. The method of use consists first in the hypodermic injection of a weak virus, that is, cultures attenuated by long cultivation at a high temperature (39° C)., and following this later, in five days, with a virulent culture. More recently Kolle has used cultures killed by heating for one hour at 58° C., which has given good results in numerous tests made during a cholera epidemic in Japan. Pfeiffer and others have experimented extensively with cholera-immune serum and have demonstrated that this has marked lyctic properties. The cholera bacilli when placed into the serum first lose motility, then swell up into coccus-like forms and finally dissolve. This property is said to be due to two substances, one found in

normal serum and the other found in immune serum. Neither substance alone can destroy the cholera bacilli but the two acting together are strongly bacteriolytic. The immunity produced by the Haffkine and Kolle bacterins is temporary only.

J. **Yellow Fever.**—This highly infectious, but in no wise contagious disease, is peculiar to tropical and subtropical countries. The primary cause is as yet unknown but it is supposed to be a protozoan. The sole carrier of the infection is a mosquito, *Aedes calopus.* The disease has been highly epidemical in the southern states but since the discovery of the part played by the mosquito the mortality rate has been lowered to a marked degree. In fact the disease is now under complete control. No *Aedes* mosquitos, no yellow fever.

It has been observed for a long time that a frost checked the disease at once, which as is now known, was due to the fact that the frost killed the carriers of the infection. In a general way the statements made under malaria prophylaxis also apply here. Caucasians, especially those not acclimated in the yellow-fever countries, are very susceptible to the disease; Negroes and Latin races are far less susceptible.

The history of the establishment of complete yellow fever control is of intense interest. As early as 1881 Dr. Finlay of Havana suggested that the mosquito was responsible for yellow fever, based upon the observation that the disease disappeared as soon as the colder weather killed the animal. In 1900 the United States appointed the Yellow Fever Commission placed under the direction of Dr. Walter Reed, with James Caroll, Jesse W. Lazear and Aristides Agramonte as associates. Of these Lazear died of yellow fever and Caroll took the disease but recovered. Dr. Reed has since also died, of spotted fever, which he contracted while investigating this fatal cattle disease. The commission was stationed at Cuba (Havana) and the outcome of their investigation may be summarized as follows.

1. The specific primary cause was not found, but it was supposed to be an organism similar to that which causes malaria.

2. The fever is transmitted by the bite of a mosquito.

3. The mosquito must harbor the infecting agent about 12 days before it becomes actively transmissible to man. The earlier bites (1–8 and 10 days) are harmless.

4. The mosquito *Aedes calopus* is the intermediary host of the primary cause of yellow fever, while man is the definitive host.

5. Yellow fever has an incubation period of from 41 hours to 5 days.

6. Yellow fever is not carried or spread by fomites. The use of disinfectants is of no avail against the spreading of yellow fever.

7. One attack of yellow fever establishes immunity to subsequent attacks.

8. Yellow fever can be prevented by destroying the specific carriers of the disease, namely the *Aides calopus.*

9. The *Aides calopus* (formerly *Stegomyia calopus*) must feed upon a yellow fever patient during the first few days of the disease in order that said mosquito may become a carrier of the disease.

Insect powder (Pyrethrum) is employed to destroy mosquitoes in houses. Sprays, crude oil, etc., are used on ponds, pools, and stagnant water in yellow fever districts to destroy the mosquito larvæ. Drainage of wet lands, of swamps, of pools, etc., prevents the development of mosquitoes. Rain barrels and cisterns may breed the yellow fever mosquito. Cold weather and frosts check yellow fever because the mosquitoes are killed.

The work of the yellow fever commission made possible the digging of the Panama canal and yellow fever epidemics are a thing of the past. The work of the commission is far better known and better appreciated in Europe than it is in the United States. It should also be mentioned that there were many others attached to the commission whose names are not generally mentioned, as soldiers, nurses and attendants, who are deserving of much credit for the success of the remarkable work done.

K. Pellagra.—Pellagra is a disease which has created great havoc in Italy and other Eastern countries, and which first appeared in the United States about 1907. It spread very rapidly and up to 1911 numerous cases have been reported from the Southeastern United States and from Illinois, with a few scattering cases from Kansas, Virginia, Pennsylvania, New York, Massachusetts, California, and other states. The disease is said to be caused by eating moldy corn (*Zea mays*) or foods prepared from such corn. Ceni and others declare that the primary cause is a species of Aspergillus (*A. flavescens* and perhaps also *A. fumigatus*). It is also believed that the ordinary household mold (*Penicillium glaucum*) is a primary cause. The mortality rate is very high, and the disease is said to be terrible in its effects. It first manifests itself as an eruption of the skin usually appearing in the early spring, February or March, after some variable prodromal symptoms. The skin becomes darkened and blotchy. Eczematous eruptions next appear, with desquamation. Gradually, as the older eruptions heal, while new ones form, the skin becomes rough, from which the name, *pell' agra*—rough skin—is derived. The symptoms increase from year to year. The nervous manifestations are varied and are accompanied by great suffering.

Pellagra is not contagious or infectious, though the tendency is transmitted from one generation to another. Childern of pellagrins are often born with asymmetrical heads and various other deformities. They may be idiotic or stupid and defective generally.

Acute pellagra runs a rapid course, but more generally it is chronic, the suffering continuing for years in an ever increasing ratio. The sufferers simply degenerate from year to year and die a slow terrible death.

Lombrosa, Ceni and others recognized the fact that pellagrins are mostly of the poorer class, whose principal diet is polenta, a mush made from corn meal. This much is usually prepared in large potfuls, sufficient for a week's eating, and set away, exposed to dust, dirt, flies, etc., so that these ignorant peasants often eat polenta which is more or less moldy and otherwise spoiled. Efforts were at once made to correct these conditions, but proved only partially successful as far as checking the ravages of the disease was concerned. The primary cause of pellagra is not yet discovered. Dr. Louis W. Sambon of the London School for tropical medicine asserts that maize, either sound or spoilt, is not the cause of the disease, that it is decidedly endemic in its tendencies, that its stations are closely associated with streams of running water, and suggests that a small blood sucking fly belonging to the genus Simulium is the agent by which pellegra is conveyed. Others suggest that it is a dietary disease indicated by the fact that the disease does not develop in those who use a well mixed and well balanced diet.

L. Syphilis.—The primary cause of syphilis is the *Treponema pallidum* (*Spirochaeta pallida*), belonging to the group of protozoa known as the Zoömastigophora (Flagellata). The life history is still unknown. The full life cycle appears to be far more complex than was originally supposed. The male organism is the form usually recognized as the Treponema of syphilis. The female cell is supposed to develop in certain body cells, as the lymphocytes and the endothelial cells. The idea of the bisexual nature of the organism is gaining more and more credence among bacteriologists. These matters cannot be entered into in a work of this kind.

Syphilis as well as gonorrhea are filth diseases in the sense that with absolute physical cleanliness, as well as moral cleanliness, these diseases could not exist. Sanitarians have made the interesting observation that cities and towns that were subjected to a thorough cleaning up as a safeguard against the spreading of some infectious disease, such as cholera or typhoid fever, also showed a decrease in cases of the so-called social diseases. It is known that in those establishments for prostitutes where physical cleanliness is required and strictly enforced (primarily for business reasons only), the case rate for the two diseases is much reduced. While it is true that the great majority of cases are traceable to promiscuous intercourse, this factor *per se* plays no part in the dissemination of the two diseases, excepting in so far as this promiscuity increases the chances for contact with physical uncleanliness on the part of both sexes, the female in particular. A decrease or increase in promiscuity has no pri-

mary influence as to any increase or decrease in cases. The reason why the morally clean are quite free from the diseases is because they do not expose themselves. The immoral men and women (that is, sexually highly promiscuous) might be equally free from infection provided they were themselves physically clean and kept away from those who were infected.

It is essentially chronic in its course, the effects being apparent even in the third and fourth generations. Primitive races are said to have been free from this disease until the advent of civilization, yet the disease is of great antiquity having been widespread in ancient Rome and Greece. It is very infectious *via* abrasions, cuts and all breaks in the continuity of the skin and mucous membranes. The infection is carried by all manner of exposed objects, as clothing, dentist's instruments, pipes, dishes, drinking vessels, etc., in fact anything and everything which may have been in contact with a syphilitic. The primary lesions of the patients are very infectious.

The disease is readily preventable. All that is necessary is to keep away from the carriers of the infection. Syphilitics should be isolated until cured. The disease is very readily kept under control by the proper remedial agents, but persistency in the use of medicines is necessary to effect a cure. Ehrlich's 606 (Salvarsan), is considered in the nature of a specific, given in hypodermic, intramuscular or intravenous injections.

M. Gonorrhea.—This is also a filth disease. The primary cause is the non-sporogenous *Micrococcus* (*Diplococcus*) *gonorrheæ*. It is not infectious but exceedingly contagious to mucous membranes. As *Ophthalmia neonatorum* (ophthalmia of the new-born) it is a very fruitful cause of blindness. The suppurative discharges from patients are highly contagious. The contagion is carried by patients and by the articles touched or handled by them. The disease is difficult to eradicate from the system. It is not so frequently localized in urethra and vagina as is generally supposed, but it may travel to the bladder, kidneys, joints, etc., and it may be general upon nearly all mucous membranes of the body. It is very apt to become chronic, giving rise to very serious after effects. Syphilis and gonorrhea have the following in common.

1. Both are highly contagious by direct contact, but particularly so to mucous membranes. They are in no sense infectious and are epidemic or general only in proportion to the number of contact inoculations. The chief carriers and disseminators of the contagions are the women in public houses and the male frequenters of such houses. Lack of personal cleanliness is a very fruitful source of spreading the infection.

2. The innocent (infants, children and adults) are occasionally infected through contact with those afflicted with the diseases, as in shaking hands, kissing, contact with clothing and other articles used by those already in-

fected. Physicians, dentists, and nurses may become accidentally infected. Physicians and dentists may inoculate patients accidentally, through the use of improperly disinfected instruments; this is, however, quite rare. Contaminated drinking vessels, spoons, forks, etc., may transmit the infection.

3. In both diseases the primary causes are readily destroyed by the use of disinfectants. With absolute cleanliness the diseases could not exist. In brief, the two diseases could not exist if moral and physical cleanliness prevailed.

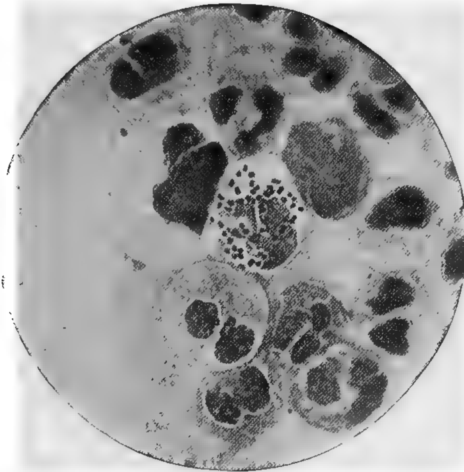


FIG. 96.—Gonococcus and pus cells from theurethral discharges of acute gonorrhea The organism is readily demonstrated by the usual staining methods, using monhylene blue or Gram's method. The Gonococcus is cultured with some difficulty (use blood serum-agar in incubator at 37°C.). There are several other cocci resembling the Gonococcus in form, but these differ in that they can be cultured in ordinary media at the room temperature. (*Williams.*)

4. Both diseases are difficult to cure as already stated. Both are and do become general or systemic in character, and are not local as is generally supposed. Those suffering from these diseases should be isolated and should never be allowed to come in close contact with the innocent.

5. Physicians, pharmacists and nurses should act as public agents in giving information regarding the transmissibility of, and the difficulty of curing syphilis and gonorrhea and pointing to clandestine prostitution as the most active source of the contagion. It should be made a criminal offense for a syphilitic to convey the contagion to an innocent person. In the army and navy the men receive careful instruction as to preventive measures. This was found necessary as the prevalence of these diseases incapacitated a large percentage of the men from active duty.

In the treatment and cure of syphilis mercurial and arsenical preparations and the iodides play a very important part. In the treatment of gonorrhea, disinfectants, especially silver nitrate and protargol, play a very important part. The antigonorrheic bacterin has been used with some success as a prophylactic and as a cure in chronic cases. Only competent physicians can treat these diseases properly. All advertised and patented "quick cure" remedies are fakes.

Ehrlich and Hata have discovered what appears to be a specific in the treatment of syphilis, namely, intramuscular and intravenous injections of dioxydia-amidoarsenobenzol (Salvarsan, or "No. 606"). The tests thus far made have yielded astonishing results. Many of the most severe forms of the disease have been promptly cured by a single dose of this remedy.

The Wassermann or Wassermann-Noguchi test for syphilis is now generally applied to determine whether or not the Spirochæta is in the system. The reaction is due to certain bodies in the blood serum of syphilitic persons that display a marked affinity for lipoids and in particular, lecithin. Many workers now use, as antigen, an emulsion of lecithin or guinea-pig heart, in place of the watery emulsion of the liver obtained from a syphilitic fetus as described by the originators of the reaction; the advantages being that lecithin and guinea-pig's heart are always on hand and alcoholic extracts are more stable than watery extracts.

The following is an outline of the method of procedure as given by George Gillman of San Francisco.

1. Antigen (a) (original Wassermann); the liver of a syphilitic fetus is cut into very small pieces and an emulsion made of it by shaking with normal salt solution (0.85 per cent.) in the proportion of one (1) part of the liver to five (5) parts of the salt solution. After the shaking is completed, the supernatant liquid is removed and clarified by centrifugalization, after which the clear liquid is pipetted off, one-half of 1 per cent. of phenol added and stored on ice until wanted for use.

(b) If lecithin is to be used as the antigen, it is prepared as follows: Make up a solution of pure lecithin in alcohol; of this alcoholic solution, a quantity equal to 0.1 gm. of lecithin, is added to 100 cc. of normal salt solution. This is also stored on ice.

(c) Guinea-pig heart extract is prepared as follows: The heart is rubbed up very fine in a mortar (containing ground glass) with absolute alcohol in the proportion of one (1) gram of the heart to 25 cc. of absolute alcohol. It is then heated to 60° C. for an hour, filtered through filter-paper and kept in the refrigerator ready for use.

As the strength of the antigen will vary in different preparations, it must be standardized before being used. It should be of such strength

that the quantity used will not hemolyze 1.0 cc. of a 5 per cent. suspension of washed lamb's blood-corpuscles in the presence of 0.2 cc. of a known positive serum, 0.1 cc. of complement, and 2 minimal units of the hemolytic serum. The unit is determined as follows: A series of test-tubes are prepared each containing the same quantities of the reagents mentioned above and varying amounts of the antigen. The usual technic is followed and the unit determined by the quantity of antigen that inhibited hemolysis. After this determination the same antigen must be tested with a known negative serum used in place of the positive serum and using double the unit of antigen. This double unit should not inhibit hemolysis of the blood cells. The unit being determined, the antigen is so diluted that 1.0 cc. will contain the unit.

2. *Antibody.*—The blood serum or cerebrospinal fluid of the syphilitic person. A sufficient quantity of the patient's blood is collected from the lobe of the ear or finger tip, in any sterile vial (best in a Wright's capsule), aseptic precautions, of course, being observed. The blood is then centrifugalized and the serum used. The spinal fluid is obtained in the usual manner by lumbar puncture.

3. *Complement.*—The normal blood serum of a guinea-pig. The blood from one guinea-pig is required, thus making it necessary to sacrifice one animal for each test. The blood must be used fresh, as the serum loses its complementing value if kept over twenty-four hours. The blood is defibrinated, centrifugalized, and the serum used. If stored, it should be frozen.

4. *Hemolytic Serum.*—The blood serum of a rabbit that has been injected with washed lamb's blood-corpuscles. The rabbit is immunized as follows: The lamb's blood is first obtained, best by cutting its ear and allowing 10 cc. of blood to run into 30 cc. of a 1 per cent. sodium citrate solution in normal salt solution. (This will prevent the blood from clotting). It is then centrifugalized, the supernatant fluid pipetted off, and the blood-corpuscles washed with normal salt solution by repeated centrifugalization and dejection of the supernatant fluid. Five cc. of the washed blood-corpuscles are injected into the rabbit five (5) or six (6) times at repeated intervals of five (5) days. On about the tenth day after the last injection, blood is taken from the rabbit, centrifugalized, and the serum used. Before using this serum, it is necessary to test its power after being inactivated (heated for threequarters of an hour at 56° C. to destroy complement). The test is made to determine the minimum quantity of the serum that will hemolyze 1 cc. of the 5 perfcent. suspension of lamb's blood-corpuscles, with 0.1 cc. of complement (normal guinea-pig serum). Various quantities of the serum to be tested are put in a series of test-tubes with 1 cc. of the suspension of lamb's blood corpuscles and 0.1 cc. of the complement in each tube. The tubes are put in the

incubator at 37° C., for an hour and then examined to determine the smallest quantity of serum that produced hemolysis. (The proper quantity is usually 1 cc. of a 1 in 2000 dilution, in normal salt solution. The quantity necessary for the reaction is two minimal units, thus 1 cc. of a 1000 dilution is used for the reaction). The dilution used should never be lower than 1:1000. If it happens to be lower it will be necessary to give the rabbit a few more injections of blood-corpuscles, before using its serum.

5. *Lamb's Blood Corpuscles.*—Five cc. of defibrinated lamb's blood are collected and washed with normal salt solution in the same way as the rabbit's blood. Then a 5 per cent. suspension in normal salt solution is made.

The antigen, the patient's serum and the hemolytic serum must be inactivated (to destroy complement) before using, by heating them for three-quarters of an hour at 56° C. The two sera should be inactivated as soon as made.

The antigen, antibody (patient's serum) complement, and hemolytic serum should each be so diluted with normal salt solution that 1 cc. of the dilution will contain the necessary quantities needed for the reaction.

Technic for Performing the Reaction.—Into a test-tube place 0.2 cc. of the antigen, 0.2 cc. of the patient's serum (antibody), and 0.1 cc. of the complement. Incubate at 37° C. for three-quarters of an hour and then add 1.0 cc. of the solution of hemolytic serum, containing two minimal doses and 1.0 cc. of the 5 per cent. suspension of lamb's blood-corpuscles. Incubate the whole for two hours, place in the refrigerator over night, and then note if hemolysis has occurred. If the antibody of syphilis is present in the suspected blood serum, hemolysis will not occur because the complement is "fixed" to the immune body by the aid of the antigen and the reaction is positive. Should the suspected blood serum not contain the specific antibody, hemolysis will occur because there is no immune body to "fix" the complement, therefore causing the hemolytic amboceptor (hemolytic serum), by the aid of the red corpuscles, to fix the complement, producing hemolysis and the reaction is then negative.

The substances employed are subject to many external influences, and it is, therefore, necessary to control their action. The controls made are necessary in order to demonstrate that none of the employed substances alone "fix" the complement, and that the occurrence of either a positive or a negative reaction, when testing a suspected serum, is due to and dependent upon the fixation or non-fixation of the complement by means of the immune body.

The quantity of antigen used for the reaction may have to be either increased or decreased. The controls will indicate when a change is required and the proper quantity necessary is determined by the method given under the preparation of the antigen.

There are many other tests to demonstrate the presence in the human organism of syphilis; as the chloride of gold test which within recent years has gained much favor among clinicians; the gum mastic test is considered excellent in the hands of some workers; etc. For a time laboratory workers recommended a microanalytical method for the demonstration of syphilis but it never met with favor.

There are many other communicable diseases as measles, mumps, scarlet fever, and whooping cough, besides the diseases due to the attacks of higher parasites, as itch, trichinosis, tapeworm, roundworm, liver flukes, hookworm, etc., which we will, however, not discuss more fully. The suggestions given under the diseases described will also apply, in a measure to other communicable diseases. Summed up briefly, preventive medicine direct and indirect, consists of giving heed to the following.

1. Living in accord with the most approved methods of hygiene. This is direct preventive medicine.

2. Treating disease in accord with the most approved modern methods. This is indirect preventive medicine because it protects the well against infection from the sick.

The following table of communicable diseases giving the average period of incubation (also known as latent period), the primary cause, nature of communicability and principle carriers or sources of infection, will be found useful.

Name of disease	Incubation period, day	Primary cause	Nature of communicability	Carrier or suorces of infection
Anthrax or wool sorter's disease.	2	Bacillus anthracis	Infectious and contagious.	Cattle, sheep.
Bubonic plague	4–6	Bacillus pestis	Infectious	Rats, mice fleas, filth.
Asiatic cholera	2–4	Bacillus choleræ	Infectious and contagious.	Flies, polluted water and food.
Diphtheria	2–3	Bacillus diphtheria	Infectious and contagious.	Animals, foods, the sick.
Erysipelas	4–6	Streptococcus pyogenes.	Very contagious to wounds.	Dirt, perhaps flies, mosquitoes.
Influenza, Grippe	1–4	Bacillus inflenzæ.	Infectious, not contagious.	Air, and exposed objects.
Glanders	3–5	Bacillus mallei	Contagious and infectious.	Horse and horse-like animals.
Gonorrhea	3–5	Micrococcus gonrheæ.	Very contagious, not infectious.	All contaminated objects.
Mumps	10–16	Unknown	Very infectious	Air and the sick.
Malaria	6–10	Plasmodium malariæ.	Infectious, not contagious.	Mosquitos (Anopheles.)
Relapsing fever	5–6	Spirochæta Obermieeri.	Infectious	Insects, as bed bugs, etc.
Measles	8–9	Unknown	Very contagious,	Exposed objects.

Name of disease	Incubation period, day	Primary cause	Nature of communicability	Carriers or source of infection
			also infectious.	
Hydrophobia............	20–60	Unknown........	Contagious to wounds.	Mad dogs, wolves and other canines
Rubeola, rubella.........	18	Unknown........	Infectious and contagious.	Exposed objects.
Scarlatina..............	2–5	Unknown, perhaps Protozoa.	Infectious and contagious.	Exposed objects.
Small-pox..............	12	Unknown........	Infectious and very contagious.	Exposed objects.
Syphilis.......	14–30	Spirochæta pallida	Very contagious, especially to lesions.	Exposed objects. A filth disease.
Tetanus, lock-jaw........	2–3	Bacillus tetani ..	Contagious to lesions only.	Dirt, infected objects of all kinds.
Typhoid fever...........	14	Bacillus typhosus	Infectious and contagious.	Polluted water and food. Flies.
Vaccinia................	3–6	Unknown........	Contagious by inoculation only.	Cow virus, human vaccinia.
Varicella, chicken-pox. ..	14–15	Unknown........	Contagious	Those affected.
Whooping cough.........	8	Unknown........	Very infectious...	Exposure to those affected.
Yellow fever............	3–4	Unknown, plasmodium?	Infectious, not contagious.	Mosquitos (Aedes-calopus).
Leprosy................	Weeks, months, years.	Bacillus lepræ....	Infectious and contagious.	The patients.
Tuberculosis............	Weeks and longer.	Bacillus tuberculosis.	Infectious........	Sputum, milk from tubercular cows.
Dengue.................	4	Protozoa?........	Infectious........	Mosquito (culex fatigans).
Pneumonia..............	1–2	Micrococcus pneumoniæ (Diplococus.	Infectious........	Carried by persons.
Dysentery (bacillary	8	Amæba dysenteviæ	Infectious........	Pollusted water supply.
Malta fever.............	6–10	Micrococcus melitensis.	Infectious........	Goats' milk, stings of insects.
Beri-beri...............	Months	Micrococcus?	Infectious........	A tropical disease.
Pellagra...............	?	?	Neither infectious nor contagious.	The fly *Simulium* (?)

In some diseases the *mortality rate* is very high, as in yellow fever, beri-beri, tetanus, cholera, plague and leprosy. In others it is low, as in syphilis, gonorrhea, malaria, whooping cough, mumps and varicella. In certain diseases the prognosis is rather uncertain, the mortality rate being high at times and again low, as in scarlatina, small-pox, measles and grippe. In the recent grippe or influenza pandemic the mortality rate was unusually high. In this disease the mortality rate is high at the early periods of the invasion, gradually growing less and less severe. It may be that the causative organism, whatever it may be, loses in virulency in its passage through the hosts. The hemorrhagic form of smallpox has a mortality rate of nearly 100 per cent. whereas some epidemic forms of this disease are very mild, showing practically 100 per cent. recoveries. The so called black scarlatina shows a very high mortality rate. Some epidemics of measles show a comparatively high mortality rate. Some diseases run a somewhat variably rapid course as pneumonia, diphtheria, spinal meningitis, bubonic plague and Asiatic cholera, ending either in death or recovery. Other diseases, as scarlet fever, measles and diphtheria may have after-effects or sequelæ which often assume a chronic course and may finally result in death. Certain diseases run a regular course which varies but little as to the sequence of symptoms and duration, as typhoid fever (five weeks). Others run a variably chronic course, ending either in death or recovery, as pellagra and malaria. Some diseases are very persistent, difficult to eradicate from the system, showing certain effects even to the third and fourth generations, as tuberculosis and syphilis Malaria leaves certain after-effects, as enlarged spleen ("ague cake"), which may persist through life.

Savage races are peculiarly susceptible to certain diseases, as tuberculosis, small-pox, gonorrhea and syphilis and peculiarly enough these diseases did not originate with primitive peoples, but with advanced civilization, though of great antiquity.

DISSEMINATION OF DISEASE

The manner in which disease is spread has been indicated in the precedeing but the subject has not been fully discussed. The student of pharmacy should have a course in general sanitary science and in preventive medicine. The general principles of immunity have been discussed and the more important communicable diseases have been briefly outlined, but the vast subject of preventive medicine has not been touched upon. This must be taken up in a separate course.

The agents and agencies concerned in the spreading of communicable diseases and others, constitute the groundwork of preventive medicine.

TABLE OF THE INSECT CARRIERS OF EPIDEMIC DISEASES

(Compiled by Dr. J. J. V Manning)

COMMON NAME	SCIENTIFIC NAME	DISEASE (Proven)	DISEASE (Suspected)	VICTIM
PASSIVE CARRIERS				
1. House Fly, Tyhpoid Fly, Septic Fly	Musca domestica	Tyhpoid, Summer Diarrhoea, Asiatic Cholera, Tuberculosis, Bacillus of Green Pus, Yaws (Staggers)	Gangrene, Hookworm, Bubonic Plague, Egyptian Ophthalaia (Sore Eyes, Anthrax	Man. Cattle and Sheep.
2. Little Fly, or Gnat	Hippelates flavipes	Pink Eye		School Children.
3. Itch Mite	Sarcoptes scabei	Seven-Year Itch, Suppurations, Running Sores	Leprosy	Man. Children.
ACTIVE CARRIERS Which may be Intermediary Host of Disease Germ or Necessary Host, in which the Organism Undergoes a Developmental Cycle.				
4. Mosquito	Anopheles maculipennis, Stegomyia calopus, Culex fatigans, Stegomyia fasciata	Malaria (Ague or Chills and Fever, Yellow Fever, Dengue or Breakbone Fever, Elephantiasis, Kala Azar, Dumdum Fever of India	Rocky Mountain or Spotted Fever Delhi Boil, Leprosy, Malta Fever, Chorea	Man, Children, Bat Wild Mammals Goat.
5. Ticks	Margaropus annulatus, Dermacentor venustus, Ornithodorus moubata	Texas Fever, Rocky Mountain Fever or Spotted Fever, Relapsing Fever, African Tick Fever	Bugonic, Surra	Cattle, Man, Small Wild Mammals, Rodents, Horse, Mule, Camel, Elephant, Dog.
6. Lice	Pediculi capitis and vestimenti	Tyhpus Fever, Mexican Typhus, Relapsing Fever	Surra	Man and Rodents.
7. Flea of Man, Rat, Mouse, Cat, Dog, Ground Squirrel	Pulex irritans, Laemopsylla cheopis, P. fasciatus, P. serraticeps, C. musculi	Bubonic Plague or Black Death	Surra, Infantile Paralysis Inf. Kala Azar, Leprosy	Man. Infants.
8. Tsetse Fly. (Pronounced (Tset'zɛ)	Gloaainffl palpalis, Glossina morsitans	Sleeping Sickness of Africa	Nagana (an epidemic disease of Africa identical with Surra of Asia	Man. Antelope and Wild Pig. Horse. Camel. Dog.
9. Sand Fly	Phlobotomus, Simulium reptans (Italy), Simulium vittatum	Dengue or Breakbone Fever, Pappa taci Fever of Italy, a Fatal Malaria	Pellagra	Man.
10. Buffalo Gnat, or Black Fly	Simulium sp		Infantile Paralysis (Iowa State Board of Health	Children.
11. Biting Stable Fly	Stomoxys calcitrans	Epidemic Poliomyelitis (Infantile paralysis	Surra	Man, Horse, Monkey, Mule, Camel, Elephant. Cat, Dog.
12. Bedbug	Cimex lectularius	Bubonic Plague, Kala Azar, the Black or Dumdum Fever of India and Italy, Tubercylosis, Leprosy	Epidemic Poliomyelitis (Infantile Paralysis, Beri-Beri Relapsing Fever	Man.
TWO FLIES OF MANY WHOSE LARVAE FEED ON MAN.				
13. Screw-worm Fly, or Gray Fly	Chrysomyia macelaria	Deposits eggs on wounds or in ears or nostrils of sleeping children or adults. Larvae burrow on hatching, causing great pain, and sometimes death		Man.
14. Congo Floor Maggot	Ochromyia anthropophagi	Fly deposits eggs in cracks of floor. Hatched larvae suck blood of sleepers at night		Man.

Of almost equal importance is a knowledge of the agencies which man may make use of in the control of the agencies of disease dissemination. The proper dissemination of a knowledge of sanitary science is of even greater importance than is sanitary legislation.

The table on opposite page and the classification of disease carriers and the outline of a course in sanitary science will serve to indicate the ground that is to be covered in a course for students of pharmacy.

CHAPTER XVIII

SUGGESTIONS ON A MICROANALYTICAL AND BACTERIOLOGICAL LABORATORY FOR THE PHARMACIST

1. The Qualifications of the Analyst

What type and grade of scientific or analytical work should the properly trained pharmacist be prepared to do? What should his special training and qualifications be in order that he may do special work? These questions have been discussed within recent years by the leading pharmacists in the United States. It is not within the province of a work of this kind to discuss pharmaceutical education, nevertheless the following suggestions are in place.

1. The pharmacist who has had a thorough training in an adequately equipped college, assuming that he has mental aptitude, should be prepared to do special work along the following lines.

(*a*) Chemistry and toxicology.
(*b*) Pharmacy and pharmaceutical manufacture.
(*c*) Pharmacognosy and microanalysis.
(*d*) Bacteriology.

2. For some time to come the specialists in pharmacy will tend to work in more than one branch; or rather, the special endeavor will be developed through correlated branches of science. Thus the toxicologist will advance through chemistry and pharmacology. The pharmacognosist through botany and materia medica. The bacteriologist through sanitary science and microanalysis.

3. The present arrangement of courses in our leading colleges of pharmacy is such that the following specialists will arise from the ranks of the limited number of promising students.

(*a*). Toxicologists, rather than chemists or pharmacologists.

(*b*). Pharmacognosists, rather than botanists or specialists in materia medica.

(*c*.) Microanalysts, rather than bacteriologists or sanitary officers.

It is true that a number of specialists employed in state and municipal laboratories and in pharmaceutical laboratories (pharmaceutical manufacture) have arisen from the ranks of the students of pharmacy, and pharmaceutical chemistry, but it is nevertheless a fact that the college curriculum is such as to train and qualify especially along the lines indicated. Our authoritative pharmacologists are without exception from

the ranks of those holding higher university degrees. Our eminent bacteriologists and sanitarians are largely men with medical and university degrees. Our leading botanists are from universities. The college of pharmacy alone (but not by any means all of them) specialize along the lines of pharmacy, pharmacognosy, toxicology and microanalysis. To a lesser degree also in materia medica and in the chemical testing and assaying of medicamenta. A few of the colleges prepare fairly well qualified sanitary assistants, if not sanitary directors. Other branches of science which may be taught in a college of pharmacy, such as botany, physiology, pharmacology, sanitary science, and bacteriology, are simplified and specially modified presentations of like course as given in medical schools and in universities. The common every day practices of pharmacy, such as filling prescriptions, pill rolling and tablet making, cannot be rated as science. These operations come under the head of art.

4. As to microanalysis and bacteriology considered as practical working specialties, the following is offered.

(*a*). The two specialties go together and merge into one another. The microanalyst should be qualified to do bacteriological work, and *vice versa*, the bacteriologist must be prepared to do microscopical work.

(*b*). The college course in microanalysis must be comprehensive and should serve as a basis for the bacteriological work (the direct bacteriological methods).

(*c*). The course in bacteriology should be well flanked by the correlated sciences, zymology, parasitology, serology, immunology and general sanitary science.

The following outline will serve to explain the scope of the correlated sciences, microanalysis, bacteriology and sanitary science, and the preparation which is necessary to do efficient laboratory work. The subjects outlined are to be taught in the third and fourth years of a college of pharmacy having the usual university entrance requirements.

Course I. General Microanalysis.—Four hours of laboratory work each week, during the entire college year. This course is to be given during the third year of the full four year course and is to follow the laboratory course in pharmacognosy and in plant histology.

A. THE MICROSCOPICAL EXAMINATION OF FIBER, FOODS AND DRUGS

I. Examination of Fiber.

1. Vegetable Fiber.
   - (*a*) Cotton. Cotton cloth. Mercerized cotton cloth
   - (*b*) Paste board. Wrapping paper. Tissue paper.
   - (*c*) News-paper. Filter paper. Etc.
   - (*d*) Book paper. Bank note. Etc.
   - (*e*) Writing paper.

(*f*) Cordage. Thread. Etc.
(*g*) Hemp fiber and cloth.
(*h*) Linen cloth. (Linen tester.)
(*i*) Artificial (viscose) silk.

2. Animal Fiber.
(*a*) Human hair.
(*b*) Hair of other animals. Wool. Bristle.
(*c*) Woolen cloth.
(*d*) Camel's hair. Alapaca. Mohair.
(*e*) Silk fiber. Silk cloth.
(*f*) Artificial (gelatine) silk.

3. Mixed Fiber. Animal and Vegetable.
(*a*) Government note. Bank note.
(*b*) Felt paper.
(*c*) Shoddy.
(*d*) Mixed cloth (wool and cotton).

4. Inorganic Fiber.
(*a*) Glass fiber. Glass wool.
(*b*) Asbestos.
(*c*) Metal fiber.

II. Commercial Starches.
1. Corn starch. Rice starch. Wheat starch.
2. Potato starch. Sweet potato starch. Banana starch.
3. Arrowroot starches, etc.

III. Dextrins. A comparative study should be made of the different kinds of dextrins in order to determine the source of the starch used, the degree and character of the dextrinization, etc.

IV. Starch Fillers. A study of starch fillers used in sausage meats.

V. Ice Cream Fillers. Kinds. Starch fillers. Gum arabic. Tragacanth, etc. Fillers combined with milk coagulants (rennet).

VI. Flours and Meals.
1. Cereal flours. A critical comparative study should be made of wheat, rye, rice and barley grains and the flours made therefrom. The hand gluten test. The Bamihl test and the Winton modification of the Bamihl test. Processed flours and chemical tests for bleached flours. Polished rice.
2. Oat meal and corn meal. Make a comparative study. Note the distinct polarizing bands in the corn starch.
3. Buckwheat flour. Italian Buckeye meal, etc.
4. Pancake flours. Mixed flours. Make estimates of the percentages of the different flours in the compound or mixture.
5. Banana meal. Squash meal, etc.

VII. Comparative Study of Brans.—Wheat, rye, rice, barley and corn brans. Middlings.

VIII. Cotton Seed Cake Linseed Cake. A comparative study.

IX. Prepared Starches, Flours, and Meals.
1. Spaghetti, macaroni, noodles.
2. Sago, etc.

X. Bread and Pastry. Examine as to the identity of the materials used, as to kind of flour, etc.
1. Breads, biscuits, rolls, etc. Examine as to the identity of starch, use of mixed flours, absence or presence of yeast cells, etc.
2. Cake, cookies, "Arrowroot biscuits," etc.

XI. Breakfast Foods. These are to be examined as to the material used, ascertaining manner of manufacture and the absence or presence of substances declared on the label.

1. Flaked corn and wheat.
2. Rolled corn and wheat. Mixtures.
3. Puffed rice and wheat. Manner of manufacture. Chinese and Hindoo puffed rice.
4. "Cream of wheat," "Carnation mush," etc.
5. Shredded wheat, grape nuts, etc.

XII. Baby and Invalid Foods. These are to be examined as to the presence of flours, of unaltered starch, as to the identity of starch, use of milk or cane sugar, presence of dried milk, of casein, etc.

1. Dried milk and casein. Pure and mixed.
2. Starchy baby foods.
3. Horlick's malted milk.
4. Borden's condensed and malted milk.
5. Eskay's food.
6. Peptogenic milk powder, etc.

XIII. Spices and Condiments. These are to be examined as to identity and quality (organoleptic testing) and as to absence or presence of adulterants.

1. Pepper.—Black and white. Processed white pepper (bleached).
2. Capsicums.—Hungarian, Mexican, American, etc.
3. Allspice and allspice stems.
4. Cloves and clove stems. Exhausted cloves.
5. Nutmeg, mace. False mace. Adulterants.
6. Cinnamons. Adulterants and quality.
7. Mustard. Prepared mustards. Mustard hulls.
8. Herbaceous condiments. Marjoram, sage, thyme, etc.
9. Umbelliferous spices. Curry powders, etc.

XIV. Dairying Products.

1. Milk. Make a critical comparative study of normal cow's milk, pasteurized milk, boilt miik, evaporated milk and condensed milk, including bacterial content, presence of blood corpuscles, pus corpuscles, sediment, etc.
2. Sour milk, klabbered milk, buttermilk. Examine as to bacteria and other organisms present. Dutch hydrogen peroxide test. Examination of washed and centrifugalized samples.
3. Cream. Whole milk. Half milk. Skimmed milk.
4. Butter and butter substitutes.
5. Cheese and cheese parasites.

XV. Home Drinks.

1. Coffee. The normal and roasted bean. Ground coffee. Coffee substitutes. Dekofa. Cereal coffee. A careful study of ground coffees and their more common admixtures and adulterants. A study of coffee substitutes as to composition.
2. Teas. Qualities and grades. Government standards and tests. Tea culture in the United States.
   (a) Coloring substances. Reed and West tests for color.
   (b) Exhausted teas. Tea adulterants. Japanese teas.
   (c) Tea substitutes.
3. Cocoas and Chocolates.

(*a*) Cocoas and chocolates. Method of manufacture.
(*b*) Cocoa shells.
(*c*) "Soluble cocoas."
(*d*) Cocoa butter.
(*e*) Adulterants of cocoa and chocolates.

XVI. Food Products, Animal and Vegetable. Samples may be secured from private homes, grocers and canneries. They are to be examined as to identity, quality and purity and the findings recorded on special report cards. In the examination of these substances the polarizes, the micrometer scale, the Thoma-Zeiss hemacytometer (Turck ruling) and other necessary apparatus are used. The Lagerheim sublimation tests for benzoic acid and salicylic acid and the Curcuma thread test for boric acid and the starch paper test for sulphurous acid are used.

1. Canned meats. Canned fish. Anchovy pastes, etc. Examine for mold and bacterial contamination and the presence of preservatives.
2. Sausage meats. Examine for starches and starch fillers, preservatives and added coloring substances.
3. Jams and jellies. Examine as to identity, use of green fruit, fruit refuse, preservatives, yeast, bacteria, mold. Presence of agar or other fillers.
4. Catsups and tomato pastes. Examine for preservatives, mold, bacteria and yeast cells, tomato refuse, starch, etc.
5. Preserved and pickled fruits. Examine for bacteria and yeast, for sulphurous acid (bleached fruits) and for preservatives. (Leaks and swells).

XVII. Candies. Qualities and Grades. They are to be examined for various fillers (starch, flour, gums, etc.), nature of coating, coloring matter, impurities, etc.

XVIII. Vegetable Drugs, Crude and Powdered. Compound Powders, Pills, Tablets, Extracts. Examine as to quality and purity, ash content (including test for acid insoluble ash), fineness of powders, organoleptics tests, etc.

1. Powdered vegetable drugs.
2. Compound powders. Dusting powders. Face powders.
3. Cattle powders.
4. Poultry powders.
5. Extracts, solid and fluid.
6. Medicinal teas.
7. Pills.
8. Tablets.
9. Crude drugs. Pressed herbs.
10. Patent and proprietary preparations of an organic nature.
11. Calomel, charcoal, mercury, sulphur.
12. Pastes, plasters, ointments.
13. Snuffs, cigarettes, tobaccos.
14. Unknowns.

The sequence of the several operations of the complete analysis of a sample of powdered vegetable drug may be given as follows:

1. Noting the condition of the seals of the sample or package. Breaking the seals.
2. Thoroughly mixing the sample. Selecting an average sample.
3. Organoleptic testing (consistency or feel, color, odor, taste).
4. Determining the fineness by means of a suitable nest of sieves.
5. Preliminary examination of the average sample and of the samples upon the different sieves, using pocket lens, tweezers, etc. Organoleptic testing of individual fragments, etc.

6. Special examination (macroscopical and microscopical) of the several portions on the different sieves if thought desirable or necessary.

7. Again mixing the several portions on the several sieves and reducing to uniform fineness, if thought desirable or necessary.

8. Complete and thorough microscopical examination.

9. Ash determination if thought desirable.

10. Acid insoluble ash determination if thought desirable.

11. Special tests if thought desirable.

12. Recording the results of the analysis.

B. THE MICROSCOPICAL EXAMINATION OF THE BODY

I. External Tegument.
- 1. Hair and scalp.
  - (*a*) Macroscopic parasites. Eggs, larvæ.
  - (*b*) Microscopic parasites.
  - (*c*) Sebaceous deposits and dirt.
  - (*d*) Epithelial cells and other tissue cells.
  - (*e*) Evidences of diseased tissues.
  - (*f*) Powders used, etc.
  - (*g*) Hair tonics, ointments, hair oils, etc.
- 2. Face.
  - (*a*) Eye secretions, normal and abnormal.
  - (*b*) Secretions of the ear.
  - (*c*) Facial hair.
  - (*d*) Face lotions, ointments, etc.
  - (*e*) Face powders, etc.
  - (*f*) Eruptions and other abnormal conditions.
- 3. Skin—Normal and abnormal. Proceed much as for Hair and Scalp.
- 4. Finger Nail Deposits.
  - (*a*) Nature of deposits.
  - (*b*) Interpretations of deposits.

II. Internal Tissue.—Normal and Abnormal.
- 1. Muscular Tissue.
  - (*a*) Normal.
  - (*b*) Parasite—Trichinæ, etc.
  - (*c*) Pathological conditions.
- 2. Nervous Tissue.
  - (*a*) Spinal cord—Negri bodies in rabies.
  - (*b*) Brain.
  - (*c*) Fibers and ganglia.
  - (*d*) Terminal nerve elements.
- 3. Osseous Tissue.
  - (*a*) Osseous elements.
  - (*b*) Periosteum.
  - (*c*) Marrow.
- 4. Cartilaginous Tissue. Kinds.
- 5. Connective Tissue.
- 6. Adipose Tissue.

III. Digestive Tract.
  1. Mouth and Teeth.
    (*a*) Epithelium and other normal tissue elements.
    (*b*) Food particles.
    (*c*) Bacterial flora.
    (*d*) Pathological conditions.
  2. Stomach.
    (*a*) Normal contents, digestion, action of ferments.
    (*b*) Vomited material.
    (*c*) Pathological conditions.
  3. Intestines.
    (*a*) Small intestines.
    (*b*) Large intestines. Colon. Fæces.
    (*c*) Parasites.
    (*d*) Pathological conditions.
    (*e*) Test diets, Value and significance of.
IV. Genito-urinary Tract. Male and Female. Normal and Abnormal.
  1. Epithelial cells, etc.
  2. Urinary sediments.
  3. Pathological secretions.
V. Blood Work.
  1. Normal.
  2. Pathological
  3. Blood counting.
VI. Respiratory Tract.
  1. Nasal secretions.
  2. Expectorations and Secretions.
    (*a*) Buccal and pharyngeal. Throat.
    (*b*) Bronchial sputum.
    (*c*) Pulmonary sputum.

C. URINARY SEDIMENT. MICROSCOPICAL EXAMINATION

This outline includes both the organized and the unorganized sediments and deposits.
I. Crystalline and Amorphous Chemical Deposits and Sediment.
  1. Uric acid, crystalline.
  2. Uric acid compounds.
    (*a*) Acid sodium urate, (generally amorphous, occasionally crystalline).
    (*b*) Acid potassium urate (amorphous).
    (*c*) Acid calcium urate (amorphous).
    (*d*) Acid ammonium urate (crystalline).
  3. Calcium oxalate (crystalline).
  4. Earthy phosphates.
    (*a*) Ammonium-magnesium phoshate (crystalline).
    (*b*) Calcium phosphate (amorphous and crystalline).
  5. Calcium carbonate (crystalline).
  6. Calcium sulphate (crystalline).
  7. Leucin, tyrosin, cystin (crystalline).
  8. Cholesterin (crystalline).

II. Accidental Urinary Inclusions.
  1. Starch granules.
  2. Vegetable cells and tissues. Fæcal matter, etc.
    (*a*) Ducts and vessels.
    (*b*) Sclerenchyma cells.
    (*c*) Parenchymatous tissue.
    (*d*) Bast fibers.
    (*e*) Cork cells.
    (*f*) Cotton fibers.
    (*g*) Lycopodium.
    (*h*) Linen fiber.
    (*i*) Seeds and seed tissue.
  3. Animal fiber and elements.
    (*a*) Hair and wool.
    (*b*) Fragments of feathers.
    (*c*) Scales of moths.
    (*d*) Cartilage cells.
    (*e*) Fat globules.
    (*f*) Muscle fiber.
    (*g*) Fibrous tissue.

III. Urinary Concretions.
  1. Uric acid calculi.
  2. Calcium oxalate calculi.
  3. Mixed calculi.
  4. Platinum foil tests.
    (*a*) Ignition.
    (*b*) Behavior with HCl.

IV. Casts.
  1. Hyaline.
    (*a*) Pure hyaline.
    (*b*) Fibrinous.
    (*c*) Waxy.
  2. Granular.
    (*a*) Fine.
    (*b*) Coarse.
    (*c*) Pigmented.
  3. Epithelial.
  4. Fatty.
  5. Blood.
  6. Pus.
  7. Bacterial.
  8. Mixed.
  9. Crystalline (organic base).
    (*a*) Urates.
    (*b*) Oxalates.
    (*c*) Cystin.

V. False casts or cylindroids.

VI. Mucus Threads.

VII. Prostatic Plugs.

VIII. Blood. Smoky Urine. Hematuria.
 1. Normal blood corpuscles.
 2. Crenated corpuscles.
 3. Phantom corpuscles.
 4. Corpuscles associated with casts.
 5. Hemin crystals.
 6. Blood clots.

IX. Epithelial Cells. Normal and Abnormal.
 1. In order of size.
  (*a*) Vaginal (flat cuboidal and columnar. Usually associated with bacteria).
  (*b*) Bladder.
  (*c*) Cervix.
  (*d*) Urethra.
  (*e*) Renal pelvis.
  (*f*) Ureters.
  (*g*) Prostate.
  (*h*) Kidney tubules.
 2. As to form.
  (*a*) Flat or squamous.
  (*b*) Cuboidal (with or without fat granules and analogous to put corpuscles).
  (*c*) Columnar or cylindric.
  (*d*) Irregular.
 3. As to quantity or numbers.
  (*a*) Normal.
  (*b*) Excess of squamous (as in irritation and inflammation).
  (*c*) Excess of cuboidal (as in chronic inflammation. Ulceration, with pus and blood corpuscles).

X. Pus Corpuscles—Glycogenic Reaction.
 1. Normal.
 2. Amœboid.
 3. Inclusions of the cells.
 4. Decomposition changes, etc.

XI. Mucus Corpuscles.

XII. Amyloid Bodies.

XIII. Spermatozoa.

XIV. Micro-Organisms. Normal and Pathological.
 1. Urethral and vaginal bacteria—Normal.
 2. Air infection of the urine.
 3. Pathological infection of the urinary tract.
 4. Micrococcus ureæ.
 5. Yeasts.
 6. Molds.
 7. Pathogenic bacteria.
  (*a*) Streptococcus (Staphylococcus) pyogenes.
   variety albus.
   variety citreus.
   variety aureus.
  (*b*) Typhoid bacillus.
  (*c*) Anthrax bacillus.
  (*d*) Erysipelas
  (*e*) Tuberculosis.

(*f*) Colon bacillus.
(*g*) Gonococcus.

XV. Animal Parasites. Rare.
1. Mites.
2. Echinococcus.
3. Filaria sanguinis hominis.
4. Distoma hæmatobium.
5. Ascarides.

XVI. Tumor elements.

XVII. Toxic Substances. Urotoxic Coefficient.
1. Toxins.
2. Ptomaines.
3. Leucomaines.

D. EXAMINATION OF GONORRHEAL DISCHARGES FOR THE GONOCOCCUS

I. Securing the Sample.

II. Mounting and Staining the Sample.

III. Examining the Slide Mount.
1. Intercellular diplococci.
2. Intracellular diplococci.

E. EXAMINATION OF TUBERCULAR SPUTUM

I. Securing the Sample.

II. Mounting and Staining the Sample.

III. Examining the Slide.
1. Tubercle bacilli.
2. Associated organisms—Mixed infections.

F. EXAMINATION OF FÆCES

I. Preparing the Sample.
1. Diluting and washing.
2. Centrifugalizing.

II. Normal Fæces.
1. Undigested Food Particles.
   (*a*) Vegetable tissues.
   (*b*) Fruit seeds.
   (*c*) Fat cells.
   (*d*) Muscular tissue.
   (*e*) Starch, etc.
2. Bacteria.
3. Epithelial cells.

III. Abnormal Fæces.
1. Dysentery.
2. Typhoid fever.
3. Ulcerations and inflammations. Blood and pus corpuscles, etc.
4. Intestinal parasites.

G. BLOOD COUNTING

I. The Thoma-Zeiss Hemacytometer. How Used. Making the Dilutions.
II. Securing the Blood Samples.
III. Mounting the Sample.
IV. Counting the Corpuscles.
  1. The red corpuscles.
  2. The white corpuscles.
V. Examining the Fresh Blood.
  1. Manson Method.
  2. Ross method.
VI. Preparing and Staining dried Blood Films.
VII. Blood Glycogen Test. Iodophilia.
VIII. Pathogenic Organisms in the Blood.

The following blank report sheet should be used. The sample reports given will indicate how these are to be filled out, based upon the results of the analysis:

Form No. 1. Blank report sheet for the microscopical examination of organic drugs and dry food substances.

No. (Laboratory or other serial number).
Label ....
....
Sample received.......................Sample examined......................
Conditions of wrappings and seals..
Organoleptic tests..
  Consistency of Feel...
  Color...
  Odor..
  Taste...
Adjunct Tests...
  Ash...%
  Acid-insoluble ash..%
  Sand (beaker test)..%
Special Tests...
....
....
Microscopical Findings..
....
....
....
....
Conclusions...
....
....
....
....
..................................Analyst.

The following is a sample report analysis using the proposed report card:

No.: 5432.
Label: *Broken Senna, U. S. P., John Smith & Co., Kalamazoo, Michigan.*
Sample received: *August* 15, 1912. Sample examined: *August* 20, 1912.
Conditions of wrappings and seals: *Good.*
Organoleptic Tests...

Consistency or Feel: *Dry, gritty, sandy, dirty.*
Color: *Not unusual.*
Odor: *Senna-like.*
Taste: *Sandy, gritty.*

Adjunct Tests...

Ash: 19.6%.
Acid-insoluble: 9.4%.
Sand (beaker test): 9%, *sand and small pebbles.*

Special Tests: *Pebbles picked out by hand. About* 4% *senna seeds and pod fragments and stems present.*
Microscopical Findings: *The histological characters of African senna. Stem tissue excessive. Sand and dirt excessive. Senna seeds and pods present in considerable quantity.*
Conclusions: *Adulterated with sand, pebbles, senna seeds, senna pods and stems* 25%. *Misbranded becauses labeled U. S. P., whereas it is below the U S. P. standard.*

RICHARD ROE, Analyst.

Form No. II. Blank report sheet for the microscopical examination of catsups, jams, jellies, etc.:

(No., label, dates, condition of seal and organoleptic tests, as for Form No. 1.)

Adjunct Tests.
Sublimation tests for...
Benzoic acid...
Salicylic acid...
Boric acid (curcuma thread)...
Iodine reaction...
Intracellular...
Extracellular...
Special Tests...
....
Microscopical Findings.
General...
....
....
Cytometric counts.
Dead yeast cells..per cc.
Living yeast cells..per cc.
Bacteria [1]..per cc.
Mold (hyphal fragments and clusters)..per cc.
Mold spores..per cc.
Living yeasts..per cc.

[1] The total count, inclusive of rod shaped forms, coccus forms, etc., should be given.

Bacteria..per cc.
Mold (hyphal fragments)........................per cc.
Mold spores....................................per cc.
Smut spores....................................per cc.

Conclusions..
....
....

..............................Analyst.

We may give an example of a report as follows:

FORM No. II

Lab. No. 462.
Label: *Pure currant jelly. Made by Smith, Jones & Co., Nantucket, Wis.*
Sample received *August* 5, 1914. Sample examined *August* 5, 1914.
Condition of seals: *Good, unbroken sample.*
Organoleptic tests: *Not conclusive.*
Consistency or feel: *Poorly jellied.*
Color: *Normal for Currant jelly.*
Odor: *Faint, somewhat disagreeable.*
Taste: *Not characteristic, bitterish, quite acid.*
Adjunct tests.
Sublimation tests for
Benzoic acid: *Negative.*
Salicylic acid: *Very marked.*
Boric acid (curcuma thread): *Negative.*
Iodine reaction: *Very marked.*
Intracellular: *Negative.*
Extracellular: *Positive, very marked.*
Special tests: *Salicylic acid color reaction, with ferric chloride very marked.*
Microscopical examination.
General. *Some apple tissue (window cells and pulp cells) and currant tissue sclerenchyma present. Added wheat starch about 5 per cent.*
Cytometric counts.
Dead yeast cells, 80,000,000........................per cc.
Living yeast cells, *none*..........................per cc.
Bacteria, 600,000,000...............................per cc.
Mold (hyphal fragments and clusters), 84,000........per cc.
Mold spores, 5,000,000..............................per cc.
Smut spores, *none*.................................per cc.
Conclusions: *Misbranded. Adulterated with apple and with wheat starch and made from fermented and decomposed material, preserved with salicylic acid. Not fit for human consumption because of the quantity of yeast, mold and bacteria present.*

JOHN DOE, Analyst.

Course II. Bacteriological—Quantitative and Qualitative Determinations of Organisms in Foods and Drugs.

A course in general laboratory technic is the necessary preparation to this course. Such preparatory course should be given during the third year of the full four year course, which would mean that the present course must be given during the fourth year.

A laboratory course of at least one hour each day extending throughout the entire college year. The time necessary to do the laboratory work will vary from day to day. The work is to be supplemented by lectures, special readings and seminar work. The laboratory methods employed are those of the Laboratory Section of the American Public Health Association, The U. S. Public Health Service and the Bureau of Chemistry of the U. S. Department of Agriculture, in-so-far as these methods are applicable.

I. Substances to be analyzed.
  1. Liquids of all kinds.
  2. Semiliquids and semisolids miscible with water.
  3. Solids of all kinds.
II. Numerical and quantitative limits of comtamination in different substances.
  1. For mold—quantity of spores and hyphæ.
  2. For yeasts—number and kind.
  3. For bacteria—number and kind.
  4. For pus, dirt, sand, etc.
III Methods.
  1. Making concentrations.
  2. Making dilutions.
  3. Making the counts and estimates.
    (*a*) Bacteria.
    (*b*) Yeasts.
    (*c*) Mold spores and mold hyphæ.
    (*d*) Algæ, in drinking waters, etc.
    (*e*) Protozoa.
    (*f*) Pus cells, in milk, etc.
    (*g*) Dirt, sand, etc.
  4. Plate counts—Petri dish cultures.
    (*a*) Culture media used.
    (*b*) Optimum temperature.
    (*c*) Time of incubation.
IV. Qualitative determinations.
  1. Apparatus.
  2. Culture media.
  3. Stains.
  4. Special methods.
    (*a*) Colon group of bacilli.
    (*b*) Presumptive colon bacillus test.
    (*c*) Sewage streptococci.
    (*d*) Dysentery bacilli and amoebæ.
    (*e*) Bacillus typhosus.
    (*f*) Paratyphoid group.
    (*g*) Cholera vibrio.
    (*h*) Yeasts.
    (*i*) Molds.
    (1) Animal parasites.
    (*k*) Larvæ, ovæ, etc.

V. Biological water analysis.
  1. Bacteria, number and kind.
  2. Diatoms.
  3. Desmids.
  4. Nostoc.
  5. Other algæ.
  6. Molds; significance of.
  7. Evidence of soil and sewage contamination.

VI. Bacteriological milk analysis.
  1. Quantitative.
    (*a*) Standards for different geographic areas.
    (*b*) Summer and winter standards—temperature standards.
  2. Qualitative.
  3. Pus and blood corpuscles; significance of.
  4. Milk diseases.
    (*a*) Blue milk
    (*b*) Ropy milk.
    (*c*) Bad odors, bad taste, etc.
  5. Sour milk.
  6. "Buttermilk" tablets.
  7. Kefir, koumys, etc.

VII. Bacteriological Examination of Shellfish.
  1. Selection of sample.
  2. Making a record of the sample.
  3. Transportation of the sample.
  4. Laboratory procedure.
  5. Bacterial counts.
  6. Determining bacteria of the colon bacillus group.
  7. Statement of results. Rating.

VIII. The Bacteriological and Toxicological Examination of Meat and Meat Products.
  1. Direct microscopical examination of meats.
    (*a*) Bacteria on the surface of meats.
    (*b*) Mold and mold spores, as in moldy bacon, pork, fish, etc.
    (*c*) Presence of bladder worm, larvæ of parasites, etc.
    (*d*) Trichinæ in pork and examination for trichinæ.
    (*e*) Cereal fillers and starches in sausage meats.
    (*f*) Preservatives and coloring substances in meats.
  2. Plate cultures.
    (*a*) Numerical counts of bacteria.
    (*b*) Number of gas formers and of acid formers.
    (*c*) Bacillus botulinus in pork and in vegetables. Botulism.
  3. Toxicological tests.
    (*a*) Inoculation tests (guinea pigs) to prove the absence or presence of toxins or ptomaines.
    (*b*) Tests for tuberculous meats and for the tubercle bacillus.
  4. Biological Tests. Determining the source of the meat.
    (*a*) Sugar test for horse meat.
    (*b*) The precipitin test for meats from different animals.
    (*c*) Microscopical examination of tissues, fats, fat crystals, etc.

IX. The Bacteriological Examination of Eggs and Egg Products.
   1. Direct microscopical examination.
      (*a*) Bacteria.
      (*b*) Molds.
      (*c*) Mold spores.
   2. Plating methods.
   3. Egg tests.
      (*a*) Candling.
      (*b*) Brine test.
      (*c*) Organoleptic tests, etc.
   4. Evaporated eggs.
   5. Cold storage eggs, etc.

X. Bacteriological Examination of Pharmaceutical Products.
   1. Direct microscopical examination.
      (*a*) Bacteria.
      (*b*) Molds.
      (*c*) Mold spores.
      (*d*) Yeasts.
   2. Plating methods.
   3. Colon bacillus test.
   4. Tetanus bacillus test.
   5. Tests for the staphylococcus and streptococccus groups.

XI. The Microscopical and Bacteriological Examination of Syrups.
   1. Medicinal syrups.
      (*a*) Official, simple and medicated.
      (*b*) Patent and proprietary medicated syrups.
      (*c*) Medicinal preparations containing syrup.
   2. Soda fountain syrups.
   3. Fruit juices containing sugar. Fruit juice concentrates.
   4. Syrups, molasses, treacle, corn syrup, etc.

XII. The Microscopical and Bacteriological Examination of Fermented Foods and Drinks.
   1. Whiskey and brandy.
   2. Beer. Beer diseases.
   3. Wines. Wine diseases.
   4. Other fermented drinks.
      (*a*) Sake or Japanese rice wine.
      (*b*) Arrak.
      (*c*) Yoghurt.
      (*d*) Kephir.
      (*e*) Koumiss.
      (*f*) Soja sauce.
      (*g*) Mazun.
      (*h*) Leban.
      (*i*) Ginger beer.
      (*j*) Beebe wine.

XIII. The Bacteriological Examination of Mineral Waters.
   1. Examination of centrifugalized sediments.
   2. Plating methods.
   3. Presumptive colon bacillus test.

XIV. Determining the Efficiency Value of Disinfectants.
  1. Phenol germ destroying coefficient.
  2. Toxic Coefficient.
  3. Albumen coagulating coefficient.
  4. Comparative cost.

XV. Determining the Purity and Quality of Sera, Bacterins and of Related Products.
  1. Purity and freedom from bacteriological contamination.
  2. The purity of smallpox vaccines.
  3. Purity of bacterial vaccines.

XVI. Special Biological and Toxicological Tests.
  1. Arsenic in foods. Biological test for arsenic.
  2. Toxicity tests with defibrinated blood.
    (a) Toxalbumins and toxins.
    (b) Saponins.
    (c) Chemical hemolysis.
  3. Frog tests for the presence of alkaloids.

The following blank will be found useful in making reports of bacteriological examinations. In many instances however, it will be found necessary to supplement the report or to make a special report.

FORM NO. III

Bacteriological Examination

(No., label, dates, condition of seals as for Form I.)

I. Direct count. (Thoma-Zeiss hemacytometer with Turck ruling.)
  1. Bacilli per cc..
  2. Cocci per cc ....

II. Plate and tube cultures. (Lactose-litmus-agar.)
  1. Temperature differential test.
    (a) (20° C.) colonies per cc..
    (b) (38° C.) colonies per cc..
  2. Color differential test.
    (a) Pink or yellow colonies per cc ....
    (b) Not pink or yellow colonies per cc....................................
  3. Gelatine liquefying colonies per cc......................................
  4. Indol reaction (±)...
  5. Neutral red reduction (±)..
  6. Gas (hydrogen) formula...
  7. Gram-stain behavior (±)..
  8. Presumptive colon bacillus test (±),
    (a) Amounts used...
    (b) Number of tests..
    (c) Rating...

III. Special tests...
....
....

IV. Conclusions...
....
....

.................................Analyst.

Course III. Sanitary Science, Including Parasitology.—This course is to be given during the fourth year of the full four year course in a properly equipped college of pharmacy. A standard textbook should be used, supplemented by lectures, laboratory work and practical demonstrations, and extending throughout the entire college year. Under the present arrangements of the college curriculum this course must be given concurrently with the course in Food Bacteriology (Course II).

I. Symbiology. Parasitology.
  1. Beneficent symbioses.
    (a) Mutualism.
    (b) Individualism.
  2. Commensalism. Nutricism.
  3. Antagonistic symbioses. Parasitism. Parasitology (exclusive of pathogenic bacteria).
    (a) Exo-parasites—Lice, etc.
    (b) Skin parasites—Itch, etc.
    (c) Intestinal parasites—Tape worm, etc.
    (d) Blood parasites—Malaria, etc.
  4. Compound symbioses. Paracytoses. Patrocytoses.

II. Sanitary Rules, Laws and Regulations.
  1. National, State and city.
  2. Quarantineable diseases.
    (a) The national quarantine.
    (b) The state quarantine.
    (c) The city or community quarantine.
    (d) The family or house quarantine.
  3. Reportable diseases.
  4. Occupational diseases.
  5. Water supplies and purification of drinking water.
  6. Sewage disposal.
  7. Disease prevention.

III. Sterilization and Disinfection.
  1. The more important disinfectants.
  2. Disinfection and fumigation.
    (a) Public buildings.
    (b) Railway disinfection.
    (c) Street car disinfection.
    (d) Private dwellings.
    (e) Sick room disinfection.

IV. Epidemiology.
  1. The more important pandemical, epidemical and endemical diseases.
    (a) Influenza or La Grippe.
    (b) Asiatic cholera.
    (c) Bubonic plague.
    (d) Smallpox.
    (e) Yellow fever.
    (f) Malaria.

(*g*) Typhoid fever.
(*h*) Diphtheria.
2. The prevention and control of epidemics.
(*a*) Malarial control.
(*b*) Typhoid control.
(*c*) Smallpox control.
(*d*) Plague control.
(*e*) Yellow fever control.
(*f*) The social diseases.

V. Pure Food and Drug Laws, Rules and Regulations.
1. National, state and city.
2. Standards of quality and purity.
3. The enforcement of the laws.

2. The Laboratory

A. *Location of Laboratory.*—It may be in a separate building, as the home, but as a rule a corner room in the pharmacy is best suited for the purpose. This room may be in the basement, or on the first, second or other floor. Do not select a room with a through passage for obvious reasons. It may adjoin a chemical or pharmaceutical laboratory, though it should not be a part of such laboratories. Chemicals and chemical fumes interfere with bacteriological and microscopical work. It should have one door and two or more windows. There must be good light and the environment should be favorable for bacteriological work, for which reason a room in the basement is not, as a rule, desirable,

The walls and ceiling of this room should be absolutely plain and well protected by white enamel paint. The floor may be cement, slate, or hard wood, well oiled with boiled linseed oil, or it may be painted, or covered with linoleum. The entire room (walls, ceiling, floor) should be washed, scrubbed and disinfected from time to time. That is, it should be kept bacteriologically clean.

B. *Furnishings.*—All windows exposed to direct sunlight should have white translucent roller shades. The laboratory should be well supplied with gas; water, both hot and cold; and means for lighting (gas, electricity, acetylene). There should be just enough furniture and shelving, no more. One table with slate top or lined with linoleum; one stool, shelves for samples, apparatus and reagents. A case for chemicals, cotton, culture media, etc. A case, with lock and key, for samples to be examined. The plumbing must be of the best and the fixtures must be of safe construction. The sink should be large and deep and should be lined with porcelain and supplied with an ample drain board. A hood or ventilator should be provided to carry off steam vapors. Near the table for microscopical work should be a shelf or case for the works of reference.

C. *Apparatus.*—There will be required:

(*a*) A good simple lens.

(*b*) Compound microscope.
Ocular with micrometer scale.
Oculars, Nos. 2 and 3.
Objectives, Nos. 3, 5, 7 and 1/12 in. oil immersion.

(*c*) Slides and covers.

(*d*) Section knife or razor, and strop.

(*e*) Polarizer, for the study of starches, crystals, etc. Should be convenient to use. This is very important. The selenite plates which are usually supplied with the polarizer are useful.

(*f*) Thoma-Zeiss hemacytometer with Turck ruling, for counting bacteria spores and yeast cells in vinegar, jams, jellies and other like substances. Other special counting chambers.

(*g*) Accurately ruled metal or hard rubber millimeter ruler for measuring seeds in fruit products, etc.

(*h*) One Arnold steam sterilizer (copper). A vegetable steam cooker will serve.

(*i*) One hot air sterilizer. The ordinary double walled baking ovens which may be secured from any hardware dealer, will serve the purpose. Cut in a small opening at top for the thermometer.

(*j*) One rice cooker in which to prepare culture media, etc.

(*k*) One small incubator with thermo-regulator.

(*l*) Centrifuge (electric).

In addition to the above there will be required the necessary chemicals, reagents, etc., good quality commercial cotton for plugging test-tubes, medium size Petri dishes, flasks (½ liter and 1 liter), several evaporating dishes, one or two moist chambers, a quantity of medium size test-tubes, slide boxes, test-tube brushes, dissecting needles, scalpels, labels, pencils, etc. Get the necessary things only. There must be a liberal supply of clean towels. No one but the analyst and his assistants should have access to the laboratory. On entering, the analyst should remove coat and hat and put on a white clean linen apron and coat, such as are worn by soda fountain dispensers. This white suit should remain in the laboratory and should be changed for a clean one as often as may be necessary.

Special equipment and apparatus may be indicated as the work progresses. For instance, it may prove desirable to have an incubator for opsonic work, for the use of physicians, used either by the physicians or by the pharmacist. A water filtering equipment may be installed, likewise a water still. An autoclave may prove desirable. There are matters which must be left to the individual pharmacist. The following is an outline of such work as the pharmacist may do in the microscopical and bacteriological laboratory.

D. *Micro-analytical Work.*—The practical work which may be done will depend upon the special preparation and qualification of the pharmacist, as has been indicated in the preceding pages, and also upon the opportunities which may offer themselves. The following is a partial list of substances which may be examined bacteriologically or microscopically, or both. Other work is also indicated.

(*a*) Vegetable drugs, crude and powdered.

(*b*) Spices and condiments, whole, ground and powdered. Prepared spices and condiments.

(*c*) Coffee, tea, cocoa, chocolate, confections, candies.

(*d*) Tobacco, including smoking tobacco, cigars, cigarettes, snuff.

(*e*) Compound powders, pharmacopœial and others.

(*f*) Tablets, pills, simple powders.

(*g*) Meats; raw, cooked, canned, sausage meats, mince meats, etc.

(*h*) Dairying products as milk, cream, cheese, butter, ice creams, cream fillers.

(*i*) Cosmetics, dusting powders, insect powders.

(*j*) Cattle and poultry powders.

(*k*) Starches, dextrins, sausage meat binders (starchy).

(*l*) Vegetable foods; as jams, jellies, fresh, pickled, cooked, canned and preserved.

(*m*) Flours and meals.

(*n*) Breakfast foods, baby food, invalid foods.

(*o*) Breads, cakes, pies, crackers, etc.

(*p*) Catsups, tomato pastes, etc.

(*q*) Macaroni, spaghetti, noodles, etc.

(*r*) Nuts, and nut-like fruits.

(*s*) Cloth material, textile fabrics generally, cordage, papers, etc.

It is assumed that the pharmacist has had the necessary training to undertake the microscopical examination of the substances above classified, with the aid of such standard works of reference as may be required. The micro-analyses should also include:

1. Gross and net weight determination of all samples that require it, for which purpose an accurate balance is necessary.

2. Moisture determinations of such substances as may require it. There should be no difficulty in constructing the necessary apparatus for making moisture determinations.

3. Ash determinations of substances which require it. This calls for a special equipment including a platinum dish, ignition furnace with burners, etc.

4. Use of special tests, as sublimation tests for benzoic acid and salicylic acid, the hand wheat gluten test, Bamihl gluten test, Grahe's cin-

chona test, color reaction tests for boric acid, salicylic acid, morphine, and opium; tests for phytosterol and cholesterol crystals, etc., etc. These and other tests are explained in the several reference works cited above. In the examination of liquids or semi-liquids as wines, beers, cider, vinegar, milk, cream, sewage, extracts, tinctures, etc., a centrifuge is desirable.

E. *Bacteriological Work.*—The pharmacist should be prepared to do the following work in the bacteriological laboratory.

(*a*) Prepare culture media for use of physicians, as may be required.

(*b*) Prepare sterile throat swabs for the use of physicians.

(*c*) Prepare stains and do staining for physicians, as may be required.

(*d*) Make bacteriological determinations of milk, jams, jellies, impure drinking water, vinegar, wine, sera, vaccines, antitoxins, contamiated foods and drinks, sewage, etc.

(*e*) Sterilize pharmaceuticals, surgical supplies, etc.

(*f*) Assist the physician in opsonic work, as may be arranged or agreed upon.

(*g*) Do bacterial culture incubation work for the physician, make subcultures, Wassermann test for syphilis, etc.

(*h*) Filter and sterilize drinking water to be supplied to customers.

F. The Cabinet of Microscopic Exibits.—In every laboratory where bacteriological and microanalytical work is being done, there should be an exhibit of all of the substances which are likely to come under observation, in order that any desirable comparison may be immediately made. The following suggestions are for the cabinet intended for general microanalytical work. Those interested can readily prepare a special, more limited cabinet, from the suggestions hereby presented.

The exhibit is to consist of objects and materials which may prove of analytical value and use in making comparisons and for purposes of check work and re-verification. The exhibit is not to include samples of the materials secured for the purpose of study or examination as to identity, quality or purity. Such materials may be kept in a separate cabinet, and for such periods of time only, as they may or might be of use for further study and comparison. If the examination shows that the article is of the quality for which it was purchased, then no sample need be kept. If it proved to be adulterated or of inferior quality, then a sample should be retained until the matter is finally settled or disposed of. In case of a retail pharmacy, the stock of drugs on hand is the exhibit of the articles which have been examined and compared with the articles in the microscopic cabinet. Should the microanalyst devote his entire effort to food products, then the cabinet will be stocked with pure and representative food products spices, flours, meals, etc.

The greatest care must be observed in the preparation of the microscopic cabinet, particularly as to the identity of the samples and the labels attached thereto. Nothing is to be included of which the source is in any way questionable. All samples must be obtained from absolutely reliable sources and even then each and every sample must be carefully examined in order to make absolutely certain that it is genuine. Drug samples must be secured from reliable dealers, food and spice samples from reliable merchants; samples of cloth, of furs, of paper, of cordage, etc., from specialists in the several products. The statements of the inexperienced lay man must not be accepted. To illustrate, the owner of a rug may emphatically declare it to be a genuine Bokhara, basing this emphatic declaration upon the misstatements of an unscrupulous dealer. A sample of ground black pepper may be declared of prime grade or quality by an experienced dealer in spices, whereas it may be made of "grinding peppers." In the case of articles which are believed to be of special importance, the source or identity of which is not clearly known, a commercially non-prejudiced and non-interested expert, or several such experts, should be consulted. If the article cannot be identified for a certainity, it must be discarded and may not be used for purposes of comparison. It may be filed in a special case for samples of this kind, with the hope that its exact identity may at some future time, be ascertained. The following are suggestions for the formation of a general microanalytical exhibit.

It is most important to guard against any excess in the size or bulk of the exhibit; the smallest quantities in the most compact groups, should be the guide. Use the smallest containers which will serve the purpose, and have them of uniform size in so far as possible and practicable. The containers must be easily accessible, the caps, stoppers or other sealing, readily removable and replaceable, without danger of becoming mixed or misplaced or displaced. Too much care cannot be given to the labeling. The legends thereon must be distinct and legible and sufficiently full and concise, so that the intelligent observer may at once know the meaning or full significance. On the other hand, meaningless and wholly useless and mentally confusing details must be omitted from the labels.

1. *Crude and Powdered or Ground Samples. Bulk Samples.*—A hard wood cabinet, similar to those which are in use in food and drug laboratories for holding samples of pure foods, spices, vegetable drugs, fiber, powders, chemicals, etc., will serve the purpose excellently. The containers are usually of glass, with screw tops, and rest horizontally in suitable hollow grooves of the drawers. Small rubber stoppered or cork stoppered Homeopathic vials are excellent, if the regulation containers are not available. The cabinet should hold several thousand articles, each

and every one distinctly labelled. Containers with liquids generally, and oils, should not be placed horizontally, but vertically, and the sealing must be perfect, for reasons which are self-evident. The samples must be representative and in many instances they must be reduced and comminuted so as to get them into the containers. A few samples may require the use of wide-mouthed containers, such as the ounce quinine vials. Not more of these should be used than is absolutely necessary, as they take up too much space. Many of the articles may be filed away in book form, or pasted in book form, or placed in small envelopes which in turn are pasted in blank books which are equal in size and form to the other sample books. The substances to be placed in the cabinet may be grouped as follows:

a. For the containers which are horizontally placed; solids and powders of all kinds, broken, cut and trimmed substances. Gums, resins, waxes, some pastes and other semisolids. Solid chemicals, most metals, etc.

b. For the containers which are to be placed vertically; liquids generally, oils, syrups, chemicals in solution, etc.

c. For the books all of uniform size; papers of all kinds, cloth of all kinds, cordage, animal hair, samples of furs, vegetable fiber generally, etc.

These physical groups may be made to include foods of all kinds excepting those which it is not necessary or desirable to keep on hand, drugs, poisons, narcotics, cloth, silks, metals, minerals, etc. It is impracticable and usually wholly unnecessary to preserve the readily perishable articles, such as fresh meats, fresh vegetables, cheese, bread and pastries. All materials in the cabinet must be non-decomposable, either naturally so or rendered so through the addition of some suitable preservative. Some food substances may be artificially desiccated before placing them into the containers.

The sample books are to be placed in the bottom drawer of the cabinet, in suitable compartments, and may include the following:

(*a*) Books of samples of commercial papers (book paper, note paper, writing paper, etc.)

(*b*) Books of wrapping paper, tissue paper, newspaper, etc.

(*c*) Book of felt papers of all kinds.

(*d*) Book of filter papers of all kinds.

(*e*) Books of cloth of all kinds.

(*f*) Books of cordage, threads, twines, etc.

(*g*) Book of parchments, bank notes, paper currency, etc.

(*h*) Book of samples of furs of all kinds.

(*i*) Book of silk samples, natural as well as artificial.

(*j*) Book of miscellaneous fiber and cloth material, etc.

Most of the books mentioned may be obtained from the special dealers

and manufacturers, but they do not come in uniform sizes. They may be trimmed to uniform size and the materials rearranged, or the articles may be removed and repasted into the blank books of uniform size. Such work must be very carefully done to avoid mistakes and confusion in the placing of labels and the descriptions.

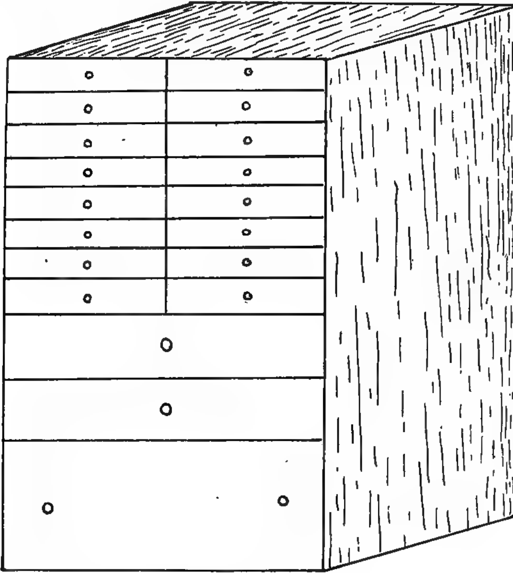


FIG. 96.—Plan for a food and drug exhibit cabinet. The outside dimensions are four feet high, two and one-half feet wide, and two feet deep. It is to be made of well seasoned hard wood, and all of the drawers must work easily and smoothly and so fastened that they will not drop out when drawn to full distance. A cabinet of the above dimensions can be made to contain sixteen shallow drawers for the horizontally placed screw top containers, each drawer holding 120 such containers placed in tiers and lying in suitable grooves; two drawers for the vertically placed containers for the liquid substances which are set in rows in holes in a deck shelf and a shallow base well; and the large bottom drawer for the exhibits in book form. The drawers may be lettered *a*, *b*, *c*, etc., and the articles in each drawer numbered seriatim 1, 2, 3, etc.; thus, "Oil, whale," might be located by "*R*–143." The case may be provided with lock and key.

The above articles may be cared for in a cabinet, the outside measurements of which do not exceed 4 feet in height, 2½ feet in width and 2 feet in depth. Such a cabinet may have sixteen shallow drawers for the horizontally placed containers (about 1,000 containers); two drawers five inches deep for the liquids (about 500 samples); and a single deep bottom drawer for the sample books placed on edge, suitably grouped in secondary compartments (altogether containing from 1,000 to 2,000 samples).

The articles in the cabinet above described are for microscopical examination, as has been indicated, but before this can be done, each and every

substance must be properly mounted upon a slide. There are many substances which it is convenient to have on hand ready mounted for immediate microscopic examination. These are to be kept in a second smaller cabinet, known as the cabinet for microscopic slides.

2. *Cabinets of Microscope Slides.*—The cabinets of various sizes can be obtained from dealers in microscopic supplies and are intended to hold slide mounts for microscopic examination. The slide mounts are obtained from various sources. Many will be prepared by the analyst, from times to time. Others will be secured from dealers in scientific instruments and supplies who generally furnish lists of the slides which they offer for sale, more especially representing the following groups of organisms and tissues.

(*a*) Bacteria. Molds and other fungi. Protozoa.
(*b*) Desmids, diatoms, and other low forms of algæ.
(*c*) Embryological slides.
(*d*) Vegetable tissues of all kinds.
(*e*) Animal tissues, normal and pathological.
(*f*) Crystals, minerals, earths, etc.

The State and Local Boards of Health may furnish slides of the principle disease germs, etc., free for demonstration purposes, and such a cabinet should be added to the exhibit. The ready prepared sets of slides should be carefully selected so as not to encumber the exhibits with things of little or not practical use. The slides will be of inestimable value in some of the analytical work. One medium size slide cabinet will be sufficient as a beginning. A complete index to the slides must be prepared and such index must be conveniently usable.

3. *Photo-micrographs.*—There should be a photo-micrograph of each and every article mounted upon a microscope slide. The prints should be mounted upon suitable cards and every photograph should be very carefully and accurately labelled. It would be desirable to have two sets of these photographs. One set in a filing case, in alphabetical sequence according to common English names. The other set pasted into a book, in groups following the appended outline. The book would be very desirable for quick reference and for comparative demonstration purposes.

It is suggested that the articles in the three divisions of the exhibit, above explained, should be arranged in alphabetical sequence according to well established and generally recognized common English names, excepting that a certain amount of special grouping is desirable or necessary, as already explained. As the exhibit gains in size it may become necessary to adopt some other system of arrangement.

For the benefit of those who are entrusted with the building up of an

exhibit of the kind above specified, the following brief instructions in making microscopic mounts, are given.

1. Smears.—Slide mounts for microscopical examination, generally known as smears, may be made from a great variety of substances, more especially from liquid, semiliquid and pasty materials, including blood, sputum, pus, urinary sediment, feces, excretions and secretions, bacteria and bacterial cultures and all substances containing bacteria, etc. Temporary smears may be made of fats, butter, oleomargarine, oils, cream, and oily substances generally. Permanent smear slides intended for the exhibit are to be made as follows. Clean a slide very carefully by means of alcohol and a clean cloth, passing it through the flame of a Bunsen burner after the wiping. Spread the substance very thinly and evenly upon the middle portion of the cleaned slide and allow it to air dry (very moderate heat may be used, not to exceed 70°C.). Next "fix" the smear upon the slide by passing it through the flame of the Bunsen burner four or five times, or, by adding a drop of alcohol. The heat (or the alcohol) coagulates the albuminous matter which may be present, and sticks the objects firmly to the surface of the slide.

The air dried and "fixed" smear may now be examined and if the mount proves satisfactory (spread sufficiently thin to make details distinct, etc.), it may be labeled and filed away. No cover glass or mounting medium is used. Or, it may be stained by means of methylene blue, Loeffler's methylene violet, Fuchsin, Safranin, Bismarck brown, Wright's stain, Giemsa's stain, etc. The selection of the stain will depend upon the object it is desired to attain. The staining should be omitted until it is known for a certainty that staining is desirable and the operator knows how to use it. The unstained mounts will keep indefinitely and may be stained any time. The only difficulty lies in the fact that since the smear mounts are to be examined by means of the 1/12 oil immersion lens, the oil used (cedar oil) would interfere more or less with the staining.

A blood smear requires special manipulation. Place a droplet of the blood (or the liquid containing the blood) upon a thoroughly cleaned slide, nearer one end. Now place one end of a second cleaned slide just beyond the droplet, at an angle of 30°–40°, lower the end until the surface of the tilted slide comes in contact with the droplet, and then gently draw it forward over about 1/2 of the slide surface (that is, the slide holding the droplet). Another method for spreading is as follows. Place the second tilted slide on the other side of the droplet, lower until it comes in capillary contact with the droplet and then push it over the surface of the first slide, instead of drawing it forward, as in the first method. After spreading, the smear is allowed to air dry and then fixed by means of a drop of alcohol, instead of heat. The stains generally used for blood smears are

Wrigth's and Giemsa's. (Consult some modern work on Bacteriology or Parasitology as to the methods for preparing any of the desired stains).

2. Balsam Mounts.—Canada balsam, suitably diltued with xylol, benzol, oil of cloves, ether, etc., is used for making permanent mounts. The balsam should be diluted to the consistence of thin syrup, or rather thin oil. In fact it should be thin enough so that when a droplet is placed near the edge of a cover glass upon a slide, capillarity should draw it under and spread it evenly. Xylol is one of the best diluents for Canada balsam. Oil of cloves is objectionable because it destroys more or less of the color of the stained mounts. Otherwise it is excellent.

The thing of special importance is to remember that all substances which are to be mounted in Canada balsam must be entirely free from moisture: if any considerable moisture is present the mounts become worthless, because the water will not mix with the balsam, forming opaque emulsions. All substances which contain moisture may be dehydrated by placing them in alcohol for a short time, until the water has become diffused into the alcohol and evaporated. After dehydrating, the material should be placed into xylol (or the same substance in which the balsam is dissolved) for a few seconds and then mounted in the balsam. Balsam mounts are of course permanent.

3. *Glycerine Mounts.*—Glycerine, or equal parts of glycerine and water, makes an excellent mounting medium and has the advantage over balsam in that moisture and water does not interfere with its use. Such mounts will keep for months and even for longer periods of time, but require careful handling, as they are easily removed from the slides. If care is observed in mounting (using just enough of the mounting medium) and placing the cover glass carefully) and the mounts are placed in suitable slide boxes, they may be kept indefinitely. The glycerine does not evaporate, or if it does vanish partially with time, more may readily be added.

Balsam mounts as well as glycerine mounts require the use of cover glasses. The operator should bear in mind the effects produced by the mounting processes. Heating and dehydrating causes a reduction in size and some slight distortion in form. The reduction in size may be considerable, a fact which must be kept in mind when comparing one and the same organic substance examined in the fresh state and in the permanent mounts.

A cabinet may be perfect in arrangement and it may be completely stocked and each and every article contained therein may be fully and accurately labeled, and yet such a cabinet would be of little practical use is there is no way of locating the articles therein contained. Just as it should be made a criminal offence to write a book (especially a book of

science) without a complete index, just so should it be made a criminal offence to prepare an exhibbit of the kind above explained without a complete index. In the matter of indices, it may be explained that some are excellent and some are so incomplete as to be of little value.

For the purpose of the above exhibit, the following classification as a basis for preparing the complete list of articles, is suggested. To the right of each group and each individual article, is given the number of the drawer and the series in the drawer in which the article is placed.

Groups of the Articles of the Exhibit

I. Bacteria.
II. Beverages.
  1. Alcoholic.
  2. Non-alcoholic.
III. Blood.
  1. Normal (human).
  2. Pathological (human).
  3. Animal (normal and pathological).
IV. Chemicals.
  1. Solid.
  2. Liquid and solutes.
V. Cloth.
  1. Animal.
  2. Vegetable.
  3. Mixed.
VI. Cordage and Thread.
VII. Earths. (see soils)
VIII. Fiber. (not woven)
  1. Animal.
  2. Vegetable.
  3. Mineral.
IX. Flours and Meals.
X. Foods.
  1. Animal.
  2. Vegetable.
  3. Mixed.
XI. Inks and Pigments.
XII. Minerals (uncombined elements).
XIII. Parasites (non-bacterial and non-protozoal).
XIV. Protozoa (parasitic and non-parasitic).
XV. Secretions and excretions.
  1. Normal.
  2. Pathologic.
XVI. Soils.
XVII. Starches.
XVIII. Tissues and Tissue Elements. (animal hair not included).
  1. Animal.
  2. Vegetable.

It will be found that everything which it may be desired to file may be classified under one or the other of the groups above named. In so far as practicable, every article should be listed under its more common English name, with exceptions. For example, the various slides of the group bacteria, may be arranged in the alphabetical sequence of the true scientific names. A like classification suggests itself for the groups protozoa and perhaps parasites.

A properly arranged and well stocked exhibit, as above suggested, is of equal importance with the laboratory equipment and the working reference library. The entire exhibit should be gone over carefully from time to time to see if labels are still in place, if still complete according to the full alphabetical list, etc. Substances which have undergone decomposition or other spoilage, should be discarded and replaced by fresh samples. Leakage must be corrected.

The following is a diagram of a bacteriological and microscopical laboratory:

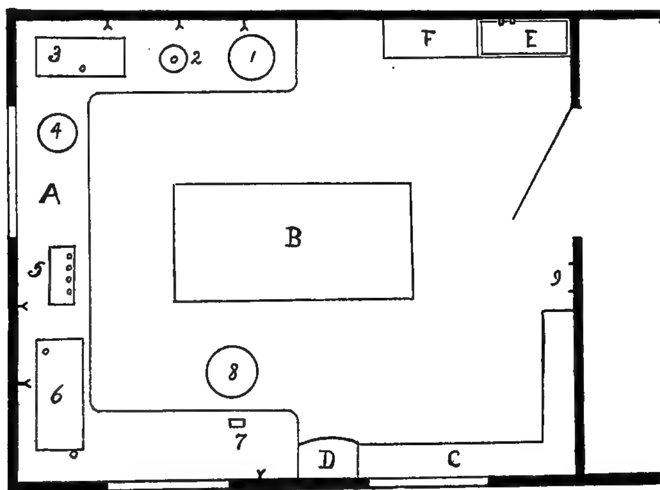


FIG. 97.—Plan of bacteriological and microscopical laboratory, using corner room in the pharmacy. (Scale 4 feet to the inch.) *A*, shelves on three sides of the room, *B*, work table. *C*, cases and shelves for reagents, chemicals, glassware, etc. *D*, case for reference books. *E*, sink. *F*, drain board. 1, Arnold steam sterilizer; 2 hot waste filter; 3, hot air sterilizer; 4, rice cooker; 5, opsonic incubator; 6, incubator; 7, compound microscope; 8, stool; 9, hat and coat books.

The following books will make an excellent nucleus to which additions are to be made from time to time.

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15. Wiley, H. W.—Food and Their Aduleration. P. Blakiston's Son and Company. 1907.

16. Winton, Andrew L.—The Microscopy of Vegetable Foods. John Wiley and Sons. 1906.

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