STUDIES ON *POLYSTOMELLA* LAMARCK (FORAMINIFERA)

By Margaret W. Jepps

Lecturer in Zoology in the University of Glasgow,
late Leverhulme Research Fellow

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Part I. Introduction and Methods, with an Account of Feeding

Introduction

There must be a vast number of biologists who, like myself, were taught as students a life history of *Polystomella*[1] based on the careful observations of J. J. Lister (1895, 1906) with the additions which he accepted from the work of Schaudinn (1895 *a*, p. 59; 1903, p. 500 in the *Arbeiten*[2]). To others beside myself it must have come as somewhat of a shock to realize later on the incompleteness of these observations; and most particularly the slender basis on

[1] I am not convinced that the last word has been said concerning the most acceptable name for this organism, and therefore continue to use the name so familiar in Great Britain, in spite of the fact that American and some other writers are now calling it *Elphidium* Montfort as advocated by Meek & Hayden (1864).

[2] All the page references in this paper to the works of Schaudinn refer to the collection known as the *Arbeiten*, and not to the isolated papers as they originally appeared in various periodicals.

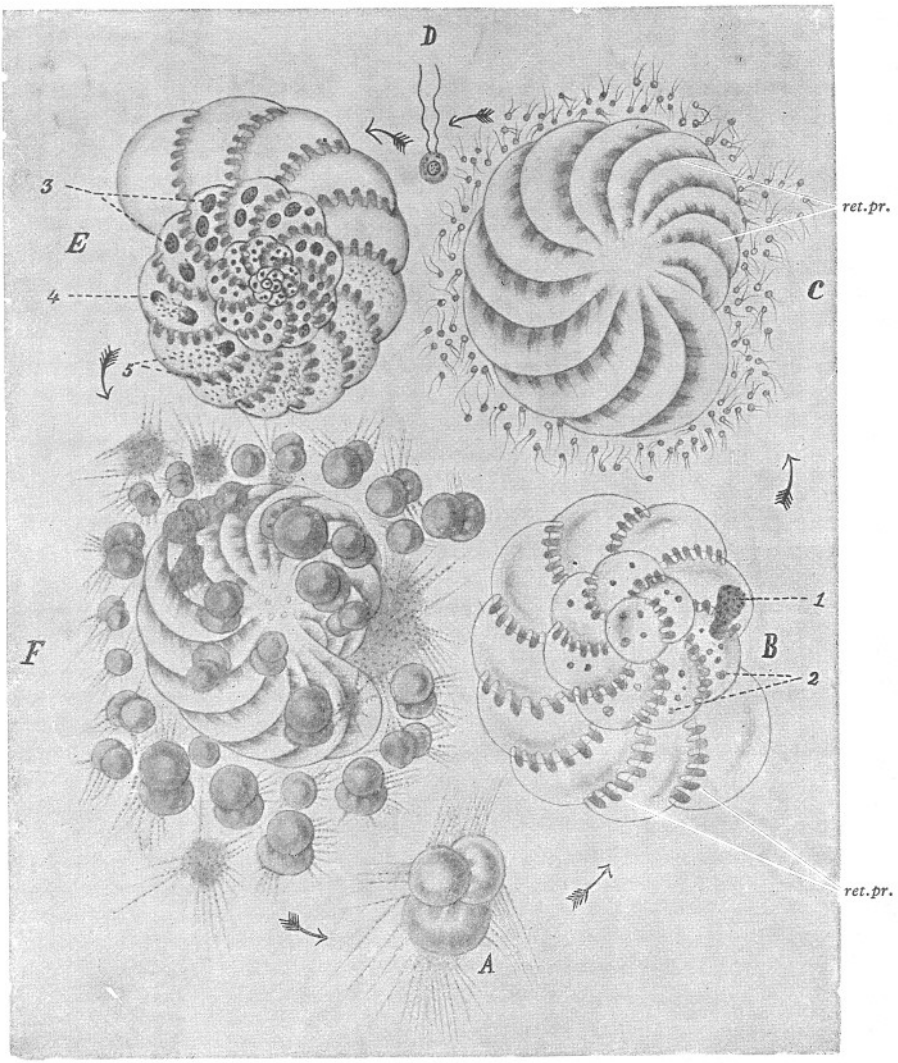
which rests the special contribution of Schaudinn, including what might be called the crisis of the life history, the process described by him as a form of sexual reproduction.

The story of the life cycle of *Polystomella* is to be found in numerous text-books of zoology, notably in the excellent short account of the Foraminifera contributed by J. J. Lister himself in 1903 to the *Treatise of Zoology* edited by E. Ray Lankester, and still more briefly in Graham Kerr (1921). In the latter, of course, the whole story is given, including Schaudinn's addition of 1903. In Text-fig. 1 is reproduced from Arnold Lang's *Lehrbuch* (1901, p. 208) an illustration which according to its legend was sketched for this text-book by Schaudinn. This figure shows a series of stages in the life history of *Polystomella*. At A is a young megalospheric form, with its large spherical initial chamber, the megalosphere, and two others, which grows into the adult at B, still having only a single nucleus. At C the protoplasm has produced a large number of flagellate swarm spores, each provided with a nucleus and two flagella. These leave the parental shell and give rise to the microspheric form at E, with a number of nuclei and a small initial chamber, the microsphere. At F its protoplasm has crept out of the shell and divided up to form a comparatively small number of megalospheric offspring, as at A, thus completing a life history which includes a sexual (megalospheric) followed by an asexual (microspheric) generation. According to Schaudinn (1895*a*, p. 62) the alternation of the two generations is not always quite regular, for the offspring of a microspheric form may occasionally be multinucleate, and produce another asexual generation. Schaudinn claims to have observed this in three out of 4300 specimens of *Polystomella*, and points out that similar irregularity occurs more frequently in other Foraminifera, quoting *Orbitolites* and *Peneroplis*. This is believed[1] to be the case in some few Foraminifera (e.g. *Orbitolites*, *Cornuspira* and 'several other genera', Lister, 1903, p. 74: in *Rotalia* and *Cristellaria*, Lister, 1895, p. 442: also reported in other Miliolidae by Hofker, see 1930*b*, p. 28) and it may happen in *Polystomella*. But Schaudinn did not say how he made this observation—it would be a matter of special difficulty in this genus, as pointed out by Lister (1903, footnote to p. 74), and nobody has repeated it since. It might be added that once the protoplasm has left the shell it is very rarely possible to distinguish between microspheric and megalospheric individuals. I have seen in a very old emptied shell at Plymouth the initial chamber (a megalosphere) indicated by the rich growth of diatoms inside the membrane which persists after decalcification, but it is usually invisible.

Text-fig. 1 is especially interesting because it includes (at D) the only figure of a swarm spore of *Polystomella* ever published by Schaudinn. At E also is shown a dividing nucleus of which we shall have more to say later.

Two years afterwards in 1903 (p. 500) Schaudinn added his description of

[1] But it is by no means certain in the absence of adequate measurements, etc., in the light of Føyn's analysis of *Discorbina villardeboana* (1936*b*, 1937). In this species the ranges of size of the initial chambers of megalospheric and microspheric forms overlap, so that it might, for all we know, be possible for a microspheric (asexual) parent to produce megalospheric (sexual) young having initial chambers of an equal or even of a smaller size.



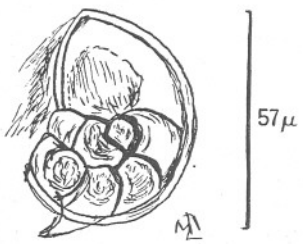
Text-fig. 1. Dimorphism and alternation of generations in *Polystomella crispa*. The arrows indicate the direction of the life cycle. A, young megalospheric individual; B, full-grown megalospheric individual, decalcified; C, megalospheric individual in the act of spore-formation, the protoplasm leaving the shell as flagellispores; D, flagellispore more highly magnified; E, microspheric individual decalcified; F, microspheric individual in the act of producing amoeboid young. 1, nucleus; 2, chromidia; 3, nuclei; 4, nucleus 'in multiple fission to produce chromidia'; 5, chromidia; *ret.pr.*, retral processes. (From Lang, after Schaudinn). After Parker and Haswell, *Textbook of Zoology*, 2nd ed., Vol. 1, p. 57. By courtesy of Messrs Macmillan and Co., London.

Three of the four guide-lines marked *ret.pr.* do not indicate the retral processes correctly. The processes are unshaded in all the figures and are projections from the concave side of each chamber.

the actual union of two swarm spores (gametes), each from a different (megalospheric) parent, to initiate the microspheric generation, which continued to develop in his culture as far as the five-chambered stage, by which time the nucleus had usually divided more than once, and then it died.

Schaudinn made extensive studies in marine aquaria which, it seems, grew up in a rather haphazard way and were, when they succeeded, probably more natural than if more elaborate and controlled methods had been employed. It is possible that by some lucky set of chances he had the good fortune to witness what so many others have striven in vain to see in more than one form of protozoon. There is, however, a disconcerting lack of corroborative evidence in the way of detail and good figures; and we find, for example, on the preceding page of the *Arbeiten* categorical statements which now appear to us almost preposterous about the 'Kopulation' of well-known intestinal flagellates. The question then arises whether he might not also have been mistaken in his interpretation of the observations under review. Further, a young microspheric *Polystomella* at the five-chambered stage might not show easily recognizable features by which it could readily be distinguished from the young of other Foraminifera. It has already been noticed, for example, that the retral processes (see Text-fig. 1 B, C) which are so characteristic of this genus are not produced by the earliest chambers in the microspheric phase (Schaudinn, 1895*a*, p. 61; Lister, 1895, p. 418). In my whole mounts of decalcified older specimens I find that one or two retral processes begin to appear on the protoplasm about the 9th–12th chamber (Schaudinn gives the 20th–25th chamber as the first of the series to show the processes; yet he refers to young *microspheric Polystomella* of 8–15 chambers without the least description of their appearance (1895*a*, p. 59)).

In April 1940, in the course of some experiments at Plymouth with the flagellate swarm spores of *Polystomella*, I found in one culture the little shell shown in Text-fig. 2. It was watched for 2 days, but unfortunately showed no sign of life. The circumstances in which it was found were such that it might quite well have been a microspheric *Polystomella* of some eight or nine chambers—a much earlier stage than any so far figured (Lister (1895) shows a young specimen of about twenty chambers in his pl. vi, fig. 7). A study of stained decalcified older specimens shows that it is quite comparable as regards its size and shape with the first eight chambers of an adult microspheric *Polystomella* (but also of some other species of Foraminifera). It was during the season of the supposed sexual reproduction, and early on the previous day three *Polystomella* had emitted their swarms of flagellate spores in a Petri dish under conditions which were as normal as possible, excepting that no food was supplied, so that one might have expected



Text-fig. 2. From a sketch by Dr M. V. Lebour of a small (? *Polystomella*) shell seen in culture. See p. 660.

to obtain some early stages of the next generation. There might have been, as can be said after making the experiments already referred to, an even greater likelihood of a very young microspheric *Polystomella* being brought in from the sea on the shell of an adult. Although these were always carefully washed, and the resulting cultures indicated that a surprisingly high degree of cleanliness was obtained, it was found that a small organism (usually a ciliate, flagellate, or small amoeba) might occasionally remain on the shell and multiply in the culture. (No other Foraminifera, as a matter of fact, had ever appeared in a long series of cultures of this kind). On the whole it does not seem justifiable, however, to identify the little shell confidently as a young *Polystomella* in our present state of ignorance of the appearance of the early stages of the microspheric phase. It is quite conceivable that it was the young of some other species with spirally arranged chambers.

On 15 March 1895, Lister wrote, 'there is no direct evidence of the conjugation of zoospores or the mode of origin of the microspheric form in a dimorphic species'; and this may perhaps still be said of *Polystomella*, although the story usually told seems very likely to be true. The only other Foraminiferan in which observed fusion of flagellate gametes has been described, leading to the development of the asexual generation, is also recorded by Schaudinn (in *Trichosphaerium*, 1899, p. 163). Again he describes the act of union of the flagellate gametes of *Gromia dujardinii* (1894*a*, p. 49; 1899, fig. on p. 163), although he did not succeed in rearing the offspring. Winter (1907, p. 19) claims to have seen the union of flagellate 'gametes' in *Peneroplis*, and le Calvez gives an account of the fusion of the 'flagellispores' in *Iridia lucida* (1938, p. 207); but neither followed the development of the zygote although the latter actually obtained a new generation in his culture. These few records constitute the only 'direct evidence of the conjugation of zoospores' in the group in spite of a great deal of work by competent workers on a number of forms of which the published life histories seem otherwise complete. This point will be further discussed.

There is, it appears, an unexpected diversity in the stages of the life history in the Foraminifera, since Myers has brilliantly demonstrated the fusion of *amoeboid* gametes in *Patellina corrugata* (1935*a*) and in *Spirillina vivipara* (1936), to give rise to the asexual generations of these two species. From his studies (1935*b*, 1936) it seems that the terms *megalospheric* and *microspheric* are not universally applicable to the sexual and asexual phases respectively even of the more typical dimorphic Foraminifera. In these two, for instance, the initial chamber (or proloculum) is not clearly divided off from the rest of the shell; and the diameter at the beginning of the spiral is actually somewhat less in the sexual than in the asexual form. For general purposes, then, it is preferable to call them by some other names such as *sporont* and *schizont*. In the present work the terms *megalospheric* and *microspheric* are retained, as they are eminently suitable in the case of *Polystomella*, though even here they have lost their widest implication.

The present studies, which it is hoped to continue when circumstances again become favourable, were undertaken with the idea of contributing something towards the demonstration of the life history of *P. crispa*, the species studied by so many generations of students in the laboratories of Great Britain. The possibility of more intensive work was provided when the Court of the University of Glasgow granted me leave of absence for the academic year 1938–9, thus enabling me to avail myself of the award of a Leverhulme Fellowship for the year. I am very glad to have this opportunity of recording my sincere gratitude to the University Court; and to the Leverhulme Trustees who have, through their Secretary Dr L. Haden Guest, M.P., so graciously expedited the work. The year was spent in the laboratory of the Marine Biological Association of the United Kingdom at Plymouth, beside the Sound where lie the famous beds of *Polystomella* whence J. J. Lister obtained much of his material, and which supply the teaching establishments of the British Isles. I am indebted to a number of benefactors who kindly bestowed on me the use of their tables in the course of the year; to a Committee of the British Association for a total period of six months; to the following Worshipful Companies of the City of London—the Clothworkers (2 months), the Fishmongers, Goldsmiths, Mercers, and Grocers (1 month each); and to the Fishmongers for another month in the Spring Vacation of 1940 when I was again at Plymouth. I offer my best thanks to Dr Stanley Kemp, F.R.S., Director of the laboratory at Plymouth, and to all those on his staff who helped me in so many ways; their friendly welcome added a great deal to the achievement and to the pleasure of the time I spent with them. Almost all my material has been collected by Mr William Searle, and I owe much to his skill and long experience for supplies in all weathers. Mr Searle continues to collect under the shadow of war in the English Channel and over Plymouth Sound. I am very grateful also to those other friends and colleagues who made it possible for me to be freed for the year from my usual routine duties, and even encouraged me to make a serious attack on a piece of work so many would have put aside as a forlorn hope. Amongst them I would especially mention the late Head of this Department, Sir John Graham Kerr, F.R.S., M.P.; Dr E. J. Allen, F.R.S., lately Director of the Plymouth Laboratory; and Mr Edward Heron-Allen, F.R.S., who gave me much material and moral support from the British Museum of Natural History. To Prof. Edward Hindle I am also grateful for some apparatus and special facilities for the continuation of the work at Glasgow, where I have in addition the ready assistance of the Director of the Marine Station at Millport (Firth of Clyde) in collecting the local material.

Material

Of the several good collecting grounds for *Polystomella* in the vicinity of Plymouth I found the material from the Drake's Island ground the cleanest and richest (Heron-Allen and Earland's Station I; see the Marine Biological Association's *Plymouth Marine Fauna*, 2nd edition, p. 34). All the catches

brought in for me during the year I was at Plymouth came from there. After the middle of March 1939 the '*Gammarus*' went out specially and brought the material straight back early in the morning. Since this ground ceased to be available in September 1939 my *Polystomella* have been collected at the station known as 'White Patch', nearer the east end of the Breakwater.

A widespread and much the most plentiful species in this neighbourhood is *P. crispa* L., and, without claiming any special qualifications as a systematist on the shells of the Foraminifera, I believe that all my work has been done with it. This is probably only of importance in the experiments with the flagellate spores, and in order to be reassured particularly at this point I have called in the skilled assistance of Mr Arthur Earland, F.R.M.S. I shall refer to his opinions in the course of these studies; and gladly take this opportunity of thanking him for his ready help.

Fixation of Material

Experience had shown that the usual methods of fixation give very poor results with *Polystomella*. This is presumably due, at least in part, to the heavy calcareous shell of complicated form, inside which most of the massive protoplasm is only freely accessible through very small openings until the calcium carbonate is dissolved away; this takes quite an appreciable time—several hours if the reaction is not to go on with such violence as to break the structure up. So that apart from the old antithesis between good fixation of nucleus and of cytoplasm respectively there are further difficulties to be overcome, and these increase with the size of the specimen to be fixed. One such difficulty is only too obvious after fixation: a more or less violent protoplasmic movement is apt to occur, the cytoplasm surging from one chamber to the next, the nuclei often becoming drawn through the foramina between the chambers and fixed there in a great variety of bizarre shapes—even though their inner structure may be quite well preserved. That these distorted nuclei were not all caught in the act of passing through from chamber to chamber, as has been suggested, is clear because of their constant relative incidence according to the method employed. It may be that the difficulty in obtaining an undisturbed fixation is also partly due to an extreme sensitivity and very quick movement of the protoplasm. A living *Polystomella* may move very swiftly, the long fine pseudopodia being very active outside the shell, while the cytoplasm in the chambers may be seen at times to be in rapid circulation.

A great many trials were made with most of the usual fixatives and variants of them, including corrosive acetic mixtures, with and without alcohol, Zenker, Susa, trichloracetic acid, Bouin, with and without urea and chromic acid, Gilson, Carnoy, Flemming and other osmic acid methods. It is not proposed to describe them all in detail. None of them gave perfectly reliable results with large *Polystomella*, and each has its fairly constant defects; but a very great improvement has been found possible, at any rate for some stages of the life history.

Cold fixatives, i.e. up to room temperatures, are as a rule hopeless for the nuclei. After such the nuclei often show no internal structure at all, being fixed only on the surface at one side, thus producing a figure more or less like that of an open empty tow-net. The fixation is much improved by heat, especially if the degree is carefully selected to reduce protoplasmic streaming to a minimum. Hot fixatives, on the other hand, have the disadvantage that their application dislodges more of the foraminal plugs ('bouchons' of le Calvez, 1938, p. 236, see p. 625 and Text-figs. 6, 7), sometimes causing them to be found lying loose in the cytoplasm of the terminal chambers, and sometimes to be thrown right out of the creature.

Corrosive acetic mixtures have been the classical fixatives for Foraminifera ever since both Schaudinn and Lister chose them for this purpose, using them 'warm' (Lister, 1895, p. 414). Lister's figures show the limitations of the method, as do his remarks in the text about protoplasmic disturbances (p. 416), the radial arrangement of the cytoplasm round about the nuclei (p. 418), and the absence of a nuclear membrane (p. 419). After trying many modifications I prefer a 6% solution of corrosive sublimate in sea water, to which I add 5% of glacial acetic acid, used at a temperature of 60–65° C. The specimens are put with a pipette into the fixative previously heated in a closed dish on a water bath, and after 5–10 min. the dish is removed to the bench and left to cool for an hour or so. Decalcification proceeds, and the *Polystomella* may be lifted towards the surface by the bubbles coming off the shells. Inspection shows, however, that they do not actually rise to the surface because as soon as the bubble touches the surface film it is stopped, even if it is not discharged; there is no risk of the specimen drying up as it hangs below in the liquid, and it is perhaps better to leave these apparently floating specimens alone. If decalcification is not completed in the fixative it is usually finished off in 70% alcohol with 3% of nitric acid, after thorough washing in 30, 50 and 70% alcohols in succession, a few drops of iodine solution being added to the last. Fixation by this method is not free from the imperfections already mentioned, though they are reduced as far as possible. The result is fairly good and of course excellent for staining. I do not find the addition of alcohol any advantage, nor the increase of the acetic acid to 25%. Higher and lower temperatures give worse results. I have not been so successful with the method which gave Myers (1935*a*) such good preparations of *Patellina*[1]—although I

[1] I had the great pleasure of seeing some of Dr Myers's fine preparations at Plymouth, as he and Mrs Myers arrived from California quite unexpectedly in the summer of 1938. They decided to work at *Polystomella* while they were at Plymouth, and we agreed to continue our separate researches, which lay along such different lines, quite independently, although for a time under the same roof. It is a pity that our records cover the same year—but our coincidence was purely fortuitous. I greatly appreciated their offer to show me their special paraffin embedding bath before its publication—part of the technique they had evolved in their long experience with the Foraminifera, and which enables them to deal with the astronomical numbers of *Polystomella* on which their results are based. I found the apparatus very useful in sectioning my much smaller sets of specimens, although for special individuals, of course, solitary embedding is to be preferred.

found that his Schaudinn's solution (corrosive acetic with alcohol, containing 25% acetic acid instead of the usual 4–5%) gives slightly better results at higher temperatures (80–90° C.) than at 60–65° C.; my less drastic mixture has the advantage also at the higher temperature.

Better effects were obtained with a modified Zenker's solution, viz. sea water, 100 c.c.; corrosive sublimate, 5 g.; potassium bichromate, 2·5 g.; with 5% glacial acetic acid added immediately before use (and omitting the usual sodium sulphate).[1]

This solution was best used at 40–50° C. for about 10 min. and then left for about 2 hr. on the bench to cool. Care must be taken afterwards to wash very thoroughly in water to avoid a precipitate in alcohol. The excess sublimate is removed with iodine in the usual way. On the whole the cytoplasm and nuclei look less disturbed after this fixation. Most of the nuclei, especially in the megalospheric form, are more often nicely rounded, showing a distinct membrane. Radiations in the surrounding cytoplasm are usually absent at this temperature, but in a few cases there is a small shrinkage space about the nucleus.

The only other useful results with large *Polystomella* were obtained with osmic acid methods. 'Flemming' was used by Lister with some success, though he barely mentions it in his published work; and from his results it was probably used warm, though he does not say so. Various modifications of these solutions were therefore given a thorough trial, the acetic acid always being added at the time of using.[2] They all produced much the same result.

In the cold, i.e. at room temperature (about 14° C.) or using ice (about 2–3° C.), after several hours' application, the cytoplasm was excellently fixed, the fatty constituents being preserved and giving it the crowded appearance it has in life;[3] but the nuclei are very bad, often showing the townet shape and no internal structure at all. There was unfortunately a tendency to violent surging of the protoplasm from one chamber to the next, no doubt rendered very conspicuous by the subsequent good fixation. When the solutions were used hot (35–40° C.) the nuclei were as usual greatly improved, but although a few good ones were seen, on the whole they are wrinkled and not so well fixed as with Zenker or corrosive acetic. Once or twice I noticed a slight exudation of protoplasm from the shell similar to that which occurs when a living *Polystomella* is put into fresh water (see p. 620, footnote 4). It was seen after the use of strong Flemming, made up in fresh water and used at room temperatures or higher.

[1] After an interesting talk with Mr J. Z. Young on the speed of ions and fixation (Young, 1935) I doubt whether the sea water is any improvement with corrosive acetic or Zenker—but with the latter at least its use saves trouble.

[2] 'Strong', 'weak', and an intermediate solution of Meves (see Gatenby & Cowdry, p. 377) were tried; also other variations with more or less chromic or osmic acids, and Føyn's mixture made up in sea water (1936*a*, p. 275). Various times (1½–21 hr.) seemed alike in their effect.

[3] It is hoped to analyse the cytoplasmic inclusions in a later part of these studies.

When desirable before staining it was found quite satisfactory to bleach in hydrogen peroxide solutions either whole *Polystomella* or sections blackened by fixation in osmic acid.

It was noticed that with the osmic solutions the nuclei were not pulled through the foramina, and some experiments were made with the object of attempting to combine the advantages of these solutions with those of the corrosive acetic mixtures with which the derangement of the nuclei is a most serious drawback. The *Polystomella* were allowed to crawl on well-cleaned microscope slides; and when their pseudopodia were well extended the slide was cautiously inverted over osmic vapour. After the desired exposure the slides could be transferred face downwards to other reagents, or the *Polystomella* washed off with sea water and immediately subjected to further treatment. (Incidentally this method was used to make whole preparations of *Polystomella* with extended pseudopodia, with or without subsequent treatment to dissolve the shell or to improve the fixation of its contents.) In the cold the best results were obtained with an exposure to the vapour for 10 min., followed by immersion in 6 % corrosive sublimate with 5 % acetic acid, in sea water. But as usual there was a great improvement when the *Polystomella*, after exposure to osmic vapour, was washed into the corrosive acetic at 60–65° C. With the osmic vapour the canal system which runs through the shell (see Lister, 1903, p. 65) is particularly well shown up, being well fixed and slightly darkened; also the cytoplasm, particularly in the outer chambers. Sometimes the whole effect, including that on the nuclei, was very good, notably in the case of one or two large microspheric *Polystomella*, which are usually very difficult subjects. The protoplasmic disturbances seem to be reduced, more foraminal plugs remain in situ, but on the whole the fixation of the nuclear structure is not improved by osmication before hot corrosive acetic and indeed may be rendered worse. It seems likely that the exposure is rarely just right, and it may be unequal in different parts of a single large specimen.

The rather complicated mixture known as Heidenhain's 'Susa',[1] used at 40° C., produced some good nuclei, but tended to give a washed out general appearance to the preparation. Small *Polystomella*, below twenty chambers or so in a megalospheric specimen, or thirty in a microspheric one, are more easily fixed. With these the best results have on the whole been obtained with 'Susa' at 40° C.

If for any reason it is not possible to use the fixative hot, a preliminary treatment with osmic vapour may be advised before corrosive acetic. Next to corrosive acetic with or without osmication, perhaps Zenker gives the best results in the cold.

Most of my material was fixed in Zenker or corrosive acetic, both used hot,

[1] See Brontë Gatenby (1937, p. 74). Water, 80 c.c.; corrosive sublimate, 4–5 g.; sodium chloride, 0·5 g.; trichloracetic acid, 2 g.; glacial acetic acid, 4 c.c.; formol, 20 c.c.; or Romeis's simplification of the same (Romeis, 1928, p. 80), saturated aqueous corrosive sublimate, 25 c.c.; 5 % trichloracetic acid, 20 c.c.; formalin, 5 c.c. The instruction is to proceed direct from these fixatives to several changes of 80–90 % alcohol.

excepting small specimens for which I often used 'Susa', also hot. The fixation was quite satisfactory as regards staining properties, which were still good after a year or more in 70% alcohol when it was unfortunately necessary to keep the specimens unstained for so long. An interval not longer than a month was aimed at in the case of Feulgen's stain, however, and when this was exceeded the fact will be noted. For staining whole mounts borax carmine, well differentiated, gives good results; and picrocarmine even better, especially for osmic acid preparations. Ehrlich's haematoxylin may be used for very small specimens only. Sections are generally stained with Ehrlich's haematoxylin and eosin. Mann's stain gives very beautiful results with thin sections (8μ), the foraminal plugs coming out yellow, while the chromatophores, to which the colour of the living animal is due, are pink.

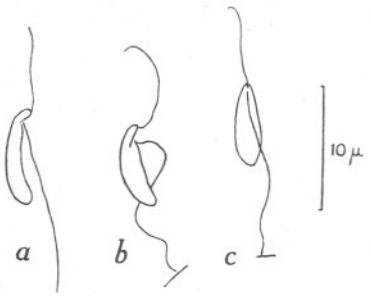
Cultures

It was obviously desirable to have *Polystomella* growing in cultures, and this has been achieved with a fair measure of success. Further experiments are contemplated with a view to improving the results already obtained. *Polystomella* lives, grows, apparently remains in good condition, and proceeds with its life history as long as it is kept in a pasture of flourishing diatoms. To this end I used the medium known as 'Føyn's Erdschreiber'—sterile sea water + salts to which an earth extract is added—with excellent results.[1] I did not attempt to free my cultures from bacteria, nor from small flagellates, both of which might have been useful in the feeding of the Foraminifera. The diatoms were grown in Petri dishes, and small numbers of *Polystomella* (up to four or five adults or about twenty young) lived here as long as the diatoms were plentiful and of a good healthy brown colour—usually 3 or 4 weeks. Larger numbers or growing up families were kept in cultures grown in larger receptacles, glass pneumatic troughs measuring 9–10 in. in diameter and 4–5 in. high being found very suitable for cultures 2–3 in. deep; and flat-sided glass museum jars with at least one polished side for purposes of observation with a horizontal microscope. The diatoms were usually grown at room temperatures, excepting in the hottest summer weather at Plymouth, but the *Polystomella* cultures were kept at various temperatures as will be noted in the course of this paper. A moderate light suits most diatoms; on dark winter days extra light was sometimes supplied from ordinary electric lamps overhead, and in the summer at Plymouth the light close to the window, even though this faced north, seemed too bright and the cultures were moved farther away. The rate of growth of the diatoms naturally varies with conditions of light, temperature, the density of the sowing and the state of the diatoms themselves,

[1] I am very glad to take this opportunity of expressing my best thanks to Dr Fabius Gross, Lecturer in Zoology in the University of Edinburgh, for so kindly showing me his technique for the culture of planktonic diatoms, both at Plymouth in 1936 and in Edinburgh in 1938. See Gross (1937), where he gives the recipe for the culture medium which I also used for bottom diatoms, viz. sterile sea water, 1000 c.c.; soil extract, 50 c.c.; $NaNO_3$, 0·1 g.; $Na_2HPO_4.12H_2O$, 0·02 g.

and it is a matter of experiment to determine the time necessary to produce an adequate feeding ground. Sometimes the life of a culture was prolonged by renewing the culture medium in the dish.

Several different diatoms were cultivated, including one or two planktonic forms, but the most suitable type was found to be the motile bottom-living Pennatae which would spread evenly over the surfaces covered by the culture medium, preferably rising up the sides of the vessel as well as covering the floor. I had the good fortune to catch an ideal form in November 1938 by crushing in a watch-glass of Erdschreiber one of the empty *Polystomella* shells from the sea, which at Plymouth sometimes contain crowded and almost pure cultures of a single diatom of this kind, often along with nematodes and various protozoa. From this source was obtained without further purification a culture containing a single species of naviculoid diatom, which was identified in a subculture of some months later as *Navicula mutica* Kutz. var. *Cohnii* (Hilse) van Huerck.[1] There was also one easily recognized flagellate which persisted unchanged, though its numbers might fluctuate, throughout the year in which the cultures were kept going. It was 8–10μ long, with a very rapid dancing motion, and no tendency to amoeboid action, keeping its shape unchanged except for slight movements of the mobile anterior end. The results of a somewhat cursory examination are shown in Text-fig. 3 and Pl. V, fig. 1, from which it is clear that our flagellate may be included in the genus *Bodo*. It does not agree very closely with the description of any of the well-known species—from freshwater habitats. Its shape and activity place it nearest to *B. celer* Klebs (1892, p. 313). No contractile vacuole was observed, nor any attempt at encystation, but this is not surprising in a marine flagellate. Neither was it seen to feed by sucking out other protozoa, but there was small opportunity of this in my cultures, unless it were extensively cannibalistic. I shall refer to it simply as *Bodo* sp.



Text-fig. 3. *Bodo* sp. living, from culture of *Navicula mutica*. *a*, swimming freely, *b* and *c*, slightly shortened and thickened as when anchored by long trailing flagellum, which also shows a tendency to wind spirally round the body.

The *Navicula*, on the other hand, unfortunately underwent a progressive deterioration in the series of subcultures. At first (December 1938) it was a plump oily diatom of the usual naviculoid shape, about 20μ in length and about 6μ wide in the middle, of a rich brown colour. It had considerable motility which enabled it to spread evenly over surfaces. The motility de-

[1] I am indebted to Mr R. Ross of the British Museum of Natural History for this identification. He says, however: 'I am not really satisfied about the *Navicula*, and it would be better to query the identification for the present.' The uncertainty is probably to be explained by the gradually increasing abnormality in the subcultures described in the text.

creased with age, and the diatom became enclosed in a thick (? mucous) coat to which bacteria adhered. When the colour of the growth changed to a yellower shade it was due for replacement as a feeding ground for *Polystomella*.[1] The most suitable temperature seemed to be about 55–60° F. (14–16° C.); at higher temperatures the growth came faster, but it was paler; lower temperatures gave a slower growth but of a richer brown. In the winter with a moderately thick sowing a Petri-dish culture at Plymouth would be ready for use after about a month, while the old one would need replacing—in the spring and early summer the plates developed in half this time or less. Almost from the beginning it was noticed that there was an admixture of shorter cells in the cultures—down to 10μ or so in length—which tended to grow in short chains or little heaps; and at times these would predominate in a patch of diatoms, the general growth habit at the same time becoming rough and not attached to the substratum. I believe, however, it was a pure culture, and that I obtained at various times a smooth growth of 18–20μ diatoms from a rough growth of short ones and vice versa. This went on throughout the summer, but I believe the original size of the longest diatoms was not regained after a while. In August 1939 it is noted at 16μ; by the following February it did not seem to exceed 10–12μ. In the spring of 1940 the cultures got much worse after a very dark winter in Glasgow, finally producing only very slowly a few cuboidal cells 5–6μ across, in little heaps, and the strain was abandoned. The shape of the cells had degenerated until they could at times not be recognized as naviculoid diatoms at all.

It was very interesting to see in February and March 1940 the *Navicula mutica* cultures which Mr D. P. Wilson had very kindly maintained for me at a lower temperature after I left Plymouth in September 1939. They appeared also to have lost their original size, but had not then declined so far as my diatoms which had been cultivated for use and much more vigorously; by the autumn, however, they seemed almost as bad as mine were in the spring.

Unfortunately, time did not allow of a closer investigation of the diatom cultures I was growing as food. I thought that the dying out might be due to an increasing unsatisfactoriness of the periodic auxospore phases owing to some imperfection in the conditions of cultivation.

The only other diatom I cultivated at all extensively for use at Plymouth was *Synedra tabulata* (Ag.) Kütz.[2] This was a beautiful golden brown form 40–50μ long, not a naviculoid and not motile. Therefore it did not spread well in the cultures but grew in heaps lying loosely here and there on the floor of the dish, excepting when it was spread about by browsing *Polystomella*. It was culti-

[1] As M. Schultze remarks (1854, pp. 20, 24), the colour of *Polystomella* depends on its state of nourishment, and agreed as far as his chemical investigation went with that of the diatoms on which it largely fed (the diatomin of Nägeli).

[2] Also identified by Mr R. Ross. This diatom appeared in a culture of *Enteromorpha* sp. (kindly named for me by Miss E. Stanbury, of the Technical College, Plymouth) which came from an aquarium taken by Dr Gross from Plymouth to Edinburgh some months earlier.

vated in Erdschreiber from November 1938 to July 1939, when it was given up since it had been showing irregularities for some time.

Latterly one or two other bottom-growing diatoms, some from the *Corallina* shore pools at Millport[1] and some at least naviculoid, have been tried at Glasgow, and have seemed more or less satisfactory as food for *Polystomella*; but I have not come across any which gives such a beautiful even growth as did *Navicula mutica* at its best. These cultures of 1939, which could be grown to any required density on any clean glass surface, gave me the opportunity for many hours of fascinating observation of living *Polystomella* going about their business, the results of which I hope to record in these studies. As Max Schultze wrote of his work in 1854, p. vii: 'Der Hauptzweck dieser Arbeit ist, durch Mittheilung treuer Beobachtungen über den Bau und die Lebenserscheinungen der beschalten Rhizopoden der Meere unsere bisher so lückenhafte Kenntniss derselben zu ergänzen.'

The *Polystomella* were taken from a D-netting which was given a preliminary washing at sea. As soon as the catch arrived in the laboratory it was put through a sieve to remove large particles, and well washed with 'outside sea water'[2] in a large flat white enamel dish until as much as possible of the remaining mud, etc., was removed. After a few hours the *Polystomella* had crept up on to the surface of the catch and some up the sides of the dish, whence they could be easily picked off with a pipette. The shells chosen for culture were again cleaned; first with a small brush[3] under a binocular, and then washed with a pipette in several changes of filtered outside sea water.[4] After this treatment it was found as a rule that no other protozoa were introduced into the cultures, the protoplasm of the *Polystomella* being withdrawn inside the shell so that nothing appeared to stick to it so long as it was kept agitated. If, however, it is left still for a few minutes the protoplasm emerges and pseudopodia are put out almost at once.

It was sometimes desirable to be able to recognize individual *Polystomella* without having each one isolated in a culture by itself; and also to know when growth had taken place, this being most clearly indicated by the formation of additional chambers to the shell. Since the shell is not normally external to the cytoplasm it seemed better not to risk an attempt to mark the shell itself in any way. Very often individuals could be put together which showed some marked difference—a set of small chambers due to irregular growth, or a protuberance,

[1] These pools support a small population of living Foraminifera of several species, including some small *Polystomella*. Mr Earland has examined a number of these and assures me they are all *P. crispa* of a small size (under 1 mm.) and often showing spines at the shell periphery, or with a tendency to make very thin shells with rather bloated chambers and no keel—probably ill-developed forms surviving under hard conditions.

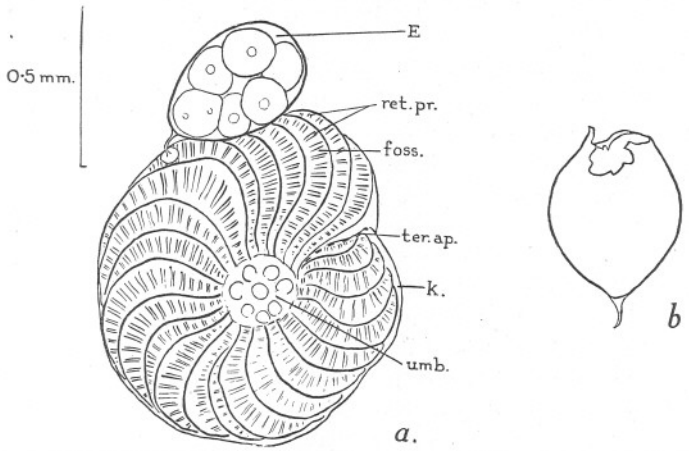
[2] Brought in from outside the Breakwater in carboys (cf. Lister, 1903, pp. 69, 70).

[3] A red sable brush no. 00, for miniatures, is very suitable. It has a fine point, can be sterilized in boiling water, and stands hard treatment well.

[4] Fresh water may not be used as it causes the protoplasm to swell and ooze out at the terminal apertures and from the fossettes (see Pl. IV). This swelling in fresh water also occurs in other Foraminifera.

for example, would serve as an identification mark, or as a landmark in a growing shell. But best of all were clear signs in the form of stalked egg cases or cocoons which occur not infrequently attached to the *Polystomella* shells at Plymouth.[1] These are almost always empty as shown in Text-fig. 4 *b*, but usually remain firmly fixed by the basal plate of the stalk.

These cocoons were found still occupied in April and May 1939, from the Drake's Island ground; in October 1940 and in June 1941 from 'White Patch'. They will continue with their development and hatch in sea water or diatom cultures in Petri dishes in the laboratory. The earliest cocoon contained 6–8 rounded objects (see Text-fig. 4 *a*) in which no movement could be detected, and no change in 10 days. Then, fearing that the contents might be



Text-fig. 4. *a*, *Polystomella* (the shell only being represented) bearing a turbellarian egg-case; *b*, emptied egg-case of turbellarian. *E*, egg case with developing embryos; *foss.*, fossette (d'Orbigny); *k.*, keel of shell; *ret.pr.*, ridges between the fossettes and over retral processes of the chambers; *ter.ap.*, terminal apertures of shell; *umb.*, umbo.

dead and disintegrating, I opened the egg case, and in it found eight little embryos with ciliated cells which gave them a slow rotation. In more developed specimens about half a dozen planarian embryos could be seen crawling over one another inside the cocoon; they were white in colour, each with two dark eyes. Hatching has occurred a few days after collection by way of an irregular hole at the distal end of the egg case, as many as eight little worms about half a millimetre long emerging. These were still quite white, and swam rapidly by means of their cilia or crept actively about the dish, while keeping constantly in the spot of brightest light. They were offered diatoms, etc., in the cultures, *Polystomella*, small copepods, alive and dead, pieces of a freshly

[1] Also on a miliolinid (*Quinqueloculina*) from 'White Patch' in June 1941. It is not suggested that they necessarily have any special relation to the Foraminifera, although they are often so firmly fastened to the organic basis of the shell that they survive decalcification by acid. When they are found adrift from their substratum they proceed with their development and hatch as usual.

killed small gasteropod, and segmenting eggs of a mollusc: but were not observed to feed on anything of a size visible with a magnification of about 40. They disappeared about a week after hatching.

There is no operculum on the egg case, and it is interesting to note in one emptied case which was mounted on a slide that the chitinous capsule seemed somewhat macerated in the vicinity of the hole, i.e. its usually obscure tesselated pattern had become very obvious, as if a process of solution had been involved in the liberation of the young turbellaria.

It is probable that the 'gestielte, ansehnlich grosse häutige Beutel mit gerissenen Öffnungen am Rucken ihrer Schale festgeheftet' found by Ehrenberg (1839, pp. 109, 133, and pl. ii, fig. 1*g*) on *Geoponus stella-borealis* (= *Polystomella striato-punctata*) from Christiania in Norway, and on *Nonionina germanica* from Cuxhaven were also turbellarian cocoons—although I never saw one with the opening near the stalk; nor one with two or three openings such as he describes (1839, p. 168). M. Schultze (1854, p. 28), on the other hand, suggests that these were tests of the ciliate *Cothurnia*, such as he saw on the same species of *Geoponus* from Cuxhaven in the spring of 1851.

These cocoons cannot at present be ascribed to any known species of turbellarian. Of those already known they look most like minute representatives of the triclad bdellourid cocoons which so far have only been found infesting the gill-books of *Limulus*, the king crab, in both its eastern and its western areas of distribution (see, for example, Wilhelmi, 1909, p. 120). The egg cases on *Polystomella* are not flattened as are the bdellourid cocoons to fit into their special habitat between the leaves of the *Limulus* gill-books.

This is the only animal in any sense parasitic on a living *Polystomella* in my material. I have seen small algae growing on a shell during life, but the *Polystomella* already seemed inactive and soon signs of life ceased altogether.

Feeding

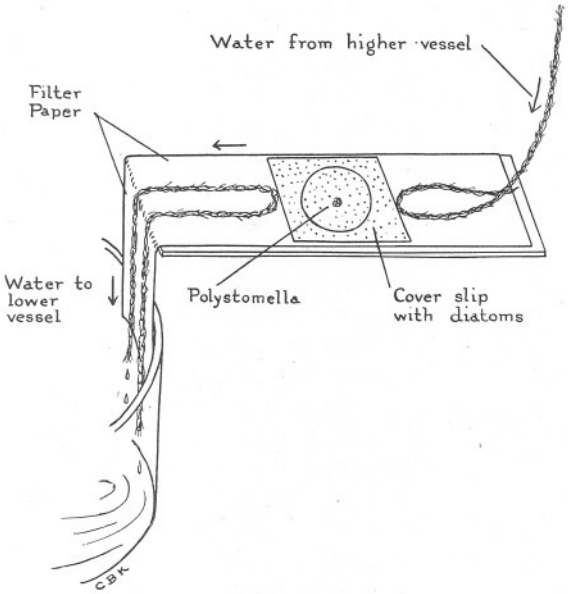
Observations were made in Petri-dish cultures with the aid of a Greenough binocular, and on the sides of taller vessels with a hand lens or a horizontal microscope. Finer details were seen by using a modification of an old method for continuously irrigating a culture over a microscope slide.[1] *Navicula mutica* was grown on cover-slips lying in a Petri dish, and when sufficiently covered, but not too thickly overgrown, the cover-slips were ready for use. A hole was punched[2] in a strip of filter paper (or two or three slips superimposed), and this was laid on a slide and moistened with sterile sea water. A clean *Polystomella*, preferably young on account of its greater transparency and activity, was placed on the slide in the middle of the hole and covered with sea water, then with a cover-slip with the diatom culture growing on its lower surface—the upper side having been previously dried off. The irrigation is very simply effected by strands of white (or 'natural') wool. I found it could easily

[1] See Schaudinn (1895*b*, p. 10). Also described by Lister in Vol. II of his laboratory notebooks (1892–1905).

[2] A sharp corkborer was used for this.

be kept going for several days at least on the microscope stage when a single strand brought the sea water from a higher level on to the slide, and two strands led it away from the opposite side of the cover-slip into a lower vessel (see Text-fig. 5).

All that is necessary is to keep the upper vessel filled, and to wash the wool about twice a day in fresh water, rinsing it in sea water before it is replaced in position. In such an arrangement I have had a young *Polystomella* feeding for a week, in the course of which two new chambers were added, while protoplasmic activity inside and outside the shell could be observed under high magnifications. This activity was seen to be speeded up when the circulation was set going.



Text-fig. 5. Irrigation of living *Polystomella* on a microscope slide.

In spite of its massive shell *Polystomella* may be fairly mobile—quite often moving along as much as 1 mm. in 10–15 min.[1] by means of its very vivacious pseudopodia, which at the time are engaged in a great deal of other activity. This restlessness is not on the whole an advantage in working with cultures. I thought at times that the *Polystomella* tended to move towards a moderate light; but such a tendency, if it really exists, is not strong enough to be useful in controlling their movements under ordinary conditions. Dujardin (1835) also found the direction of incident light to be without influence on the positions taken up by his Foraminifera; but Verworn (1889, p. 40) claims that *P. crispa* moves slowly towards the source of diffuse daylight. I could not dis-

[1] This figure agrees well with the speed of 4–8 mm. per hour recorded by Dujardin for his '*Vorticialis*' = *Polystomella* sp. (1841, p. 258).

cover that their paths bore any relation to a current, for example, during irrigation. They do not appear to seek out good feeding grounds, except by random exploration; nor to stay on them when found, excepting temporarily while actually feeding. They creep up the vertical sides of culture vessels and may spend days crawling under the surface film where their food may be scarce, and whence they are not too easily dislodged once they have established a good set of pseudopodia there.

The pseudopodia of various Foraminifera, including those of *Polystomella*, have been described several times already, and from different points of view (see e.g. Dujardin, 1841; Schultze, 1854, p. 16; Verworn, 1889; Schaudinn, 1893, 1895 *b*; Bütschli, 1894; Lister, 1903, p. 48; Winter, 1907, p. 49; Rhumbler, 1909, p. 251; de Saedeleer, 1932; Sandon, 1934; W. J. Schmidt, 1937; le Calvez, 1938).

Polystomella has pseudopodia of the *reticulose* type, like most of the group; that is to say they are narrow, almost thread-like, branched and continually anastomosing, usually by way of side streams, although occasionally two pseudopodia have been seen to flow together. Even in the finest end-twigs there is evidence of an active circulation, to and fro and across, in the lively movements of the many small granules which normally course up and down the pseudopodia of a well-fed healthy specimen. Sometimes a single granule will suddenly reverse its direction and jostle a way back a certain distance amongst the others which are in the meantime keeping on its former path.[1] This takes place even close behind the steadily advancing hyaline tip of an extending pseudopodium, or in one which is slowly contracting in the act of drawing food towards the shell; and the coloured excretory granules (to be described later), being caught up in the same movement, go to and fro on their way to rejection by the pseudopodia. The pseudopodia of *Polystomella* arise for the most part from the thin covering of cytoplasm which passes through the minute pores found all over the shell between the tubercles (Text-fig. 9*b*) and comes to lie outside it—but also directly from the protoplasm inside the shell via the terminal apertures, sometimes stretching right across the last chamber which is often not filled by the animal; and from the external openings of the canal system in the fossettes and on the umbo (Pl. IV and Text-fig. 4*a*). They may be shot out a short distance into the water like little rockets, and with the granules chasing up and down,[2] wave about like minute feelers, bending, undulating, quivering, and putting out side branches which meet and fuse and so establish the reticulum. This spreads, often in the form of a cone based on the shell, until in half an hour or so bundles of pseudopodia may lie

[1] The animation of the pseudopodia could best be shown in a film, and it was hoped to make one in collaboration with Mr A. G. Lowndes, formerly of Marlborough College, Wilts, whose ultra-rapid cinema-photomicrography (1935) has given such good results with small flagellates. A beginning was made, but the project has had to be abandoned for the present. See Pl. V 9, 10, 11.

[2] Rhumbler (1909, p. 255) ascribes a spontaneous movement to the individual granules in addition to that due to the cytoplasmic streaming.

more or less all round the shell reaching out for rather more than its diameter on every side, or for two or three times as far in one direction as the case may be (see Pls. IV, V).

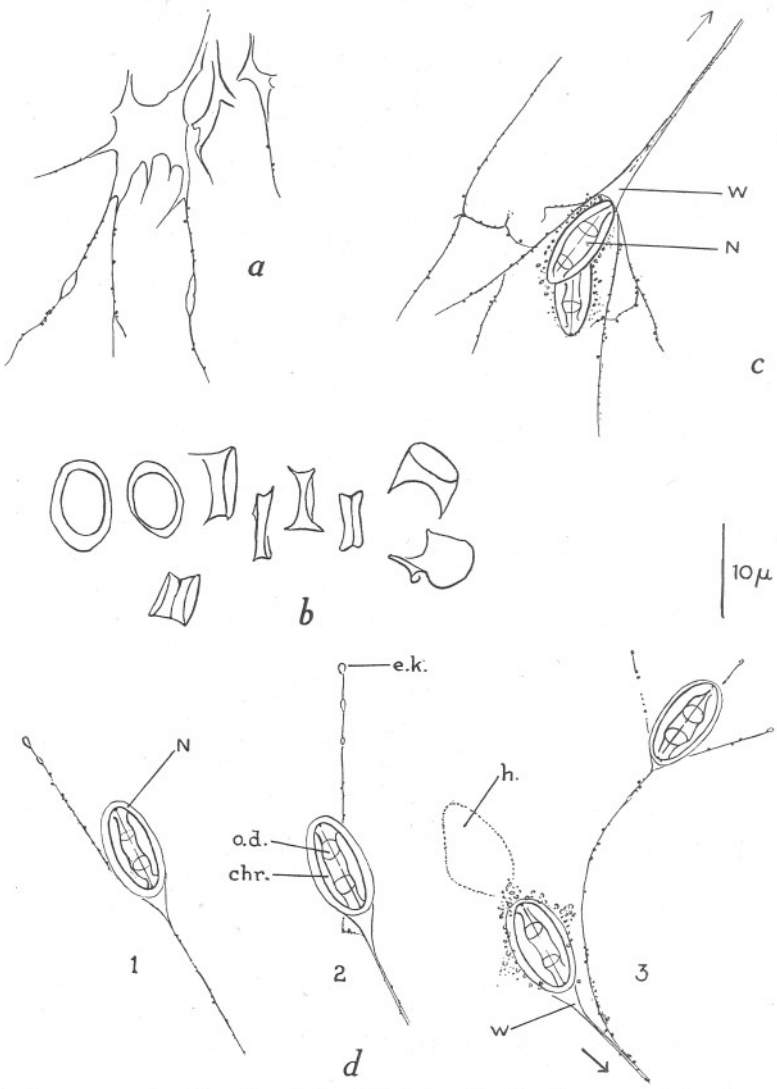
The pseudopodia seem to be bathed in mucus, which is left behind as a trail after their withdrawal. Here and there in the pseudopodia are spindle-shaped varicosities, hyaline, not very refractile, varying in size up to several μ in length. They are deformable, divisible, etc., and moved along in the general streaming of the protoplasm. Sometimes a small one may remain close to the tip of a pseudopodium forming a kind of 'end-knob' (cf. Winter, 1907, p. 50) which may be sticky (Text-fig. 6*d*). Sometimes they spread out to make small webs where the pseudopodia branch, or flattened knots where several lie near together (Text-fig. 6*a*). They become very abundant in the pseudopodia at certain times—at a particular stage of the life cycle or after injury—and may be of a mucous nature.

The circulation goes on in the pseudopodia as long as they are extended, and in a well-developed reticulum is a very wonderful sight. Normally the pseudopodia are withdrawn by a reversal of the process of extension, but when necessary at lightning speed,[1] leaving behind droplets of various sizes (? of mucus) in their tracks.

I could not make out a definite axial thread in the pseudopodia of *Polystomella*, such as is present in those of the Heliozoa for example, although it seems probable that a more fluid surface layer, in which the granules principally circulate, gradually passes over into a core of more solid protoplasm. The pseudopodia show a fairly high degree of stiffness; they extend in a straight line as a rule, and may stretch unsupported through the water for a distance at least two or three times as great as the shell diameter.

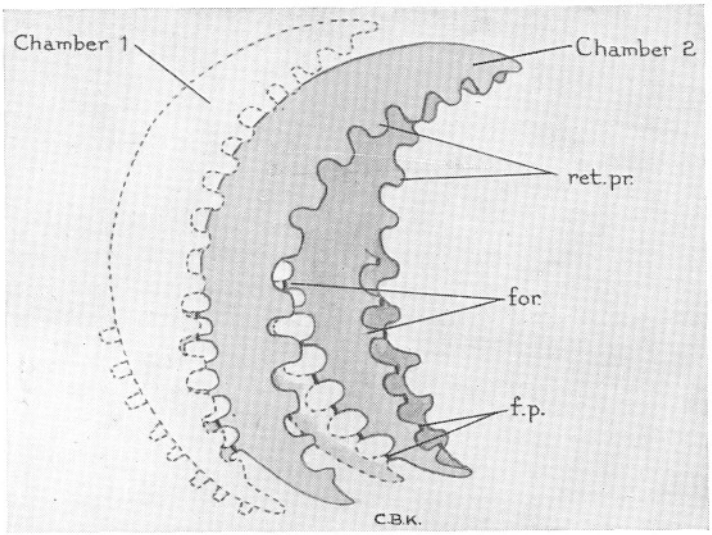
Polystomella depends for its nourishment more than usually on its pseudopodia, for not only does it come in contact with food and catch it by means of them, but the process of digestion also takes place here. No solid food is as a matter of fact ever found inside the shell. Williamson (1849, p. 166) refers to 'very minute siliceous organisms—placed in positions indicating the possibility of their occupying the interior of the segments' in his dried specimens; these were probably the same as his '*Cocconeis*' of 1852 (p. 126), 'frustules of minute Diatomaceae' which he had 'recently found' in the interior protoplasm of a *Polystomella*—none too large to have passed through the terminal apertures of the shell—and which he evidently regarded as food. They are almost certainly loose foraminal plugs (see Text-figs. 6, 7). Lister, in his laboratory notebooks (1892–1905), several times mentions 'algae', 'parasitic algae', especially in the terminal chambers, 'with thick walls' or 'collapsed', and his sketch in Vol. I (opposite p. 111) puts it beyond doubt that these were in reality foraminal plugs, cast off probably at fixation (see p. 627). In 1895 (pl. vi, fig. 10) he figures the same structures in situ, without comment. Le Calvez (1938, p. 236)

[1] These movements should be regarded as a resultant of cytoplasmic streaming and true stretching and contraction, as suggested by Schmidt (1937, p. 590).



Text-fig. 6. *a*, pseudopodia of an injured *Polystomella* showing a great development of ? mucous varicosities and webs; *b*, foraminal plugs sketched after ejection; *c*, pseudopodia showing a bundle of diatoms being drawn towards the shell in the direction of the arrow; *D*, 1, 2, 3, sketches at intervals showing the extraction of a small portion of the diatom culture on which the *Polystomella* was feeding, as described in the text, p. 628. *chr.*, chromatophore of diatom; *e.k.*, sticky end-knob; *h.*, hole in pasture; *N.*, *Navicula mutica*; *o.d.*, oil drop; *W.*, web attached to food mass.

first attempted to describe them fully, in *Planorbulina mediterranensis*. According to le Calvez the foramen is gradually closed up by a deposit which appears as a ring at the narrowest point and spreads across the opening to form a 'bouchon'. Periodically the plugs come loose and are carried out of the animal by the cytoplasmic currents. The cycle is synchronous throughout all the chambers. My observations on *Polystomella* indicate that the same thing



Text-fig. 7. Showing the protoplasmic structures filling two chambers of the shell. *Chamber* 1 (outline dotted), *chamber* 2 (shaded), protoplasm in chambers; *for.*, protoplasm in foramina before and behind chamber, forming bridges by way of which each chamber communicates with its neighbours except in so far as the bridges are closed by the foraminal plugs *f.p.*, in situ; *ret.pr.*, protoplasmic retral processes underlying transverse ridges on exterior of shell. The canal system lies in the shell substance between these structures; a spiral canal, leaving the initial chamber and following the ends of the series of chambers, runs in the umbo on either side, giving off (*a*) in the septa, meridional canals which pass out between the retral processes and the foramina and have branches opening into the fossettes, and (*b*) several vertical canals which open in depressions on the umbo. After Williamson (1849), and see Carpenter (1860).

happens here also, the plugs being composed of some 'chitinous' material, staining as le Calvez says with chromatin stains, including Feulgen, and showing in favourable specimens at all the foramina from the initial chamber outwards in both microspheric and megalospheric phases. Le Calvez does not give details of his observations on the shedding of the 'bouchons'. As already noted they are loosened by fixatives, especially when used hot, but they are certainly also cast at times in the absence of such unnatural treatment, for they appear along with the excretory granules in the dejecta of the living animal (see Text-fig. 6*b*). Their function seems at present quite obscure. (William-

son's reference in 1849 (p. 165) to a constriction seen at times in the foramina of *Polystomella* must indicate a stage in the laying down of these plugs.)

When the pseudopodia of a *Polystomella* in the course of its wanderings come across a movable solid body they may get hold of it, and by subsequent appropriate shortening gradually draw it up towards the shell, perhaps pulling it in a millimetre or so in 10–20 min. Sometimes the object may go backwards and forwards on the way as the pseudopodium stretches and contracts again, and sometimes it is abandoned altogether. A food body may be seized in either of two ways. I have seen one come against the side of a pseudopodium which suddenly bends over it—thus enclosing it—or the tip of the pseudopodium may attach itself to the food body—perhaps by the aid of a sticky 'end-knob'—spreading out against it in a fanwise manner and apparently flowing more or less around it. Then it seems as if the food is actually pulled along towards the shell, while new pseudopodia may arise beyond it and go further afield (see Text-fig. 6*c*). It was most interesting to watch pseudopodia pulling on *Navicula* growing in a carpetlike mass of bacteria, etc., until a little patch would suddenly give way and be dragged out of the carpet, leaving a hole where it had been, as shown in Text-fig. 6 *d*, 1–3, while the patch was drawn away towards the shell. When the pseudopodia are working fast the collection of food (? embedded in mucus) may be enough to form a good 'feeding cyst' completely covering the shell in an hour or two. Inside this, the pseudopodia being withdrawn so far, digestion of the meal goes on. After an interval pseudopodia again appear outside the feeding cyst and the *Polystomella* begins to emerge. Sometimes a new chamber is first formed inside the cyst; and the following laboratory notes give an account of an emergence which I watched, from a cyst which happened to be thin, mainly composed of small granules with only a few diatoms in it. As is usually the case it was somewhat thicker over the terminal face of the last chamber, and this part is referred to as the 'face-mask'. '8/8/39, 9.45 p.m. R. had moved a very little way back from 'face-mask', but still in slough. Large numbers of rather straight parallel pseudopodia over face and parts of cyst nearby—i.e. above and below 'face-mask'. Lengthened as if pushing, while great numbers of pseudopodia on opposite side [of shell] pulled, and *Polystomella* slid backwards out of thin granular slough—pseudopodia passed easily through this.'

There had probably been but little feeding in this particular cyst, but this does not affect the mode of emergence, which may, however, be above or below the 'face-mask' instead of directly opposite it. Emergence from a complete covering of food is usually at the periphery—probably a line of weakness in the feeding cyst, and the point at which the strong keel of the shell could be most useful in forcing a way through. There is another record in July 1939 of a *Polystomella* which had also made a new chamber inside its cyst, this time mainly composed of *Navicula mutica*, and then had left it backwards as usual. The cast slough was examined. The majority of the *Navicula* in the cyst looked normally healthy, but amongst them were large numbers of empty

frustules, the two valves of many of them being separated. There were also coloured excretory granules, about 2μ in diameter, especially in the 'face-mask'. This is the usual constitution of a discarded feeding cyst of *Polystomella*; inside this one the creature had evidently taken a meal, excreted, and made a new chamber. In Pl. V, figs. 4–6, are reproduced sketches made during life of another *Polystomella* which was watched as it fed on the planktonic diatom *Nitzschia*, while wandering on the side of an upright museum jar. First there was an abandoned feeding cyst (Fig. 4) composed, as could be judged from its colour, largely of diatoms; then another slough of a brighter golden hue was left behind (Fig. 5), largely made up of excretory granules. The *Polystomella* itself is seen moving off (Fig. 6). It seems that feeding may take place at any time of day or night. The interval between one meal and the next may be very short—a *Polystomella* is on record as making and discarding two feeding cysts in 7 hr.—or it may be a matter of days, according to circumstances. The actual digestion of the contents of a *Navicula* for example would seem not to take a long time, as so few are ever found in a condition intermediate between normality and the empty open frustules. These were observed mostly over the flat surfaces of the shell in one case—the undigested diatoms tending to lie near the periphery of the cast cyst. All my attempts to witness the process of digestion in detail have so far been in vain; and from what has been said it will be plain that success in this must depend on a very lucky chance, as it evidently takes place close to the outside of the shell, either in pseudopodia or in the external protoplasmic layer which is at times thick enough to enclose diatoms for example. It is interesting to note that the cast cysts of *Polystomella* feeding in *Synedra* cultures also show emptied frustules, although this genus has no open raphe. The valves may however be pierced by pores.

The 'coloured excretory granules' already mentioned are of special interest because they are such a conspicuous feature of the Foraminifera as a group. They are known as 'xanthosomes' on account of their amber colour (see Jepps, 1926; le Calvez, 1938, p. 268). They may be seen in trails (tracks of pseudopodia) and clumps in diatom cultures, etc., in which *Polystomella* have been feeding, as well as in their discarded feeding cysts. They also appear, at times in very great numbers, scattered through the cytoplasm of the living animal, or collected in certain parts of it. In *Polystomella* they vary in size up to 5μ or so across, and are often slightly angular in contour. Reference has already been made to their presence in the pseudopodia (p. 624), where they go about in the circulation for a time and are ultimately left behind by the protoplasm retreating from the mucous trail. We have seen also that they are sometimes thrown off in a more or less complete cyst-like covering. Like the foraminal plugs which are sometimes thrown out with them, they are too large to pass through the fine pores of the shell. They may be seen massed in the 'face-mask' about the terminal apertures (foramina) of the last chamber, in the fossettes between the retral processes, or in the depressions on the umbo

of the shell. Both xanthosomes and foraminal plugs are present in the protoplasm of the canal system in a number of my mounted specimens. The former can be shown in the terminal apertures as well as in the position of the fossettes; while the latter tend to collect in the latest formed chambers and perhaps usually pass out by the foramina, although in at least one preparation appearances suggest that they also may be able to leave the shell by openings into the fossettes. Excretory granules have actually been seen on their way out in strong pseudopodia emerging from the fossettes of the last two chambers and from the terminal foramina of a *Polystomella* under irrigation. Thin discarded feeding cysts often show a pattern representing the fossettes of the lower side of the shell in little heaps of excretory granules. The conclusion is that these solid bodies pass out of the shell by one or other of these two routes —and therefore that the canal system of *P. crispa* certainly has openings into the fossettes as described for the larger *P. craticulata* (up to about 5 mm. in diameter, fossilized), from the coast of Australia by W. B. Carpenter (1860), although he specifically denied it for *P. crispa*. It is easy to understand how Carpenter came to pass them over. I could not be quite certain in my preparations of more or less normal whole *Polystomella* that the meridional canals (see Text-fig. 7 legend) bore lateral branches which might open into the fossettes until I had seen them most clearly displayed in one of a set which had been living in a culture of *Synedra* for some months. Owing possibly to more than one cause, the protoplasm was badly diminished and disorganized—the nucleus had disappeared—but the canal system seemed swollen out and full of staining granular material so that it was very conspicuous indeed, even to these ultimate branches, one to each fossette, especially where it had broken away from the remains of the chambers. The canals are of course to be seen fragmentarily in sections of *Polystomella*. My observations on the canal system confirm the findings of Lister (see 1903, pp. 65, 66, with fig. 9).

It is often stated that the pseudopodia of Foraminifera have the power of killing their living prey suddenly as if by the action of some specific poison (Schultze, 1854, p. 23, for *Polystomella* and *Gromia* feeding on 'kleine Infusorien'; Winter, 1907, p. 10, for *Peneroplis* feeding on small crustacea; and others). I have seen no evidence of this in *Polystomella*; but I have not yet offered small crustacea as food, and Winter says the poison of *Peneroplis* is not so effective against 'Infusorien' and 'Flagellaten'. As already indicated my *Polystomella* fed mainly on diatoms, though there were usually plenty of small zoomastigine flagellates ('*Bodo* sp.') about. Occasionally, however, I have seen these being drawn towards the shell. They continue their movements for some time, even after they begin to look rather abnormal, and seem to die gradually in the course of their transport. This agrees with the observations of Rhumbler (1909, p. 253), although I cannot agree that the movement of the prey is necessary to its capture as he goes on to suggest (quoting Verworn, 1889, pp. 148–9). We have seen that non-motile diatoms, for example, are caught, and apparently used as food. Besides these, *Polystomella* frequently takes

inanimate objects up to its shell, such as small sand grains, filter-paper fibres in the irrigation experiments, etc., and manufactures a more or less complete covering out of them. Although I have a general impression that *Polystomella* did to a certain extent choose diatoms, and particularly *Navicula mutica* as food in the cultures, it is difficult to say anything more definite about its power of selection in the face of the probability that the function of the cyst may be partly protective (see p. 642). Further, although Cryptomonads and Chlamydomonads when available were also incorporated in the cysts, in spite of a search no evidence was obtained that they had been used as food; but even if they had been, neither would leave as conspicuous an indigestible residue behind as the frustules of a diatom. Some other Foraminifera, including the pelagic forms, are reputed to feed to a large extent on animal food, especially small copepods (see for example Rhumbler, 1900, p. 2; Winter, 1907, p. 10), and it is hoped, when opportunity offers, to try the effect of a more mixed diet on *Polystomella*.

Foraminifera have long been famous for their tenacity of life, at least in certain circumstances (Schultze, 1854, pp. 21, 31). Although at times their appetites seem large, some of them, including *Polystomella*, can survive long periods of more or less complete starvation. When short of food *Polystomella* slowly loses its brown colour, at any stage of its life history, often becoming patchy and then always paler until in 3 or 4 weeks it may be almost white. If it has not degenerated too far, the colour will return when food is again taken, a week or 10 days of good living making a noticeable difference. As pointed out by Rhumbler (1909, p. 255 footnote) the granules gradually disappear from the pseudopodia of a starved *Polystomella*; all the cytoplasm in fact seems to become thinner in its consistency, and inside the shell it is seen to decrease in extent. Lister (1895, p. 422) gives figures to show that starvation also induces a shrinkage of the nucleus relative to the cytoplasm as indicated by the number of chambers filled at the time of fixation. It is a pity that his two groups of *Polystomella* were collected at different seasons, viz. 118 fixed fresh from the sea in May and June, and forty-eight collected in October and fixed in January after 3 months in the laboratory on short rations; because, owing to the annual cycle of which Lister was unaware, there is also an age factor involved, though it is not known that this actually affects the result. The curves come out remarkably clearly for so comparatively few specimens, and in spite of the fact that the number of chambers is only a very rough indication of the *amount* of protoplasm present since they vary a good deal in size.

Part II. The Biennial Cycle, including an Account of the Life of a Megalospheric Brood in Culture and a description of the Formation of a New Chamber

The Microspheric Phase

It is well known that microspheric *Polystomella* often undergo asexual reproduction in a laboratory culture if provided with a modicum of food.[1] Schaudinn 'mentioned' the event (1894*b*, p. 42) and referred to it again with a few particulars (1895*a*, p. 59; 1903, p. 499). In 1895 Lister gave in his Postscript no. 2 (p. 445) an incomparably succinct account of the process after observing 'some hundreds of cases'. This was amplified in 1903 and illustrated with the now familiar figures (pp. 67–9). Later references (Lister, 1906, p. 7; 1907, p. 492) only added that some 200 young are commonly produced in a brood. Heron-Allen published extracts from Lister's Notebooks (1892–1905) giving further details of the actual observations, the most important being that 'in specimens whose protoplasm has begun to emerge, faintly stained round nuclei 10μ in diameter are found in the clear protoplasm of the terminal chamber' (1930, p. 7). This supports Lister's statements that there are nuclei in the young megalospheric individuals from the beginning, although, curiously, he had originally come to the same conclusion as Schaudinn that the nuclei of the microspheric parent had completely disintegrated into a chromidial form before reproduction set in. I believe that this conclusion may be due, partly at any rate, to imperfect fixation, because of a very severe reaction to disturbance, perhaps accompanied by actual injury to the protoplasmic mass in the course of securing it for fixation at a particular moment. Emergent protoplasm which was detached from the walls of a culture dish either showed no nuclei at all or nuclei somewhat altered in appearance, so as to make them even more easily concealed amongst the deeply staining chromidial bodies which are very conspicuous at this time. On the grounds of analogy and general theory one can accept complete nuclear dissolution only with the greatest reserve until it is demonstrated to be true beyond all question. There is no need for further discussion of the inaccuracies of Schaudinn's account of the process since they are fully dealt with by Lister (1903, pp. 69, 70).

Perhaps the sensitivity referred to above may account at least in part for the fact that we have as yet no description of any nuclear division in *Polystomella*. It is of course notoriously rare to come on most species of protozoa with nuclei in a state of division; and apart from the sporulation phase there are anyway only a few nuclear divisions in the long life history of a *Polystomella*. The structure of the nucleus was described by F. E. Schulze (1877, p. 17, pl. ii), and its position about midway in the series of chambers of the megalospheric phase of *P. striatopunctata* indicated. The nuclei of the microspheric phase are similar in appearance and are spread through the chambers forming approximately the middle third of the series, those in the outer chambers (as pointed

[1] And occasionally if no food is provided.

out by Lister) being larger as a rule than the others. It may be said on the whole that the nucleus of a large megalospheric *Polystomella* is more likely to be packed full of nucleoli of a smaller size, which, however, may vary a good deal inside one nucleus, while, as noticed by Lister (1895, p. 419), those of the microspheric form tend to fewer nucleoli of a larger size, especially the larger ones in the outer nucleated chambers. No positive reaction to Feulgen's stain was obtained in the nucleus of either form. Both Lister and Schaudinn believed them to increase in number by direct (amitotic) division as the chambers increased. As 'good evidence' for this Lister indicates their disposition in the chambers in pairs of similar size and appearance, sometimes united by a narrow bridge as if in the act of dividing with absolutely no visible rearrangement of their components (1895, p. 419). These observations are easily confirmed, but I believe they may be found susceptible of a different explanation. It does not, however, seem profitable to discuss the matter further at present. After examining whole mounts of some 125 and sections of over 200 microspheric *Polystomella*, many of them in April and May when reproduction is at its height, I am still unable to give the smallest indication of any division figure. Some of these specimens were fixed soon after collection from the sea, i.e. in a few hours' time during which they were kept as far as possible at sea temperatures. Others were cleaned and put to feed in diatom cultures for periods up to a fortnight, and afterwards fixed at various times of day and night. Some were starved and then fed; a further trial of this procedure is greatly to be desired.

Nuclear division in microspheric *Polystomella* seems to go on at any time of year if we may depend on size differences, etc., of the nuclei as indications of recent multiplication. Such indications are most often seen towards the middle of the series in each *Polystomella*, where the nuclei tend to be more numerous and smaller; and are evident in specimens collected in October and November as well as in April when asexual multiplication is generally imminent. If averages obtained from my comparatively small numbers can be trusted, there is not much increase in the number of chambers through the winter months, but the nuclei have multiplied from say thirty-three in the autumn to forty-four (varying $\pm$ 10) in some fifty-four chambers by the middle of April (thirty-seven specimens, 11 April 1940). About two further divisions for each nucleus are then all that are necessary to produce the number required by the brood of megalospheric young. The highest number of nuclei shown in my preparations is about 116 in a *Polystomella* of fifty-five chambers collected in March, the protoplasmic body after decalcification measuring a little over 1·5 mm. in diameter,[1] and having as many as a dozen or more nuclei in some of the

[1] My measurements refer to what is usually the greatest diameter, along a line passing lengthwise through a chamber a little before the last of the series. I regret that many were made before I realized that the official plane of measurement runs across the shell parallel to the last chamber. In counting the chambers I have reckoned the 'globular swelling' referred to by Lister (1895, p. 418), which lies next to the initial chamber as the second markedly smaller chamber of the series, which it undoubtedly is. The third chamber is roughly as big as the initial chamber, and thereafter there is generally speaking a steady increase in size in the

chambers towards the middle of the nucleated series. Another *Polystomella* of the same size (in August) had seventy-six chambers, the biggest number I ever found; and the largest specimen (in the April collection) with sixty-eight chambers measured 1·8 mm. after decalcification. Microspheric *Polystomella* tend to reach a large size on the average, although some megalospheric forms also attain the same dimensions. I have no record of asexual reproduction in a *Polystomella* that was not over 1 mm. across in the shell and probably comprised at least fifty chambers.

It seems that Lister was working with a population of a smaller size or an earlier maturity—for he quotes (1895, p. 419) as one of his largest microspheric specimens a *Polystomella* of forty-seven chambers which measured 800μ across (? after decalcification); while a few figures in his Notebooks (1892–1905) give an average of thirty-six chambers for ten reproducing microspheric individuals.

The *Polystomella*, which usually show signs of starvation by the end of winter,[1] are able to feed again as soon as the diatoms begin to increase in the spring. Their colour improves and growth again takes place. By early in March 1939 it was noted that they had begun to look a little browner in the collections, and by the end of the month some of them had taken on a rich brown colour. From now on it was possible to make a partial separation of megalospheric and microspheric forms. Apart from the fact that one may often effect a slight concentration of the microspheric *Polystomella* by selecting those of the largest size, in April they could be collected by taking those of the richest colour and those which showed the greatest recent growth. This was apparent in the shape of the creatures, because their newly formed chambers were conspicuously large, raising the contour of the shell into a high bulge as they spread over the whorl below. This feature is actually shown in Lister's figures (1903, pp. 63, 67) without comment. Though it is good enough to be useful in choosing specimens for cultures, the test is by no means infallible even at this season. Young or small megalospheric *Polystomella* which feed and grow at a greater rate, naturally tend to exhibit what we may call the microspheric figure, and there are always some larger ones of both kinds which are also exceptions to the rule. The degree of success in diagnosis of living *Polystomella* which was attained by these criteria is indicated by one or two examples. On each of two

series. It might be pointed out here that the initial chamber of the microspheric form differs from all other chambers of both forms when seen in decalcified specimens in that it is enclosed in a thickish capsule, like a cyst wall with a single pore which is the foramen. It would obviously be more significant to give measurements of this capsule where possible rather than of the more variable cytoplasm which may not fill it, although the capsule itself appears shrivelled in many preparations. As Lister (1895, p. 418) observed, the first three or four chambers are also different in that they are arranged in a spiral, which may be either right-handed or left-handed. Does this represent an ancestral form of the creature? (See Lister, 1903, pp. 135–7; Tan Sin Hok, 1935; Ovey, 1938).

[1] It will be very interesting to compare my account of the annual cycle, based on general observation and cultures, with that of Earl H. Myers derived from his statistical investigation of very large numbers of specimens, during the same year at Plymouth.

occasions in May about thirty 'microspheric' *Polystomella* were picked out, mostly by the shape, and there were some which could not be assigned to either form. Only about 10 % were correctly selected. Better results were obtained in April by choosing the darkest brown specimens, especially the largest ones, when a 60 or 70 % success was reached. It is plain that the method could not be applied to individual specimens. It is usually stated that there are only about 3 % of microspheric forms in the population, and this is true of the greater part of the year. In the month of March 1939 the percentage rose from this value to about 25 %, and in April it continued to rise to about 30 %. This was due to the fact that the megalospheric forms had been sporulating[1] from January on, and so gradually disappearing from the living population. At first only a few *Polystomella* were reproducing, the peak of sporulation occurring late in March or early in April. It was perhaps at their point of highest frequency that the shape of the microspheric forms was most distinctive; later in May the choice became confused by the presence of some megalospheric young, and perhaps by the darkening of the colour of some older ones after more prolonged feeding.

A few comparable figures are given in Table I for small collections of *Polystomella* from the 'White Patch' ground in 1940.[2]

TABLE I

Date	% microspheric form	Total collection
3. i. 40	10	250
29. ii. 40	17–18	90
5. iv. 40	about 50	94
18. vii. 40	about 7·0	about 300
21. x. 40	4·5	424

Some time in April the microspheric *Polystomella* in turn reached the peak of their season for reproduction by a form of schizogony, and in turn began to show a notable reduction of their numbers.[3] This asexual reproduction began early in February in my cultures fed on *Navicula mutica* for a little over a month, and in the sea by the middle of April there were already a large number of young megalospheric forms, 0·5 mm. or so in diameter, offspring of this process. On 26 April I selected twelve *Polystomella*, measuring 1·2–1·6 mm. in diameter, as likely to be microspheric from their colour and shape, and nine of them produced young asexually in cultures of *Navicula mutica* between 5 and 22 May (at least two of the other three were megalospheric). During this time there was unfortunately an unavoidable interval in my observations, and when I returned to them on 22 May the high season for schizogony seemed to be past, although it occurred in my cultures later on, and probably continued

[1] This process will be discussed in a later section of this series.

[2] No great value must be placed on the exact numbers given. The samples were not especially taken for this purpose, nor all in the same way.

[3] Heron-Allen (1914, p. 9, footnote) refers to the finding of 'a very large number' of two- and three-chambered megalospheric young on the Drake's Island ground in April 1929.

in the sea through the summer months.[1] From June onwards large *Polystomella* of any kind were scarce in the catches, and of these a fairly high proportion were microspheric. Of eighty specimens in August 6% were microspheric, and young of 10–20 chambers were still common in October. By the end of June some young had reached the size of the smaller specimens of the year before, i.e. a millimetre or so in diameter, but they could still often be distinguished by their browner colour. Towards the end of July a feeding experiment indicated that less food was being taken and that the rate of growth had very much slowed down, and in August I could no longer pick out 'young' and 'old' specimens amongst those of the larger sizes.

Of the *Polystomella* from 'White Patch' to which reference is made in Table I about 100 were put to feed in a culture of diatoms on 20 July 1940, and eight out of twelve microspheric individuals reproduced before 27 August, while a similar experiment in October gave no reproduction at all.

Development of the Megalospheric Phase

Some of the young megalospheric *Polystomella* produced in my cultures were reared for varying periods; and some account will now be given of the more interesting observations on two out of several families born in Petri-dish cultures of *Navicula mutica*. In both of these the parent shell was incompletely emptied—but in others all the protoplasm came out as it probably does in the wild, leaving a very clean white shell behind. Such shells often have holes in the wall of the terminal chamber, apparently made by the issuing protoplasm. The diatom cultures were of course renewed as required, and larger vessels used as the young grew up.

The largest Petri-dish brood (N 4) numbered some 150 young. On 3 and 4 February 1939 a *Polystomella* measuring a little over 1·5 mm., which had been feeding on *Navicula*[2] in sea water since 17 January on the laboratory bench at temperatures of 55–60° F., was seen under the surface film, supported by a large number of long pseudopodia. On 7 February it was still at the surface, with almost all its protoplasm outside and divided up into the family of two-chambered young, which were engaged in collecting diatoms and ? bacteria out of the surface film into their pseudopodia.

8 February. Many young have a third chamber.
9 February. Young spreading over the surface film. Some with four chambers.
10 February. Four to five chambers. Renewed the sea water carefully with a pipette.
11 February. Put a few young on the bottom of the dish where there was more food.
18 February. Parent has fallen to the bottom. The brown protoplasm remaining in the last few chambers is putting out pseudopodia. Wall of terminal chamber seen to be damaged. Some young with six chambers.

[1] J. J. Lister studied this method of reproduction in *Polystomella* from the English Channel in the months of May, June, and July.

[2] Grown in Erdschreiber, which was replaced by sterile sea water when the Foraminifera were introduced.

23 February. 4½ chambers of parent shell seen to be full of protoplasm. Young on bottom with eight to nine chambers. On surface where food is scarcer, six to seven chambers.

26 February. Young on bottom much better grown than at surface, where diatoms are very scarce.

28 February. Young on bottom with ten to eleven chambers.

3 March. Parent shell with five to six chambers full.

4 March. Young up to one dozen chambers. Some which started with irregular growth now seen to be growing regularly.

7 March. Biggest young with fifteen to sixteen chambers.

14 March. Cannot now count chambers of big young in shell. Parent shell with protoplasm in 8½ chambers.

8 April. Young up to over 0·5 mm. Fixed some, about one dozen chambers. Diatoms not very good now. On 7 March parent and some of the best young were transferred to a fresh culture of *Navicula* in Erdschreiber. These young now reach 740 μ. Parent shell has protoplasm in almost all exposed chambers.

30 April. Most young about 1 mm. across, one is 1·2 mm. One small, with only seven to eight chambers ('dwarf'). Young look well, but parent very pale and starved looking. Diatoms going off.

7 May. Taken to cooler room—at 13° C.

28 May. Some young up to 1·3 mm., with about forty chambers. Parent probably died about now.

14 June. Dwarf shell empty—about 400 μ.

23 August. Fixed sixteen young, brown in colour, and one very pale with greatly reduced protoplasm, measuring 1·1–1·5 mm.; and two smaller pale ones, also degenerate and measuring under 1 mm.

Another brood (N 2) was born in a similar culture of *N. mutica*, but kept in Erdschreiber at 55–60° F., between 17 and 23 February. The parent shell measured about 1·6 mm. across and had been in the culture since 16 January. In the interval its colour had darkened to a very rich brown shade, especially near the terminal chamber, and it had probably grown one or two new chambers. It was found up the side of the dish on 23 February, a good deal of the protoplasm remaining in the shell and part of that which had emerged undivided. Some healthy looking young were close by—but only thirty-five were ever recovered in this culture. The diatoms were especially good.

7 March. Parent shell has one to two very irregular new chambers.

14 March. A few young have fifteen to sixteen chambers, one only two to three. Many are abnormal and there are some empty shells.

16 March. Parent shell now has about eighteen chambers full of very dark protoplasm.

7 April. Parent shell now has protoplasm in all visible chambers.

30 April. Average diameter of young shells about 800 μ. There were twenty-six good young and nine very deformed. Some fixed in Susa showed about twenty-six chambers, also that individuals which appeared to have begun life as monsters came to grow quite regularly after a time, even when two nuclei were present (one pearshaped initial chamber), presumably owing to imperfect division of the parent protoplasm at schizogony.

27 May. There are twenty-five young now, up to 1·1 mm. across.

28 May. Put in cooler room, at 13° C., but kept at various temperatures (say 13–17° C.), during the summer months.

23 August. Fixed the parent. The protoplasm was a very dark colour, but the shell was highly irregular near the terminal chambers. In section the whole series of chambers was found to contain protoplasm, with about a dozen nuclei and a few shreds of chromidium. It is interesting to observe that the solitary nuclei which do not appear to have undergone recent division are quite full of small nucleoli, looking like the nuclei usually characteristic of a megalospheric form.

2 September. Young now over 1 mm. in diameter, in many cases with some irregularity towards the end of the series of chambers. Rather pale.

23 September. Taken to Glasgow in cultures of *Navicula mutica* in jam pots, and kept there at about 15–16° C. The cultures became rather poor and the *Polystomella* paler.

8 November. Moved to better cultures and improved in colour.

21 November. The *Polystomella* in about 2 l. of Erdschreiber in a large glass basin grown with *Navicula mutica*, were placed in a sink with running cold water[1] at a temperature of 11·5° C. (sea temperatures recorded at Plymouth Pier in Nov. 1939 about 13·5° C.). *Polystomella* now quite a good colour, measure up to 1·3 mm.

15 December. Temperature down to 9–10° C. (sea temperature at Plymouth fell to 10° C. by the end of the year 1939). Careful inspection showed the *Polystomella* of a good colour, but no evidence of recent growth.

18 January 1940. *Polystomella* inspected almost daily since 8 January, still brown.

20 January. One *Polystomella* looks pale. Temperature in the sink this month about 8° C.

21 January. Temperature down to 6·0° C.—very cold weather (sea temperatures at Plymouth down to about 7·5° C. by the end of January 1939). Basin was broken, and had to be left so that the culture was at about 10° C.

9 February. Two *Polystomella* now look almost white, one with brown patches.[2]

14 February. Twenty-three brown *Polystomella*, and two almost white ones which look dead.

17 February. Two very pale *Polystomella* crushed, and the protoplasm seen to be divided up into spheres of 4 μ to two or three times as big, with shining inclusions. One more *Polystomella* looks pale, the rest seem to be feeding well.

23 February. Another *Polystomella* 'white', seen next day to have brown patches on its lower side, in the fossettes.

26 February. Pale *Polystomella* of the 17th is colouring up again. 'White' one of 23rd crushed and found to contain dead rounded up flagellispores with shining inclusions. The brown patches seen to be excretory granules. Temperature this month varying 1–2° on either side of 10° C. (sea at Plymouth in January 1939 about 8° C.).

9 March. Temperature 9° C. Four 'white' *Polystomella* seen: (1) First seen to be white on 7 March and almost certainly not so the previous day. About noon the shell was observed to be full of active flagellispores, with some excretory granules. Examined at intervals all day, but no emergence seen. Next morning the activity had ceased—the little flagellates apparently having died in the shell. (2) Crushed on 10 March, cytoplasm dead, unsegmented. (3) Crushed on 10 March, cytoplasm dead, mostly segmented. (4) Crushed on 10 March, cytoplasm dead, segmented into spheres and distorted flagellispores, both showing some activity. No more 'white' *Polystomella* were seen.

19 March. Left eighteen young in culture, three rather pale, and went to Plymouth.

[1] We are fortunate in Glasgow in having a plentiful supply of water from the Trossachs which runs very cold all the year round, and shows a seasonal variation in temperature which happens to be only a little greater than the shallow water temperatures recorded from the pier at Plymouth.

[2] The significance of some details now given will appear in Pt III of this paper.

The temperature in the culture slowly rose from 9·5 to 11° C. by the time of my return (sea at Plymouth only up to about 10° C. at this date in 1939).

21 April. Eighteen *Polystomella*, no empty shells; two or three pale, but some very brown indeed in places.

30 April. Crushed three pale *Polystomella*; all dead or degenerate. The *Navicula* cultures became unsatisfactory and the *Polystomella* were transferred to cultures of other diatoms.

18 May. They were still feeding and mostly a good colour. Temperature about 13° C. (sea at Plymouth up to about 13·5° C. by the end of May 1939).

29 June. Fourteen *Polystomella* apparently still feeding, but pale or patchy in colour. Hot weather. Temperature up to 19° C. (sea at Plymouth in June 1939 about 16·5° C.).

12 July. All very pale. Left to feed in a good culture of mixed diatoms during vacation. Temperatures probably about 16–17° C. (sea temperatures recorded at Plymouth Pier July–August 1939 about 16–17° C.).

14 September. Culture now pale and patchy, though there are remains of a good growth. *Polystomella* found: six of a good colour, 1–1·5 mm.; nine very pale; two greenish, look dead. Some of the shells were preserved as dry mounts, five crushed. Contents of two pale and two greenish ones very unhealthy or dead.

These culture records show how rapidly the young megalospheric *Polystomella* may grow. Lister noted that two chambers may be formed on the first day (Notebooks, Vol. II, p. 116) and another two by the end of the second day (1903, p. 69). Actually an average rate of a new chamber almost every second day was maintained for about 3 months, by which time the young measured a millimetre or so across the shell and had some forty chambers. In June 1939 some young *Polystomella* from the sea, measuring about 400μ, formed another new chamber every other day with great regularity up to a period of 10 days in cultures of *Navicula mutica*. In July a similar experiment indicated that there was some falling off in the amount of food taken and in the rate of growth in *Polystomella* which already measured about 1 mm. Probably the average number of chambers finally attained is somewhere between forty-five and fifty; although one large megalospheric shell collected in January to keep in culture measured 1·8 mm. and was found to have contained sixty-seven chambers at the time of its collection.

No suggestion of a repeated schizogony was ever seen in my cultures.

The history of culture N 2 shows that at the end of a year some of the *Polystomella* attempted to sporulate. This seemed most surprising after so many vicissitudes in the course of their lives, and showed that it is not difficult[1] to keep these Foraminifera in cultures up to this critical stage, which as already noted will be the subject of further discussion in a later part of this series.

It is time now to refer to an unexpected imperfection in the results obtained in my cultures. In both the grown *Polystomella* taken from the sea in January 1939 and of the young reared from them at Plymouth it was found that the shells tended to become abnormally heavy, apparently owing to an extraordinary secondary deposit of shell substance which tends to fill up all the

[1] They might have done even better if they had begun life in larger vessels, for instance, or if the temperature had been more consistently controlled. It is hoped to repeat the rearing of a megalospheric family under better conditions when supplies again become freely available.

depressions, thickening the prominences and gradually obliterating the normal shell pattern. One such case is illustrated in Text-fig. 8, drawn from a sketch in a laboratory notebook in August 1939. This *Polystomella* had lived in *Navicula mutica* cultures since 17 January. It appeared as if the openings in the shell might be occluded, but several of the vertical canals opening on the umbo could still be seen and pseudopodia were put out while the sketch was being made. The same sort of disfigurement was seen in some of the young after a few months, and became worse the longer they lived in the cultures; the shells were thick from one side to the other, and did not usually reach as large a diameter as their parents, or as many wild megalospheric specimens. This general thickening is a different phenomenon from other shell abnormalities such as:

Text-fig. 8. *Polystomella* with abnormally thickened shell, after some months in culture; cf. Text-fig. 4 *a*. Some of the young reared in cultures into their second year became much worse than this.

(1) The irregular growth which often occurs in very young megalospheric offspring of schizogony, especially perhaps in cultures but not exclusively there, and which as we have seen may right itself after the formation of a few irregular chambers.

(2) The real monsters which develop when, e.g. two young grow as a twin, two initial chambers being formed.[1] Possibly such a beginning may lead to grown-up monstrous shells like those depicted by Schultze (1854, p. 30, pl. v), with more than one whorl of chambers. Other abnormal shells may also occur in the sea, e.g. with the chambers taking on a rectilinear arrangement at a certain stage (see Millett, 1904, p. 604, pl. xi) and cf. *Ozawaia* Cushman (Cushman, 1933).

(3) Shells with small irregular new chambers, such as are formed after injury to the more or less naked protoplasm at an early stage of the process of their formation.

The thickening is obviously due to some cultural condition, and at present it is not possible to account for it. Heron-Allen (1914, p. 262) relates how he got extra ridges, etc., on *Massilina* shells in cultures when he replaced the water of evaporation with hard water from his well. But thanks to Dr J. D. Robertson, of this Department, who very kindly made the necessary estimations, I can say that my culture media only contained about the average amount of calcium in sea water. Unused medium (Bearsden earth extract + Clyde sea water) contained 409 mg./l.; while after nearly 4 months

[1] Quite regular adult megalospheric specimens with more than a single nucleus occur in the sea (see also Lister, 1895, p. 425). But these have only a single initial chamber, so that if they indicate twinning it is here more complete from the outset (see also Heron-Allen, 1914, p. 249).

use by a culture of diatoms and *Polystomella*, another sample (Plymouth earth extract + Clyde sea water) contained 384 mg./l.; the salinity of the two samples being within 1 % of each other, indicated that there had been no appreciable evaporation in the used medium.

Another peculiarity of the several hundreds of young I saw in my cultures was that not one ever showed the least sign of spines on the keel at the periphery of the shell, such as are shown in three- to four-chambered young by Lister (1903, p. 69) and are commonly seen on shells of small size (under 1 mm.) from the sea usually said to be young *Polystomella* of various species (see Williamson, 1849, p. 163, on young *P. crispa* 'when obtained from deep water'; Schultze, 1854, Taf. v, on young *P. strigilata*; Brady, 1884, on young *P.? macella*, and on *P. imperatrix* at all ages). Such small spiny shells are not infrequently present in the Drake's Island collections (down to about 5 fathoms) and are very common amongst the small *Polystomella* found in shallow *Corallina* pools on the shores of the Firth of Clyde (at Millport).

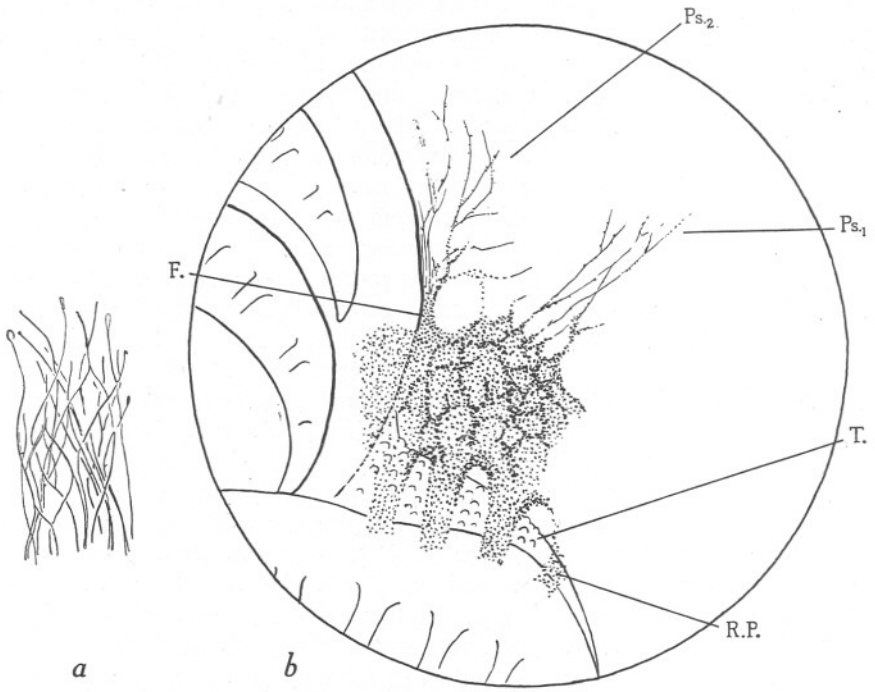
Regarding the actual emergence of the protoplasm and its subsequent fission, my observations, as far as they go, confirm the account given by Lister. As he reports the 'premonitory halo' of numerous colourless pseudopodia (1903, fig. 10, p. 67) is as a rule developed early in the day, between 6 and 10 a.m., the *Polystomella* often having crawled up the side of the culture vessel beforehand. Just as when a portion of the protoplasm emerges to form a new chamber, so here the pseudopodia are soon followed by the brown protoplasmic body, which appears to leave the shell by way of the canal openings near the last chamber[1] and probably by the terminal openings also. In some cases, as has already been mentioned, additional large openings are made in the wall of the terminal chamber as described in *Discorbina* (see Schlumberger, 1896) and *Peneroplis* (Winter, 1907, p. 23). As already noted Lister found small nuclei in his reproducing microspheric *Polystomella*; these went down to about 7μ in diameter (1903, p. 70). In my preparations there are none so small, all the nuclei being well over 10μ. In a few young of two to three chambers they measured 15–20μ, and at a dozen chambers about 30μ. They show nucleoli from the beginning; and indeed, as compared with a collection of young from the sea, a family likeness may be observed in the size, etc., of the nucleoli in a brood of young, as well as in the appearance of the chromidial mass, which may be very compact or more or less diffuse, scanty or plentiful. This is of course due to the protoplasmic structures being taken over from the parent. I am not sure whether the size of the initial chamber is more uniform within a family or not—there is evidence in my preparations of the measurements being affected by pressure of the cover-slip, and special observations would be necessary to determine this point. Retral processes appear on the second chamber of the young (see Text-fig. 1, F, A); the nucleus leaves the

[1] In his Laboratory Notebooks (Vol. II, p. 131) Lister says that during emergence of the protoplasm he found the small nuclei, measuring 7–10μ in diameter, unevenly distributed in the cytoplasm, some in the canal system.

initial chamber when there are about a dozen chambers and wanders out to take up its position usually a little behind the middle chamber of the series. The chromidial masses have entirely disappeared soon after this stage, and the nucleus now has the numerous nucleoli usually present in the megalospheric phase.

Formation of a New Chamber

Some observations were made on the formation of a new chamber. An unusually dense fan-shaped mass of closely set anastomosing pseudopodia make their appearance, radiating out from the terminal apertures of the last chamber of the shell (see Text-fig. 9*a*). Beyond them bundles of ordinary



Text-fig. 9. *a*, bundle of special pseudopodia emerging from pores of shell just above the foramina and at sides of last chamber where new chamber will lie, 12.30 a.m. Zeiss, $\frac{1}{6}$ in., 4. *b*, detail of new chamber in process of formation. Zeiss binocular, ×300; 11.0 p.m.–midnight. *F.*, cytoplasm in foramen of new chamber, i.e. terminal aperture; *Ps.*1, pseudopodia, gone at 11.30 p.m. leaving cytoplasm with clean outline, ? shell appearing. *Ps.*2, pseudopodia still lively at 11.45 p.m.; *R.P.*, cytoplasmic retral process, 11.30 p.m.; *T.*, tubercles on terminal face of last old chamber which will form part of the new last septum.

pseudopodia may reach out farther to collect any material that may be available (diatoms, excretory granules, sand, etc.) to make into a 'face-mask', inside which the new chamber will be formed. But the process can go on

without this cover, as in the case of a specially cleaned *Polystomella* under irrigation, and then the details are more clearly seen. Activity usually begins in the late afternoon or early evening, the protoplasm, which may be withdrawn at the time from the last chamber, passing out in the form of pseudopodia which flow through the successive sets of foramina, and also emerge at the fossettes and pores at the sides of the last chamber which will be partly covered by the new one. Some of the special pseudopodia after a time begin to arch over in a reticulum which outlines the cavity of the future chamber, usually about 10 p.m. to midnight, the 'face-mask', when present, being pushed away to the outside. They gradually swell at their bases and merge into one another there, whilst a fluid wells out amongst them and comes to fill up the space they enclose under their extremities with a uniformly granular mass of colourless protoplasm in which the pseudopodial streams fade out and ultimately disappear into the general circulation of the mass (see Text-fig. 9*b*). This comes to have a clear-cut surface, fashioned in the shape of the new cavity even to the retral processes and the projections at the future foramina, as shown in the figure. The longer and more active pseudopodia which normally collect the 'face-mask' material have disappeared, and the short pseudopodia remaining on the surface of the protoplasmic mass now appear stiff, with very sluggish movements. They may come and go, as the shell is deposited on the mass; it is impossible to know how much of their variation under observation is due to the unnatural illumination, etc., but in any case the shell seems to be porous from the beginning, and therefore bathed in protoplasm which lays it down initially and may continue to add to it (and at times to reabsorb it) throughout life. As soon as the surface is available a collection of shining granules may be seen there which gradually form a thin layer of shell. This seems to be laid down in patches like the pieces of a jigsaw puzzle, which unite, losing their separate outlines more or less completely as the shell thickens. After a time the characteristic tubercles are formed on the outside and the keel is laid down at the periphery. During the next day the *Polystomella* remains immobile, while the shell is deposited. There is a flow of brown protoplasm into the now penultimate chamber and sometimes into the base of the new one. Then the colourless mass there becomes vacuolated and may be withdrawn altogether, leaving the new chamber empty for a time, as is usual during the greater part of the life of a *Polystomella*. Pseudopodia emerge, the 'face-mask' is cast off, and the *Polystomella* moves away to begin feeding again some 24 hr. after the emergence of the protoplasmic mass. Although a new chamber may occasionally be formed at other times of day, this usually happens early in the night as described; and therefore it would perhaps be advisable when it is necessary to move *Polystomella* in cultures to do so late in the afternoon, say 5–6 p.m., when there is least chance of spoiling a new chamber with a shell too thin to stand being handled. Even if this is injured, however, or if an irregular chamber is formed for any reason, there may be restitution of the normal shape in the course of one or a few additions if circumstances are favourable.

Earlier observations on the development of new chambers are recorded for *Polystomella*, *Rotalia*, and *Miliola*, by Schultze (1854, p. 30); for *Peneroplis* by Winter (1907, p. 23); for *Quinqueloculina* by Hofker (1930*a*, p. 384); and for *Discorbina* by le Calvez (1938, p. 266). Excepting that I have seen nothing in my *Polystomella* to suggest that a new chamber increases in size after it is provided with a shell (as Schultze maintains), all the accounts agree so far as they coincide.

Schultze (1854) comes to the conclusion that *Polystomella strigilata* (also some Rotalidae) can live for several years, since he was able to keep them alive in captivity for a period of 9 months. From my experience this does not seem likely; I would rather agree with Winter's suggestion of senility ('Peneroplengreise', 1907, p. 19) in individuals which pass the sexual breeding season of their contemporaries without undergoing reproduction. The microspheric form usually appears as a comparatively youthful organism, even when it reproduces; but the megalospheric phase when it is grown up tends to become paler, grows much less, and is generally less active. Although a few may survive the season for sporulation it seems very doubtful that they ever again show great activity. If we regard the two generations as making up the life cycle then, it normally occupies a period of about 2 years.

Part III. Sporulation. Development and Emergence of the Flagellate Swarm Spores,[1] their Form and Behaviour, and attempts to obtain their Further Development

Development and Emergence of the Flagellate Swarm Spores

Living Material. Sporulation in *Polystomella* comprises the development and emission by the mature megalospheric stage of very numerous flagellate swarm spores.

All through the winter months it is possible to find undeveloped megalospheric and microspheric individuals down to twenty or thirty chambers or less. These may become pale and shrunken inside their shells during the lean months and many of them probably die. Several small *Polystomella*, somewhat under 1 mm. in diameter, of a good colour, were collected in January 1939 and placed in cultures of *Navicula*, where they fed well and grew new chambers. One reproduced asexually late in April, the other eight included one microspheric (which grew to 1·5 mm. across) and seven megalospheric individuals. But the majority of the megalospheric forms are fully developed by the onset of winter; they will feed and grow a few more chambers when food is available, but from January on more and more of them come to an end in sporulation. The brown colour fades away in a short space of time, a day or two, or sometimes overnight. Meanwhile especially long pseudopodia are put

[1] Known as *zoospores* or *flagellispores* (French)=*flagellospores* (German). The word *flagellula* is to be avoided. It has been criticized as an ill-coined word which does not really mean *a small flagellate* as intended, but *a small flagellum*.

out, often in a long curved bundle which may reach right across an ordinary Petri dish, and large quantities of xanthosomes are shed, so that the pseudopodia may look quite yellow. (This is not, however, a large factor in the loss of colour which is primarily due to an alteration of the chromatophores.)[1] The *Polystomella* walks away from the yellow tracks by means of ordinary colourless pseudopodia. The creature has become very sticky by secretion of a great deal of mucus (see p. 625). The pseudopodia are withdrawn, and the protoplasm in the shell takes on an almost translucent character, while larger or smaller masses of brown excretory granules remaining in the shell often become visible on the under-side—indicating presumably that fission of the cytoplasm has taken place, the heavy, coloured, xanthosomes falling down below the bodies of the developing flagellispores (cf. *Gromia*, Jepps, 1926). A *Polystomella* at this stage is a conspicuous object of a dazzling whiteness which gives it a remarkably plump appearance. The flagellispores usually emerge during the following night, mostly shortly after midnight. They may be seen in amazingly rapid motion inside the shell just before emergence if a thin place can be found and suitably illuminated under the microscope (Zeiss eyepiece no. 4, objective A; × about 100[2]). Presently they all rush out in a great cloud, or they may swim out in a smaller steady stream in the course of several hours, leaving the shell by the foramina and probably by the canal openings also. As a rule shells do not appear to be extensively damaged after sporulation, although some of the usual openings may be enlarged and the little flagellates naturally take advantage of any extra exits that may be available. The shell is left absolutely empty and clean after a good sporulation, apart from small collections of granules, mainly xanthosomes, which may remain in some of the empty chambers; such shells showing brown patches on the side which happens to lie below are recognizable in the collections from the sea for a long time afterwards.

In *Peneroplis* there is said to be a difference in the habit of the two phases; 'Wenn die Agamonten älter werden, hälten sie sich mehr am Boden...die Gamonten steigen höher, was im Reifestadium mir besonders characteristisch schien' (Winter, 1907, p. 20). Nothing of the kind has been detected in *Polystomella*, and it is not possible to say whether there is any special tendency for this foraminiferan to climb up to a higher place before sporulation. Certainly some of those which did so in culture vessels fell off when all the protoplasm retreated inside the shell, although others remained attached by the tough mucous trail of the long cleansing pseudopodia. Lister wrote in his Notebooks (1892–1905, Vol. II), in June 1895, that he had probably failed to find 'zoospores' in 1894 because he had only examined *Polystomella* climbing on the walls of his glass vessels, and these with 'zoospores' would have fallen down

[1] It is hoped to describe this more fully in a later part of these studies.

[2] A little care may be necessary to distinguish between this dance of the flagellispores and a somewhat similar commotion in the shell due to an invasion of a more or less decayed *Polystomella* by much smaller motile bacteria.

when the pseudopodia were withdrawn. Nor is it known exactly when they stop feeding. A few *Polystomella* sporulated in Petri-dish cultures of diatoms, and it was here that the earliest observations were made which gave the clue to the appearance of a sporulating individual. Four healthy brown *Polystomella* (1·3–1·5 mm. across), collected at Drake's Island on 13 January 1939, were cleaned and left in a good culture of *Navicula mutica*, the earth extract medium being replaced by sterile sea water, at a low temperature (10° C. or less). Up to 20 January, no note of any change of colour was made, but on 21 January, one was seen to be lying in the middle of the dish on 'good pasture' with 'many pseudopodia', and 'very white' in colour. Next day the pseudopodia had vanished, but the shell had a light covering of *Navicula*. By 24 January, the temperature being noted as 6° C., it was seen to be empty. On the other hand, one or two other *Polystomella*, which sporulated in this set of cultures later on, had fed and actually grown a few new chambers first.

Many of the details given above were recorded from *Polystomella* taken from the sea, cleaned, and kept in Petri dishes of sea water at various temperatures, and one or two lots kept in the laboratory under other conditions, during the sporulation season.

Although collections had been brought in from Drake's Island at short intervals throughout the earlier winter months, and sometimes kept for a day or two in Petri dishes before being used in experiments with various fixatives, no white *Polystomella* were noticed in them until 15 March. It is probable that they would not have been picked out before their significance was known, and the material was not kept before examination for so long an interval as is usually required for brown *Polystomella* to complete the process of sporulation after capture. It was, however, noted that microspheric forms seemed to be 'very scarce' in the collection of 25 January; and although about 380 *Polystomella* collected on 1 February, some of which proceeded to sporulation in the laboratory 3 days after capture, gave about 6% of microspheric specimens, a certain amount of selection had in fact been made by picking out those of a large size, so that in all probability the proportion was actually but little, if at all, increased (see p. 635). It is unlikely therefore that very much sporulation had occurred before the end of January. The sporulation season seems to fall during the coldest weeks of the year,[1] possibly reaching its height soon after the vernal rise of temperature sets in. According to Harvey (1928) the lowest winter temperatures for 'inshore water' of the English Channel were recorded (in 1924) about the middle of February, and for 'surface water' about a month later. In 1939 the temperatures posted on Plymouth Hoe from civic observations at the pier fell to their lowest (45–47° F.) during February—so possibly in this year the 'inshore water' where *Polystomella* lives, also passed through

[1] This is probably what Schaudinn meant when he said 'Mit dem Eintreten der kalten Jahreszeit merkte ich dass viele grosse *Polystomellen* eines Kulturglases nahe daran waren die Flagellosporen zu bilden, d.h. . . . hatten sogar schon die Sporenkerne gebildet' (1903, p. 500). Le Calvez (1938, p. 216) mentions that he found over 75% of the *Polystomella crispa* living on the *Lithothamnion* at Banyuls to be microspheric 'pendant les mois froids'.

its coldest phase somewhat earlier than in 1924. Miss M. F. Mare, who was engaged on planktonic observations at Plymouth in 1939, was kind enough to inform me that the spring diatom increase went steadily forward from about 14 February. Sporulation thus began shortly before the diatom increase was detected, when the *Polystomella* were at the end of their season of scarce food. When it was first observed the hours of daylight had been increasing for a few weeks since the shortest day of winter.

When it was discovered that a definite season of sporulation was going on, it was decided to seize the opportunity of making a serious attempt to carry the life cycle through this phase, which it was recognized would probably prove one of considerable difficulty if *Polystomella* were found to fall into line with other organisms previously investigated, such as *Gromia oviformis*, for example (Jepps, 1926). The following observations on various factors which might affect the formation of the flagellispores are therefore to be regarded as incidental to the chief object of investigation at the time; and the conclusions on the whole tentative, since so many of the experiments were on a small scale and often not repeated.

When sporulation was first observed in the collected material, the catches were still being brought into the laboratory and washed with filtered outside sea water (see p. 620) which had been standing there, so that the *Polystomella* were warmed up say from about 8° C. in the sea to laboratory temperatures, varying roughly about 13–15° C. It is possible that this stimulated some of them to sporulation, for it occurred in five catches from 25 January to 8 March in a fair proportion (up to 20 %) of the megalospheric *Polystomella*, in each case 3–4 days after capture. In one outstanding experiment 'about 100' brown *Polystomella* were washed well in the laboratory, and then placed in a glass basin containing a substratum of sand and a growth of diatoms, etc., accumulated during several weeks in the sea-water circulation of the aquarium, the temperature remaining between 10 and 11° C. After 3 days some of them were seen to have active flagellispores in their shells, and after 8 days it was found that ninety-seven out of 125 individuals had sporulated, leaving empty shells behind. Of the remainder two seemed dead, one was microspheric and twenty-five were megalospheric. This is the highest proportion of sporulating individuals out of a brown sample that ever occurred in my experiments. I do not think this result is due to the food provided in the substratum; in any case no such increase was observed when *Polystomella* had a few days earlier been placed in a Petri-dish culture of *Navicula* kept in the laboratory; nor to the lower temperature of the circulating water, since a series of samples of the same catch in Petri dishes and in another basin of similar dimensions to the above gave no more than 20 % of sporulation in a total of some 350 *Polystomella* kept at various temperatures between 10 and 15° C. after their washing at laboratory temperature. It may be provisionally attributed to the fact that the water was in circulation in this basin, as it was not in the second basin referred to above, which stood alongside the first and contained similar water.

It would be worth testing this suggestion when the opportunity again arises. Føyn (1936*b*, p. 17) put his *Discorbina* 'unter fliessendes Seewasser zur Schwärmerbildung' with success. It should be noted, however, that a previous lot of *Polystomella* collected on 1 February and treated, so far as is known, in the same way had failed altogether to sporulate in the same basin in a week. The only possible difference seems to be that they might by some chance have missed the stimulus (if it exists) of an appreciable rise of temperature beforehand. A small batch of one dozen megalospheric individuals had given two in sporulation in this basin 3 days after their capture on 25 January, so that this difference is not so likely to be a question of the advancing season.

After 8 March, with a view to avoiding unnatural conditions so far as possible, care was taken not to subject the *Polystomella* to a rise of temperature before sporulation. By getting them early in the day, and washing them in a more or less open antechamber to the constant temperature rooms it was possible to maintain their environment at about 10° C. On 15 March white *Polystomella* were for the first time found in the catch. Of seventeen such individuals thirteen sporulated during the following night, only one or two having a few flagellispores left in their shells by 10 o'clock next morning. The remaining four went translucent, but were fixed without sporulating on 21 March, the filtered outside water having been changed almost daily; only one was found to be microspheric. Of the brown *Polystomella*:

60–70 kept in a Petri dish at 10° C. Gave 3 sporulating during the night 16/17 March.
1 sporulating during the night 17/18 March.

60–70 kept in a Petri dish at 13° C. Gave 1 sporulated during 26 hr. from 21 to 22 March.[1]

(These two lots were in filtered outside water changed once a day.)

100 kept in a basin at 11° C. Gave 1 sporulating during the night 16/17 March.

(This lot was in circulation water. The *Polystomella* which sporulated had gone white by the morning of 16 March.)

It is suggested that the falling off in numbers of sporulating *Polystomella* as compared with the last catch might be due to the omission of the warming up stimulus; as also perhaps their more scattered distribution in time, since on this supposition only those underwent sporulation which would have done so if left in the sea.

By the date of the next collection, 21 March, the season was more advanced and greater numbers sporulated in spite of this omission. It was estimated that about 10 % of the living *Polystomella* in this catch were already white when brought in. The results of experiments with this collection are shown in Table II. The catch was divided into two portions, one of which was placed at approximately each of the temperatures shown in column 1, in filtered outside sea water. After washing the *Polystomella*, the water of those marked + in column 3 was changed daily. The last column shows the total number which had sporulated by 26 March or, in some of the experiments, by 27 March.

[1] Between 9.30 a.m. on 21 March 1939 and 11.30 a.m. on 22 March 1939.

These experiments suggest that once *Polystomella* has reached the 'translucent' stage the process of sporulation is not as a rule disturbed by fishing it out of the sea, even when it is placed in very much warmer surroundings. It is shown that 'white' *Polystomella* which have not reached so advanced a stage may not sporulate any sooner than those which are still 'brown' when caught. Of these it seems that a considerably smaller number may go on to sporulation

TABLE II

° C.	*Polystomella* brought in at 4.15 p.m. 21 March	Water changed	Result	Total sporulated
10	27 'white': 16 'translucent'	0	All finished sporulation before noon on 22 March	16/16
	11 'cream'	0	1 sporulating at noon on 22 March 2 sporulated 22–23 March 1 sporulated at 8.45 p.m. on 24 March 3 sporulated 24–25 March	7/11
	200 'brown': 100	+	Over 50 sporulated during the night 24–25 March, two by 9.30 p.m.	50/100
	100	0	38 sporulated during the night 24–25 March, none by 8.45 p.m.	38/100
14–20	22 'white': 12 'translucent'	0	9 sporulating at 2.30 a.m. on 22 March 2 more early on 22 March	11/12
	10 'cream'	0	3 sporulated 24–25 March	3/10
	200 'brown': 100	+	3 sporulating by 10 p.m. on 24 March	3/100
	100	0	3 sporulated during the night 24–25 March, none by 10 p.m.	3/100

if they are kept at the higher temperatures all the time. Something of the kind was hinted at in the few comparable sets of *Polystomella* in the earlier series which were given a warm up on catching and then kept at various temperatures. The small numbers placed in *Navicula* or *Synura* cultures in Petri dishes earlier in the year (see pp. 620 and 636) indicated the same thing. These were washed in the laboratory at room temperature, and then some were placed in a constant temperature room which was cooler. Out of twenty-five known megalospheric forms kept in the laboratory, only two attempted to sporulate; whereas out of twenty-one kept between 10 and 13° C. nine made the attempt. It is interesting to note that the dates of these attempts were spread over the months as shown in Table III, *L* representing cases occurring in the laboratory, and *C* those in the low-temperature room.

TABLE III

	January	February	March	April	May
	C	*C*	*C*	*L*	*C*
	—	*C*	*C*	*L*	—
	—	—	*C*	*C*	—
	—	—	*C*	—	—
Totals	1	2	4	3	1

The *Polystomella* in any one culture showed no tendency to sporulate simultaneously, and whether the sea water was changed each day or not seemed to make no difference to the number undergoing sporulation.

Table II illustrates the fact that the emission of flagellispores by *Polystomella* collected while still brown most often takes place some $3\frac{1}{2}$ days after their capture, whether they are subsequently kept at laboratory temperatures or in a cold room. Comparatively few individuals sporulated earlier or later. This was found to be true for most of the catches,[1] though for some unknown reason in one or two lots the process went rather faster or a little more slowly. The same thing was found to hold for the few observations it was possible to make at Plymouth in 1940 with *Polystomella* from the 'White Patch' ground. Lister (1895, p. 426) refers to an instance of sporulation 4 days after capture, giving some description of the process. The relative constancy of this interval and the fact that such a large proportion of the catch may sporulate almost simultaneously seem to indicate that something involved in the catching and subsequent treatment of the *Polystomella* has an effect on sporulation in individuals which have reached a certain stage of ripeness,[2] a phenomenon which is probably not peculiar here. Also after the initiation of the process it was found that the disturbance occasioned by examination at an advanced stage might precipitate the emission of the flagellispores. On 19 April a 'cream' *Polystomella* was taken from the catch and isolated in a small Petri dish of earth extract medium at a temperature of about 12° C. At 5.30 p.m. it was still 'cream', and through a rather thin shell it was plainly seen that the protoplasm was undivided. At 9.0 p.m. it was unchanged, and it was then noted that two small rounded masses of protoplasm which had oozed out near the keel of the shell still showed a few brown chromatophores near their centres. There were a few short pseudopodia out. At 11.0 p.m. the *Polystomella* was again put under the microscope. The extruded masses were at once seen to have lost their clear-cut outlines, and their surfaces to be heaving rapidly about as they divided up into smaller masses of irregular shape and then into little spheres with shining inclusions. No brown chromatophores were any longer visible. Flagella broke out before the small spheres (roughly 4μ in diameter) finally became separate. The protoplasm inside the shell was following suit, beginning at the damaged place, and by 2 a.m. the emergence of the flagellispores was in full swing. It has been repeatedly observed that great crowds of these suddenly came out when *Polystomella* which were due to sporulate were put under the microscope for examination, especially during the critical hours about midnight and soon after.[3]

[1] Apart from specimens which become established in cultures.

[2] E.g. it either retards or expedites the process.

[3] A similar experience was had with a large spherical *Gromia oviformis*, about 2 mm. in diameter, collected at Millport on 24 November 1939, and placed in a diatom culture at Glasgow next day. On 4 December there was a whitish film over the brown shell contents; and at about 5 p.m. some protoplasmic masses which had been extruded were divided up to form the uniflagellate swarm spores of this species. There was not much change by 8.0 p.m.

It was noticed quite early in this investigation that a large proportion of the shells were emptied say between 9.0 p.m. and 9 a.m., whether they had been collected before or after the brown colour of their contents had disappeared; so, in order to have a good supply of flagellispores, sporulating *Polystomella* were examined at intervals throughout the night, usually being left in darkness between the examinations. It was thus discovered that emergence of the flagellispores began in the majority at about 2 a.m., often continuing until daybreak, although a few individuals might be active an hour or two before midnight, and a few would not begin until later in the morning of the next day. The only variation in treatment which might possibly have advanced sporulation in my experiments was: (1) changing the sea water earlier in the day, or (2) placing the *Polystomella* in a diatom culture.

Both procedures might have something of the effect of a circulation of the medium, which as we have seen may perhaps facilitate sporulation. Le Calvez (1938, p. 205), after observing that the emission of flagellispores in *Iridia lucida* 'se produit à une heure avancée de la nuit', adds 'J'ai constaté cette sortie nocturne chez presque tous les Foraminifères'. Is the nocturnal habit an indication that darkness itself may also favour the emission of flagellispores? Føyn (1936*b*, p. 17) placed his *Discorbina* 'ins Dunkel' when he desired them to sporulate. On the other hand, flagellispores are quite often seen to be active and emergence to take place during the hours of daylight in the laboratory.

One or two collections taken later than 21 March in 1939 gave no results of special interest here excepting perhaps that of 26 April, which illustrates how the two forms of reproduction are going on simultaneously at this season. The temperature of the sea was recorded as about 10·5° C.; the *Polystomella* were washed at about 12° C. and left at 10° C. Microspheric forms were about as common as megalospheric. On 27 April there were sorted out twelve dark brown individuals showing the microspheric 'figure' (see p. 634) and seventeen paler brown ones. These were placed in well grown diatom cultures, and gave the results shown in Table IV.

TABLE IV

Dark brown 'Microspheric' *Polystomella*		Mid-brown *Polystomella*	Pale brown *Polystomella*
6 kept at 10° C.	6 kept at 13° C.	10 kept at 13° C.	7 kept at 13° C.
Schizogony in 5 by 22 May	Schizogony in 4 by 22 May	Schizogony in 1 by 5 May	Schizogony in 0 by 5 May
1 megalospheric	1 sporulated on 6 May	6–8 sporulated by 5 May	6 sporulated by 5 May
		1 megalospheric	1 megalospheric

One *Polystomella* was observed to sporulate as late as 28 June, but most of the megalospheric individuals surviving so long showed little activity.

until the *Gromia* was pushed with a dissecting needle, when it suddenly became quite white, and then appeared to start 'steaming'—the 'steam' gradually disappearing in crowds of dispersing flagellispores. The steaming continued at 1.0 a.m. By 9.30 next morning the shell was empty except for the mass of stercomata and xanthosomes left behind by the departed protoplasm, and the Petri dish was swarming with the still active flagellispores.

Lister seems to have made his observations on schizogony in smaller *Polystomella* than those I studied at Plymouth (see p. 634), the smaller sizes given by him for the nuclei in the megalospheric young (see p. 641) being perhaps referable to this fact. The figures he gives for the megalospheric form indicate that they all comprised fewer chambers than my large mature specimens. The highest number of chambers mentioned by Lister is forty-one (1895, table on p. 423, and cf. p. 639 of this paper), and the average number of chambers in twenty-seven reproducing individuals collected March–May 1895 is 30·5 (Notebooks, Vol. 1); whilst here and elsewhere (1895, p. 426) he refers to specimens of seventeen and eighteen chambers respectively which were in course of forming flagellate spores. I also obtained sporulation (but not schizogony) in small specimens, down to about 25–30[1] chambers at any rate. They were noted in the Drake's Island material in March and April 1939; and were much more common in the same months the following year in the collections from 'White Patch'.

I can only suggest that these *Polystomella* happened to come from less favourable grounds than the fine large specimens I have been fortunate enough to be supplied with at Plymouth since 1938. The frequent presence of spines on Lister's small specimens,[2] and the shape of some of his figures are reminiscent of the small *Polystomella* occurring at Millport (see footnote to p. 620) for example, which are probably ill-nourished and subjected to great and disturbing variations in their shallow habitat. An interesting possibility is that the small sporulating individuals may have been produced late in the season of the previous year and so may have missed the time of plentiful food—reproducing nevertheless at about the same date as their better fed and better grown elders of the same generation.

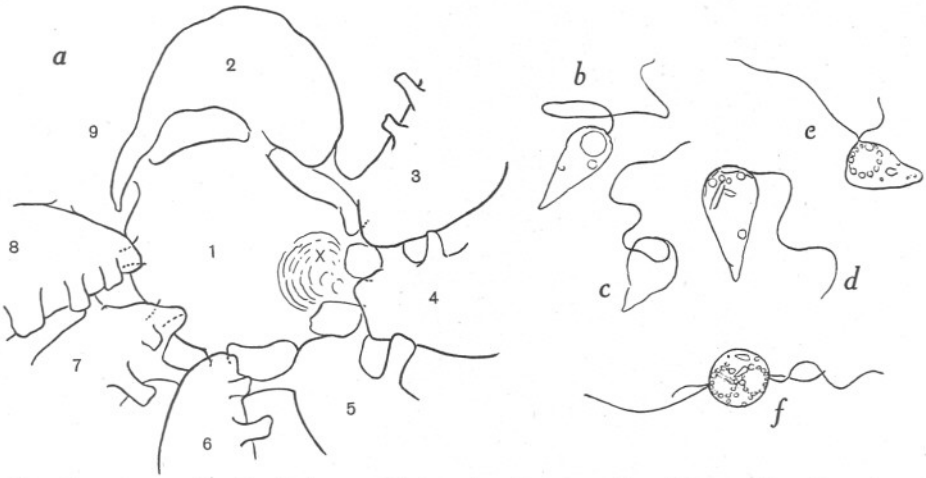
Fixed Material. The first sign of approaching sporulation seen in fixed material is the opening up of extra channels of communication between the chambers, as indicated by Lister (1895, p. 425 and pl. 8, fig. 32*a*; also 1903, p. 71). This condition was found from January onwards in specimens fixed after 24 hr. in the laboratory—which might have brought on the process as suggested on p. 647. It happened that no *Polystomella* was fixed the same day as it was collected until late in March 1939, and by that time freshly caught brown specimens after decalcification showed cytoplasmic bridges growing in from the innermost whorl of chambers to make a connexion with the large central initial chamber (see Text-fig. 10*a*). The same thing was seen in *Polystomella* freshly caught at the 'White Patch' ground at the end of February 1940, when the whole population looked rather pale and starved. In some of these modified specimens the large nucleus has disappeared, and I can see no sign of any nucleus at all, either in whole mounts or in sections.

It is instructive to compare two lots of *Polystomella* collected towards the end of March 1939, i.e. probably at about the height of the sporulation season.

[1] Estimated from the number of chambers exposed in the outermost whorl.

[2] Also seen on a certain number of the smaller specimens from Drake's Island (see p. 641.)

A sample of the catch on 24 March was cleaned and left in a small basin of filtered outside sea water at 9–10° C. Some representative specimens were taken out and fixed each day in Zenker at 40° C. Some of the catch of 30 March, on the other hand, was carefully sorted, at similar temperatures, into five categories by colour, viz. 'dark brown', 'mid-brown', 'pale brown', 'golden', and 'pale cream', which were fixed separately in Zenker as before. From whole mounts and sections of this material a rough time-table of the stages in sporulation may be constructed which agrees quite well with other more casual and fragmentary observations, and was confirmed also by a parallel set of samples from the collection of 30 March, which was allowed to



Text-fig. 10. *a*, sketch of the central cytoplasmic parts of a *Polystomella* collected on 13 January, and fixed 14 January. Zenker at 40° C. Boraxcarmine. Whole mount. 1–8, first eight chambers; 9, position of chamber 9. At *X* is an inrush of cytoplasm, due to fixation, from chamber 4 to the initial chamber (1) through one of the median secondary openings heralding sporulation. *b*, *c*, *d*, sketches illustrating living flagellispores of *P. crispa*. Only one flagellum was seen during life. *e*, the same after exposure to osmic vapour; both flagella are now visible, but there has been some shortening of the body. *f*, a motile sphere, also after osmic vapour, showing a stage in fission.

sporulate in Petri dishes. It is of course not to be expected that every sporulating *Polystomella* will adhere rigidly to any time-table.

It is not known how long before flagellispore formation one may find the opening up of the extra channels through the shell—but they appear to increase very fast during the last 48 hr. Not only does the initial chamber acquire median bridges to all the adjacent chambers at their inner ends, but these chambers come to communicate much more freely with one another, involving the canal system, which broadens out, forming wide sinuses in places, as may be clearly shown up when the system is full of xanthosomes on their way out of the shell: the foraminal openings also may become greatly enlarged. A *Polystomella* captured brown at this time of year may begin to

look pale on the second day after capture, the brown colour may all be gone by the morning of the third day, and the special extrusion of xanthosomes over. There is often a distinctly yellowish phase, 'golden' in my series, after which the shell contents come to look quite white and ultimately translucent in the course of the third day, immediately before the emergence of the swarm spores during the following night. The large nucleus of the parent megalospheric individual disappears as a rule during the time the brown colour is going. In my preparations it is still present in three out of every four individuals at the 'golden' stage, but in hardly any which were classified as 'white' or 'cream'. It might be mentioned here that the old nucleus seems to be slightly enlarged and rather empty of nucleoli towards the end of its existence; but I have seen no stages in its dissolution. There is apparently an interval during which I can find no nucleus; and then small nuclei, only a few μ across, make their appearance, scattered evenly through the cytoplasm, seeming to increase in numbers until the whole animal is stuffed full of them. One has the impression that they become a little smaller as they increase in numbers as if they were multiplied by some kind of division. Finally, as already described, the cytoplasm undergoes a multiple fission. In sections it begins to look like a fine reticulum with knots in its meshes enclosing one or several of the small nuclei surrounded by darkly staining granules and eosinophil bodies; while, in between, are intensely staining strands which in places form the walls of vacuoles. At these knots rounded bodies gradually concentrate, each of which seems ultimately to undergo two rapid divisions to form the individual flagellispores immediately before they emerge from the shell.

Lister (1895, p. 425) gives an account of his observations on sporulation in *P. crispa*, with illustrations of various stages in pl. 8. I have confirmed the main facts, but I cannot agree with his interpretation of some of the details. The latter are extremely difficult to understand in such a large mass of protoplasm full of granules and other inclusions of various kinds. Fixation also seems unusually imperfect at this stage, owing perhaps to the great amount of mucus which is present. My clearest preparations showing small nuclei were made from *Polystomella* fixed in osmic vapour for 10–15 min., followed by hot corrosive acetic as described on p. 616. I have nothing at present to say about the mode of disappearance of the large nucleus, nor concerning the origin of the small ones. Though I confess I have sometimes not been certain of the presence or absence of the latter, I have never suspected their presence as long as the large nucleus could be seen. They show up at later stages as red or blue spots after picrocarmine or Ehrlich's haematoxylin respectively.

Form and Behaviour of the Swarm Spores

The ultimate divisions giving rise to the flagellispores take place inside the shell as a rule. There is the usual kind of variation in the details of the successive fissions—a coarsely reticular appearance of the cytoplasm having been noted in some cases at a rather late stage; in others, spheres of different

dimensions separate off and divide up afterwards to the small swarm spores. These are usually fully formed and very active when they leave the shell, although after emergence figures showing that the last two fissions have not been completed are not infrequently seen (see Text-fig. 10). Pairs of flagellispores are thus not uncommon.

When swimming freely the swarm spores are more or less carrot-shaped bodies, about 6–8 μ long (see Text-fig. 10 *b–d*), proceeding with the blunt end foremost and swinging round in a wide spiral as each little flagellate rotates on its axis under the influence of the rather thick longer flagellum which appears to precede it, but is rarely seen in action owing to its rapid motion of considerable amplitude. This flagellum reaches a length about three times that of the body or even more, and the shorter second flagellum which is invisible during active progression is about body length. (See osmic preparations in Text-fig. 10 *e,f*). One gets the impression that it may be carried relatively straight ahead. The whole movement seems well calculated for the sweeping of as large an area as possible by the longer flagellum; as also is the way in which the swarm spores spread out evenly through all the available water, even in the larger vessels, showing no orientation with regard to light, gravity, etc. They swim actively, with very occasional short rests on the bottom, for some hours, say 8–10 under ordinary conditions in the laboratory,[1] and then settle down on the bottom, permanently rounded up in the form of little spheres measuring 4–5 μ across. These gradually fade away, losing their flagella, though their ghosts may be seen for some days, picked out by the shining inclusions which may remain in them. Of these inclusions two kinds have been distinguished: refractile angular bodies of a somewhat crystalline appearance, insoluble in hydrochloric acid and in sodium hydroxide solution, of which half a dozen may be present (Pl. V, fig. 2); and secondly, a small number of less shining discoid bodies which appear to lie flat under the pellicle near the anterior end of the body, soluble in sodium hydroxide, staining blue with Nile blue hydrochloride, and perhaps with methyl green and acetic acid. In osmic acid, although nothing darkens conspicuously, the flagella are well demonstrated. Iodine solutions are useless for showing up the structure.

The swarm spores may go on swimming for half an hour or so under the microscope, especially if uncovered and not too brightly illuminated or overheated. But sooner or later they begin to shorten, and finally round up and become ghostly as described above. In fixed and stained preparations there is a strong tendency to assume the spherical shape, possibly owing to swelling. Figures of selected specimens are given in Pl. V, figs. 2 and 3. The vacuoles, which in some preparations contain eosinophil bodies of various sizes, seem to arise as the swelling comes on.

[1] But see Exp. 16 (p. 660), when on 31 March 1940 some were still swimming in apparently good form after about 50 hr. in earth extract medium, at 8–10° C., in the dark excepting for three short intervals when they were under microscopic observation.

J. J. Lister (Notebooks, Vol. II, p. 10, dated 4 June 1893[1]) describes a *Polystomella* he had collected the previous day on the shore at Highcliffe (Hants), and which when he crushed it yielded 'zoospores', 'yellow masses' (=excretory granules) and 'algae in pairs' (=foraminal plugs). He did not study the zoospores in detail, stating (1906, p. 8) that he had not even counted the flagella. It seems, however, from other passages in his Notebooks that he believed there were two, and that they were perhaps unequal (see also 1895, p. 426, pl. 8). Other references to these swarm spores in the literature are made first by Schaudinn as already noticed (see pp. 610–646 and Text-fig. 1 D, which is either very diagrammatic or shows a rounded-up form); secondly by Kofoid (1934), who gives additional support to the hypothesis that they are flagellate parasites because Myers (1935*a*) had shown the products of sporulation in another foraminiferan (*Patellina*) to be amoebulae; and thirdly by le Calvez (1938, p. 300), who says in table I that they measure $1{\cdot}8 \times 3\mu$, and bear two flagella respectively 12 and 4μ in length. This last reference is difficult to explain.

Attempts to Trace the further Development of the Swarm Spores

Apart from the casual observation of swarms of the flagellate spores coming from a single *Polystomella* or from two or more individuals, some half-dozen special sets of observations were recorded when the swarm spores were watched under a Greenough binocular microscope for considerable intervals of time immediately after their liberation or a few hours later. Samples of the flagellispores from single individuals were studied swimming freely in watch glasses, and mixtures were made from these in various ways; in Petri dishes also mixed swarm spores from two to four *Polystomella* were watched, at laboratory temperatures or down to 10° C., in filtered outside water or in earth extract medium. In one case the dishes were kept in the dark when not under observation. In no instance was any sign of fusion detected, either amongst these still swimming freely in the water or amongst those which had settled down on the bottom of the dish.

Single flagellispores were seen to tumble about one spot for a while now and then; and sometimes two would tumble about together, thus appearing to be playing around one another, but they always parted company in the end. Finally, as already noted, the swarm spores ceased to move and degenerated, without ever showing the least tendency to become amoeboid.

It might be said that under microscopic observation, even with a Greenough binocular, conditions might be against the performance of syngamy. The series of experiments now to be described, where the swarm spores were not subjected to examination until some time had elapsed, afford no reason to assume that it commonly occurred here either.

[1] It is not possible to verify my references to Lister's Notebooks at the present time of disturbance as they are stored in a place of comparative safety. The references given are taken from my own notes made at the British Museum some years ago.

SERIES I

This series (Exps. 1–8) was set up with *Polystomella crispa* (P.),[1] from the Drake's Island ground. They were brought into the laboratory and washed with filtered outside sea water (F.O.S.W.) at laboratory temperatures (about 13° C.). Unless otherwise stated they were kept in about 25 c.c. F.O.S.W. in Petri dishes (P-dishes). 'Big basins' measured 9–10 × 4–5 in. and held about 2 l. of medium. Cover-slips (with or without a culture of *Navicula mutica* var. (N.) growing on them) were suspended in the latter on sewing cotton tied to a cross-thread by a fisherman's bend so that they could easily be removed for microscopic examination and replaced: or they were floated on the surface of the medium or placed on the bottom of the dish, sometimes before a culture of N. was grown therein. Earth extract medium (E.) or sterile sea water (S.S.W.) prepared as described in footnote 2 on p. 660 were also used. N. cultures were always grown in E., in P-dishes or in big basins. Sometimes P-dish cultures were placed, uncovered, in big basins of sea water, thus providing a variety of more or less well-stocked feeding grounds.

Experiment 1. Set up during the morning of 4 February 1939. Out of about 100 P. collected 1 February, three shells were found empty, active swarm spores in the dish.[2] Sea water not changed since 2 February. Other P. were removed. On 5 February added 1–2 c.c. of S.S.W. with swarm spores from Exp. 2. Sea water changed[3] for F.O.S.W. at 4.15 p.m. on 4 February, and renewed 5 and 7 February. Temp. about 13° C. Subsequent examinations 7 February (ghosts of flagellispores about 5 μ in diameter seen)[4] and 12 February.

Experiment 2. Set up 11.0 a.m. on 5 February. Two more P. from same lot looked white; washed and left in S.S.W. on 4 February. One showed active swarm spores at 3.30 p.m., both empty next morning. S.S.W. replaced by F.O.S.W., changed 7 February. Temp. about 13° C. Subsequent examinations 7, 12 February.

Experiment 3. Began at 11.30 a.m. on 4 March 1939. In dish of 30 P. collected 28 February and 1 March two found empty. Flagellispores pipetted into N. culture in P-dish at 11.30 a.m., 4.0 p.m., 9.30 p.m., and 5 March at 9.45 a.m. Temp. about 13° C. Subsequent examinations 7, 10, 18 March.

Experiment 4. Set up at 9.45 a.m. on 5 March, from same dish, in which about a dozen P. have now sporulated. Some of the deposit and ten empty shells pipetted into big basin F.O.S.W. with P-dish culture N., and with cover-slips lying on the bottom. On 12 March added 4 P. containing active flagellispores from dish of fifty collected 8 March, two at 2.0 a.m. and two at 4.40 a.m. Cover-slips suspended in basin at 10.0 a.m. Temp. 13° C. throughout. Subsequent examinations 12, 15, 17, 18 March.

Experiment 5. Set up 2.0–5.30 a.m. on 12 March 1939, with 75 P. collected 8 March, and kept at 10° C. in sea water renewed 10 and 11 March 1939. Fourteen P. white and removed to S.S.W. at 2.0 a.m. on 12 March. About ten of these with active swarm spores later transferred to big basin F.O.S.W., with P-dish culture N. and suspended cover-slips. Temp. 10° C. Subsequent examinations 12, 15, 17 March, 10 April.

Experiment 6. Set up at 2.30 a.m. on 12 March 1939, with 85 P. from same collection, kept at 13° C., otherwise treated as in Exp. 5. At 2.30 a.m. on 12 March six with flagellispores were transferred to similar basin. Temp. 13° C. Subsequent examinations 12, 15, 17 March, 17 April.

[1] See p. 613.

[2] Presumably emerged during the preceding night.

[3] By carefully pouring or pipetting it off, causing as little disturbance as possible to remaining shells, or to flagellispores rounded up on the bottom.

[4] This will not again be mentioned; it is an indication that fairly high magnifications were used in making examinations of material from all the available surfaces in the cultures.

Experiment 7. Dated 10.30 a.m. on 9 March 1939, when 'about 100 P.' from same collection were placed in basin A under the laboratory sea-water circulation system, with good growth of diatoms, etc. (see p. 647). At 4.0 a.m. on 12 March a sample of 4 P. was inspected and all had active swarm spores in shells. On 16 March ninety-seven shells out of 125 were found to be empty. Temp. after first washing 8–11° C. Subsequent examinations 15 March, 23 April.

Experiment 8. At the same time another '100 P.' were placed in basin B of filtered circulation sea water, with N. cultures in P-dishes on bottom. On 12 March 1939 there were seventeen empty shells out of 112. Temp. as in Exp. 7. Subsequent examinations 15, 17, 18 March.

SERIES II

In this series (Exps. 9–15) the *Polystomella* were not warmed before sporulation excepting where stated. Collected from Drake's Island ground.

Experiment 9. Set up at 2.0 a.m. on 22 March 1939.

(1) Three 'translucent' P. from dish of twenty-two white P., collected 21 March, washed at about 20° C., and kept at 14° C., were transferred to P-dish culture N. at 2.0 a.m., with active flagellispores emerging. Shells almost empty at 4.0 a.m. Temp. 14° C. Subsequent examinations 11.0 a.m. on 22 March, 9 April.

(2) Five similar P. kept separately in watch glasses A–E of F.O.S.W., in damp chamber. Examined 6.0 a.m., 11.0 a.m., 9.45 p.m. (no movement now) on 22 March and on 23 March.

(3) Mixed swarm spores from watch glasses A+B+C+D in P-dish culture N. Examined 11.0 a.m. on 22 March, 9 April.

(4) Mixed swarm spores from watch glasses A+B, B+D, A+D, in three separate watch-glasses in damp chamber. Examined 6.0 a.m., 11.0 a.m., 9.45 p.m., on 22 March.

Experiment 10. Set up at 3.30 a.m. on 22 March 1939. Three translucent P. from stock dish of twenty-seven white P. similar to last, but kept throughout at 10° C., with active flagellispores in shells, put into P-dish culture N. At 4.0 a.m. added some flagellispores from eight more P. which had become active in stock dish. Examined 11.0 a.m. on 22 March, 9 April.

Experiment 11. Set up at 8.30 p.m. on 27 March 1939. Out of 'about 100 P.' collected 24 March 1939, and kept at 9–10° C. in small basin, four emitting flagellispores were placed in 400 c.c. F.O.S.W. in flask in which there was a little brown algal growth.

(1) Some of this suspension was kept in P-dish F.O.S.W. at 9–10° C. Examined 11.0 p.m. on 27 March and on 28, 29, 31 March, 9 April.

(2) At 10.15 p.m. on 27 March 1939 flask emptied into big basin with two P-dish cultures N., and suspended cover-slips. Kept at 9–10° C. Subsequent examinations 28, 29, 31 March, 10, 16 April. Temp. rose to 20° C., one day[1] and fell again to 11–13° C. From 22 April kept at 13° C. See Exp. 15 for further examinations.

Experiment 12. Dated 9.50 a.m. on 1 April 1939. Two P. out of dish of three pale brown P., collected 30 March, emptied overnight. Temp. 9–10° C. throughout. Subsequent examinations 3, 4 April.

Experiment 13. Set up in the morning of 3 April 1939. One P. sporulated overnight in each of two dishes containing three and thirteen mid-brown P. respectively, collected 30 March. Swarm spores were mixed:

[1] The temperature varied a good deal in this experiment owing to the late spring sunshine on a glass roof, and it was well above that of the sea at the time, viz. 10–11° C.

(1) '1 volume' from each, in each of four watch-glasses in damp chamber. Temp. 9–10° C. Examined 1.0 p.m. on 3 April and on 4 April.

(2) '2 volumes' from each, into P-dish culture N. Kept at 9–10° C. Examined 3.30 p.m. on 3 April (but very small organisms would hardly have been distinguished in the body of the culture), 9, 16 April. Temp. rose to 20° C.[1]

(3) '2 volumes' from each, in P-dish F.O.S.W. Kept at 9–10° C. Examined 1.0 p.m. on 3 March and on 4, 5, 9 April.

(4) '2 volumes' from each, to each other in original dishes. Kept at 9–10° C., behind a screen to shade from direct light from window. Examined 1.0 p.m., 8.30 p.m. on 3 April and on 4, 5, 9 April.

Cover-slips were floated on top of medium in P-dishes (2), (3), (4).

Experiment 14. Set up at 2.10 a.m. on 20 April 1939. Pale P., collected 19 April, kept in separate P-dishes E., under black paper, at 12–14° C.[1] Three had swarm spores emerging at 2.10 a.m. on 20 April, viz. dishes A, B, and 2. Examined these dishes 5.0 a.m., 11.0 a.m., 9.30 p.m., on 20 April and on 21 April.

(1) The three shells and some emerged swarm spores from A+B+2 pipetted into P-dish E., and then to big basin E. with P-dish culture of N., suspended and floating cover-slips. Kept about 12° C. Examined 21 April, 6 May (E. replaced with F.O.S.W. on 31 May, and basin used as pasture for freshly caught P.), 24 June.

(2) Swarm spores from A+B+2 pipetted into P-dish E, kept under black paper, at about 12·5° C. Examined 5.0 a.m., 6.0 a.m., 11.0 a.m., 6.0 p.m. on 20 April and on 21, 22, 23, 24 (changed E.), 26 April.

(3) Swarm spores from A+B+2 pipetted into P-dish culture N. in E. Temp. about 12·5° C. Examined 6.0 p.m. on 20 April (obscured by *Bodo*), and on 30 April.

(4) Mixed swarm spores from A+2, B+2, A+B, respectively on slides at 2.50 a.m. Temp. 12·5° C. Examined 3.0 a.m., 5.0 a.m., on 20 April (flagellispores rounded up and motionless).

(5) Mixed swarm spores at 11.0 a.m. from A+B+2 in a fresh P-dish E. Temp. 12·5° C. Examined 6.0 p.m. on 20 April and on 21, 22 (changed E.), 23, 24 (changed E.), 26 April.

(6) Mixed swarm spores at 11.0 a.m. from A+B, A+2, B+2, A+B+2, respectively in four separate watch glasses in damp chamber. Temp. 12–14° C. Examined 9.0 p.m. on 20 April and on 21 April.

Experiment 15. Dated 9.30 a.m. on 28 April 1939. Out of seventeen rather pale P., collected 26 April, and put into big basin used in Exp. 11, after removing four empty shells, on 27 April, four were found empty.[2] Temp. 13° C. Subsequent examinations 6, 22 May.

SERIES III

In this series (Exps. 16–18) the *Polystomella* were collected at the 'White Patch' ground.

Experiment 16. Set up at 6.0 a.m. on 29 March 1940. Room darkened 28 March to 12.30 p.m. on 30 March. From seventeen white P., of various sizes, collected 28 March, washed F.O.S.W. at 5·0° C., kept in two P-dishes E. (containing 4 and 13 P. respectively) at 8–10° C., flagellispores began to emerge at 1.30 a.m. on 29 March.

(1) At 6.0 a.m. 13 P., some from each P-dish, with swarm spores, put into N. culture grown in big basin, cover-slips suspended, kept at 9·0° C. Examined 1.0 p.m., on 29 March (seven shells quite empty), and on 31 March, 4 April. And see Exp. 17.

[1] The temperature varied a good deal in this experiment owing to the late spring sunshine on a glass roof, and it was well above that of the sea at the time, viz. 10–11° C.

[2] Presumably emptied overnight.

(2) P-dish of E. which had contained thirteen white P. and had many flagellispores, kept at 8–10° C. Examined 6.30 a.m., 11.0 a.m. on 29 March and on 30, 31 March (a few carrot-shaped flagellispores still swimming),[1] 4, 10 April.

(3) P-dish which had contained four white P. and had few swarm spores, kept at 8–10° C. Examined 6.30 a.m., 11.0 a.m. on 29 March and on 30, 31 March.

Experiment 17. Set up at 11.30 p.m. on 8 April 1940. Out of about thirty brown P. collected 5 April, and kept in two P-dish cultures N., i.e. food available, at 8–10° C., three from one dish and one from the other with active swarm spores put into watch-glass, and half an hour later transferred to big basin used in Exp. 16 (1). Temp. 8–10° C. Subsequent examinations 11 April, 18 July (temp. now 13·5° C.).

Experiment 18. Dated noon on 9 April 1940. In dish of about thirty brown P., from same collection, kept in E. with no food, three small P., i.e. not over 1 mm. in diameter, emptied since 3.30 a.m. Temp. 8–10° C. Examined 10 (found small shell, see p. 610), 11 (removed other P. and put in a little N. culture), 12 April.

The series of experiments is, as will be seen, incomplete, but a fair range of conditions is covered. No further development was obtained in any, excepting perhaps the last which produced the small shell described on p. 610 (see Text-fig. 2). Nor was any later stage ever seen in the cultures of *Polystomella* started in January 1939 in diatom cultures (p. 649), where, however, it happened that there never were swarm spores from more than a single *Polystomella* at any one time. It was estimated that a single parent *Polystomella* of moderate size might produce swarm spores to the order of some 500,000; and when one considers the relatively small number of microspheric individuals developed in nature from the large proportion of megalospheric *Polystomella* in the ordinary population, which themselves originate in broods of less than 200 from each of the former, one should perhaps not be surprised at a very low measure of success in experimental cultures, even if it takes two swarm spores to produce one microspheric animal. Their natural rarity does seem to suggest some special difficulty in their development; and perhaps one may conclude from these experiments that it is probably not simply any difficulty in one swarm spore making contact with another. It might be that union can only occur between very special pairs of flagellispores, or that some very special circumstance is necessary for their further development, with or without fusion. It may be that this difficulty is not confined to *Polystomella* (see p. 611, and cf. Rhumbler, 1909, p. 325). It would therefore be well worth while to make further attempts to discover what it is.

In the meantime attention might be drawn to the following points:

(1) *Medium.* Gross (1934) reports a striking success in the culture and induction of syngamy amongst the flagellate gametes of *Noctiluca* (Cystoflagellata) in earth extract medium, a success he had failed to achieve in sea water. In my experiments sea water of various kinds[2] was used; and also earth

[1] After an unusually long interval, since all shells had been removed at 6.0 a.m. on 29 March (see p. 655).

[2] Viz. sterile sea water, i.e. boiled and kept in the laboratory for at least a month before use; outside sea water (see p. 620), filtered through a single filter paper; and sea water circulating in the aquarium tanks, which, however, since the time of Lister (1903) is known not to be satisfactory for certain stages in the development of *Polystomella*.

extract medium, the only (doubtful) success being obtained when the latter was used throughout sporulation and subsequently (Exp. 18). In this case no diatoms were present until late in the experiment, which almost certainly means that the *p*H was about 8·0. In the *p*H determinations made now and then values above 8·5 were never obtained, even in Petri-dish cultures of *Navicula* so long as they remained healthy. Nevertheless, the *p*H was always a little higher in the presence of diatoms, and in view of the results of Gross (1934) even this small rise may make a difference. The unusual survival of flagellispores noted in Exp. 16 occurred in similar circumstances.

(2) *Food.* It is hardly possible that this is important during sporulation and the earliest succeeding stages—excepting perhaps in so far as its presence affects the *p*H. In the experiments there are several instances where the flagellispores had hours together in plain sea water or earth extract medium beforehand, even if they were later placed with diatom cultures. These it was hoped would have supplied a sufficiency of suitable food of various sizes for the earliest stages, beginning with such small organisms as the bacterial growths which were always present. In other cases sporulation was at least completed in a diatom culture.

(3) *Temperature.* The experiments were carried out at various temperatures, Exp. 18 at the lowest of all (under 10° C.). It is believed that sporulation is best carried out at such temperatures (see p. 649); and arguing from natural conditions in the sea it may be expected that the early subsequent development is at any rate possible at similar low temperatures. It does not follow, however, that it is readiest under these conditions, as is indicated by the work of Maclagan (1932) on *Smynthurus* (Collembola), in which it was shown that, though this insect lays the largest number of eggs at about 7° C., development proceeds best at about 16° C.

(4) *Concentration of Swarm Spores.* This may be important in more ways than one. Apart from the well-known facts that any kind of development may be rendered abnormal, or may cease altogether, when the organisms are too thick on the ground, and that if it is a case of syngamy a certain minimum concentration is on the other hand necessary, it has been suggested that facilitating substances may be passed into the medium by one or both of the partners in such fusion (see, e.g., *Nature*, Vol. 143, 17 June 1939, p. 1036, referring to the work of Moewus, Kuhn and others). Such substances must be present in the medium in certain concentrations.

No attempt was made to count the flagellispores per unit volume, but a good range of concentrations was in fact used in the course of the experiments. Renewing the medium at various stages might also have an effect in this respect, and here again there was considerable variation in the procedure.

(5) *Light.* Light as is well known has many unexplained affects on living organisms. Reference to some of these is made in *Nature* (loc. cit.), where the liberation of the special sensitizing substances in the presence or absence of light is noticed in connexion with syngamy in *Chlamydomonas* (Phytomonad-

ina) and in fishes respectively. No special arrangements with regard to light were made excepting where mentioned in Exps. 13, 14, 16.

(6) *Maturing of the Flagellispores.* This might have been affected by the disturbance suffered by the *Polystomella* taken from the sea and subjected to experiment. Possible effects would perhaps have been varied by the use of flagellispores produced: (*a*) During the night following capture (Exps. 9, 10, 14, 16), (*b*) two to four days after capture (Exps. 1–8, 11, 12, 13, 15, 17, 18); or (*c*) by *Polystomella* which had been for some time in culture. As already explained swarm spores from more than one such *Polystomella* were never available at any one time, so that syngamy might here have been out of the question for a different reason.

It might also be that the flagellispores are only capable of syngamy during a limited period of time after they are liberated. Some attempt to circumvent such a difficulty was made by mixing the swarm spores at various intervals after emergence, and in other experiments allowing them actually to come out more or less simultaneously from two or more *Polystomella* previously placed together in the same dish.

(7) *Selective Mating of the Presumptive Gametes.* It has been established in some of the lower vegetable organisms amongst the algae and fungi that syngamy only takes place between the products of certain pairs of parents, though these may not differ in any visible characteristics. American investigators have recently claimed that there are distinct 'mating types' amongst the Ciliata, only some of which are interfertile (see Jennings, 1939). Whatever the explanation of this, if similar barriers exist in *Polystomella*, it was hoped that they might have been overcome by using as many parents as possible in the experiments. Although only two or three were usually available, flagellispores from 'about a dozen' (Exp. 4), 'about ten' (Exp. 5), six (Exp. 6), and seven (Exp. 16), were used in others. In Exps. 9 and 14 mixed flagellispores from different individual pairs were observed, and no difference in behaviour noted.

Because these experiments are only a beginning, and have not as yet given any positive result, it is thought worth while to describe them in some detail, in the belief that when this line of inquiry is resumed, perhaps by another investigator, they may provide a starting point a little further on than was available in 1938.

Summary

Part I. After a brief statement of the present unsatisfactory position regarding the life history of *Polystomella crispa*, an account is given of experiments with various fixatives on *P. crispa*, collected near Drake's Island, Plymouth. A method is described for the cultivation of *Polystomella* in diatom cultures, especially with *Navicula mutica* var., with which a flagellate (*Bodo* sp.) was constantly present. The identification of individual Foraminifera, and determination of growth, were often possible by some irregularity in the shell, or by noting the attachment of one or more stalked egg cocoons of an unknown

turbellarian worm. The feeding of *Polystomella* on diatoms seized by the pseudopodia outside the shell is described; the structures previously taken for ingested algal cells inside the shell are shown to be foraminal plugs ('bouchons' of le Calvez) which are discarded from time to time and thrown out of the shell along with the excretory granules (xanthosomes), mostly via the canal system.

Part II. Notes are given on the microspheric form and its reproduction, through the spring and summer, by schizogony. The rearing of two broods in laboratory cultures is described, with an account of the formation of a new chamber to the shell. It is concluded that a complete life cycle consisting of one microspheric and one megalospheric phase occupies a period of about two years.

Part III. Sporulation of the megalospheric form is described as seen during life; and various factors are discussed which might affect the process. The relatively small size of the reproducing *Polystomella* with which Lister worked is ascribed to their having lived under less favourable conditions. From a study of stained preparations, certain details are added concerning the opening up of the shell, presumably to facilitate the eventual escape of the flagellate swarm spores; also concerning the accompanying cytoplasmic and nuclear changes. Some account is given of the form and structure of the swarm spores, both alive and in permanent preparations. Experiments are described which constitute an attempt to carry the life cycle beyond the stage of sporulation. They are so far unsuccessful, with one possible exception, under all the various experimental conditions which are briefly discussed in their turn.

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EXPLANATION OF PLATES IV–V

I am indebted to the Carnegie Trust for a grant towards the cost of the coloured figures in Plates IV and V.

PLATE IV

Painted by Miss Cecily Brown Kelly.

Fig. 1. A living *Polystomella crispa* from Plymouth.

Fig. 2. Empty shell of same to show terminal face of last chamber. *Ch.*, chambers of the shell, the cavities of which are in communication by the foramina; *for.*, foramina in front of last chamber, which form the terminal apertures of the shell; *foss.*, fossette (d'Orbigny); *K.*, keel of the shell; *ps.* pseudopodia arising from protoplasm inside and outside the shell; *ret.pr.*, ridges over the retral processes of the chambers, between the fossettes; *sep.*, ridges over the septa between the chambers; *ter.ap.*, terminal apertures of shell; *umb.*, umbo.

PLATE V

Fig. 1. *Bodo* sp. *Corr.ac.*, iron haematoxylin. This fixative gave better results with this flagellate than did Schaudinn's mixture, Bouin, or osmic vapour and alcohol. A fair number of nuclei in the preparation show the large karyosome and peripheral granules characteristic of the genus, and in many cases the kinetoplast does not stain uniformly, sometimes appearing as a thick-walled empty sack, especially after hot Schaudinn. *N.*, nucleus; *K.*, kinetoplast.

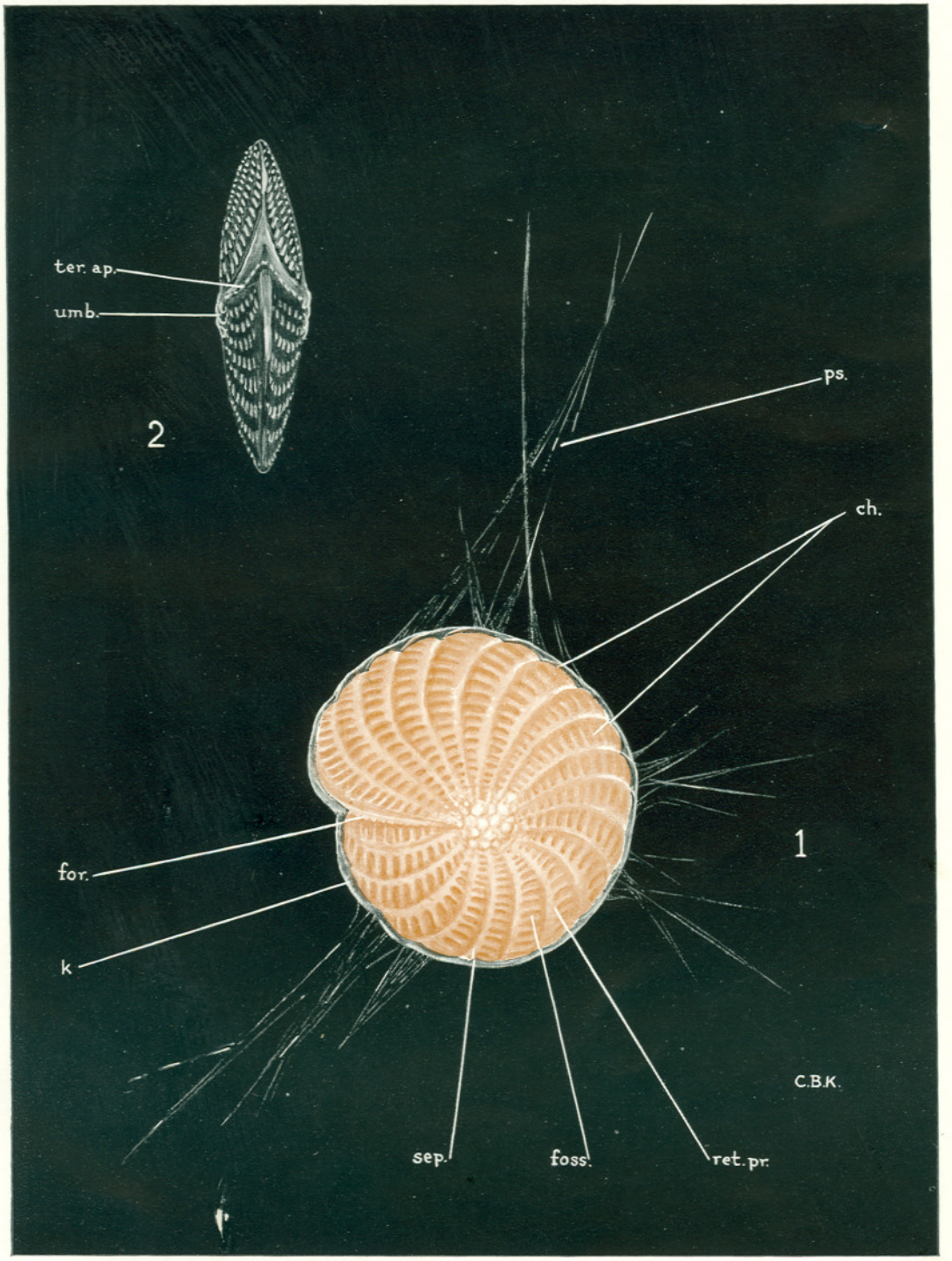
Figs. 2, 3. Flagellispores of *Polystomella crispa* stained with iron haematoxylin and eosin, after fixation in osmic vapour and alcohol and in Schaudinn's fluid respectively. In Fig. 3 the longer flagellum was about five times as long as the body. *cryst.*, refractile inclusion; *N.*, nucleus; *eos.*, eosinophil body in vacuole.

Figs. 4–6. Illustrating three successive stages in the feeding of *Polystomella*, as described in the text on p. 629.

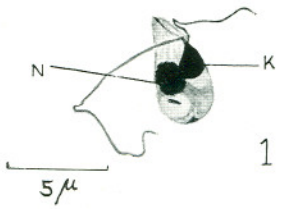
Fig. 7. Part of Fig. 5 at a higher magnification, showing undigested diatoms, and excretory granules (xanthosomes).

Fig. 8. Part of a similar cast feeding cyst showing frustules of a naviculoid diatom and excretory granules.

Figs. 9, 10, 11. Pseudopodia of *Polystomella*. From film made by A. G. Lowndes. Zeiss obj. A, C, D, resp., eyepiece × 10. The granules are well shown in Fig. 11, anastomoses in Fig. 9 and 'webs' in Fig. 10. The black segments at the margins are the edges of the shells.



Polystomella crispa L.



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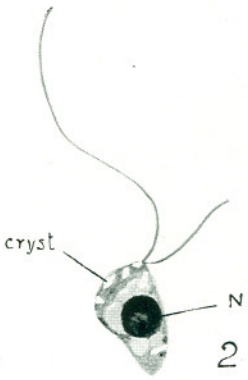

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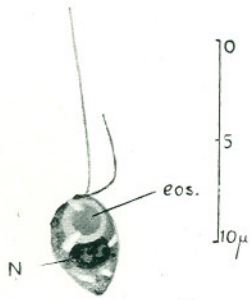

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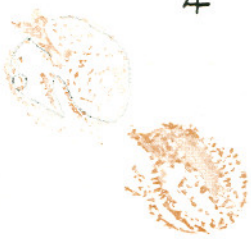

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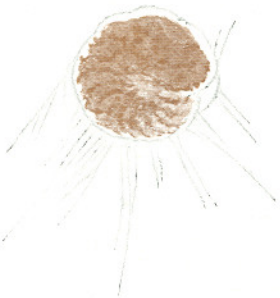

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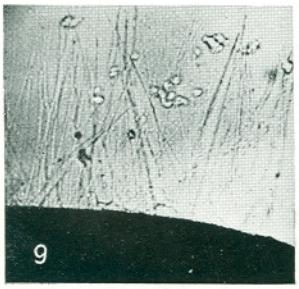

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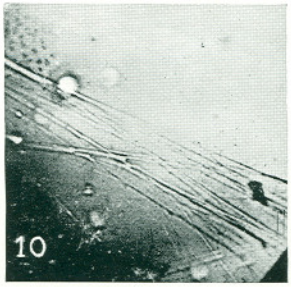

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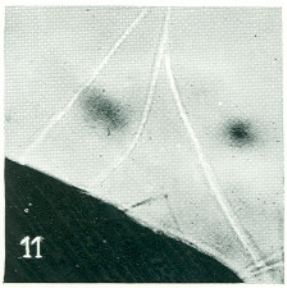

11

Polystomella crispa L.