Spongospora subterranea, (Wallroth) Johnson.[1]

BY

T. G. B. OSBORN, B.Sc.

Lecturer in Economic Botany, the Victoria University, Manchester.

With Plate XXVII.

THE organism producing the 'Corky' or 'Powdery Scab' of potatoes (*Spongospora subterranea*) has recently been described by both Johnson[2] and Massee,[3] but, as the results contained in these investigations are not in agreement, further work on the subject seemed desirable. In the present paper I shall confine myself to giving an account of the life-history and cytology of *Spongospora* so far as I have been able to trace them, together with some remarks on its affinities. Into the disagreement that has arisen between the two previous workers on the subject of nomenclature, I do not propose to enter. The name I have adopted would seem to be justified on the ground of priority,[4] and it is sufficient to refer to the discussion in the papers quoted.

The plants used in this investigation were grown in the experimental greenhouses of the Botanical Department of the University of Manchester, and I wish to thank Mr. J. M. Hector, of Leeds University, for some of the 'seed' potatoes used. The laboratory work has been carried out in the Cryptogamic Research Laboratory here, and I should like to express my thanks to Professor W. H. Lang for the facilities afforded there, and for his kindly interest and help.

INTRODUCTORY.

The disease known as 'Powdery Scab' or 'Corky Scab' of potatoes is produced by an internally living parasite, *Spongospora subterranea*, hitherto regarded as a member of the Mycetozoa and having affinities with *Plasmo-*

[1] A summary of these results was given in the January number of this Journal (p. 271), and in the same number Mr. A. S. Horne gave a preliminary account of his work on *Spongospora* (p. 272).

[2] Johnson, T.: Econ. Proc. Roy. Dublin Soc., vol. i, pt. 12, Apr., 1908; Sci. Proc. Roy. Dublin Soc., vol. xii, N. S., No. 16, July, 1909.

[3] Massee, G.: Journal of the Board of Agriculture, vol. xv, 1908, p. 592.

[4] *Erysibe subterranea*, Wallroth, 1842; redescribed by Brunchorst (who was unaware of the earlier description) in 1886 as *Spongospora Solani*.

diophora Brassicae, Woronin. It is stated by Johnson to be rampant in the West of Ireland; references to its occurrence in this country have not been infrequent during the last few years in the 'Journal of the Board of Agriculture', and I have obtained specimens from more than one locality in the neighbourhood of Manchester.

In its earliest stages, which are visible on young tubers not larger than hazel-nuts, the disease is apparent by small slightly raised pimples, and a slight discoloration of the surface. When cut open, the infected areas appear faintly purplish and extend from approximately the outermost cells of the tuber towards the deeper layers. As the organism matures the surface of the potato above the diseased portions becomes ruptured. If the soil is dry, wound cork is formed, and the extent of the injury is quickly limited. In damper soils cork formation being checked, the infected area becomes hollowed out, this hollowing being continued as the tuber develops until large cavities over an inch in diameter and of considerable depth are produced.

There is not any apparent hypertrophy of the tissues such as is caused by *Plasmodiophora* and *Sorosphaera Veronicae*.[1] The disease, moreover, would seem to be limited to the tubers, though I have occasionally found small scabs on the rhizomes as well, but never on the aerial portions of the plant.[2]

My own observations as to the transmission of the disease from infected 'seed' or soil to sound 'seed' potatoes have been entirely negative. I have planted tubers of 'Up to Date', 'Factor', and 'Conquest' in pots of infected soil and side by side with infected 'seed', under varying conditions of moisture and temperature, but in no case was I successful in inducing the disease.

It has not been a part of the present investigation to test any remedies or chemical checks to the disease.

Methods.

Material was fixed in almost every case in the weaker Flemming's solution. Acetic alcohol was tried, but this was not successful, owing to a shrinkage of the tissues and an apparent hardening of the starch grains which interfered with the section-cutting. The material was brought through ten per cent. glycerine to absolute alcohol, cleared in chloroform and embedded in wax with a melting-point of 54° C. Microtome sections were cut at thicknesses varying from 2–10 μ (4 μ was most frequently used) and

[1] Bloomfield, J. E., and Schwartz, E. J.: Annals of Botany, vol. xxiv, 1910, p. 35.

[2] Schwartz, E. J.: Annals of Botany, vol. xxiv, 1910, p. 511. *Sorosphaera Junci* is limited to the roots of certain species of the Juncaceae, and does not produce hypertrophy of their tissues. I am much indebted to Mr. Schwartz for giving me material of *S. Veronicae* and *S. Junci* for comparison with *Spongospora*.

were stained with Flemming's triple stain, gentian violet and orange, or Heidenhain's iron haematoxylin. As counterstains with the last-named, orange G, erythrosin, or light green (*Lichtgrün*) dissolved in clove oil were tried, also one per cent. aqueous Congo red. In spite of its poor keeping qualities, light green was found to be the most generally useful, as it clearly differentiated the host protoplasm from that of the parasite.

Summary of Present Knowledge.

Briefly stated, our present knowledge of *Spongospora* is as follows. Uninucleate myxamoebae are observed in young potato cells,[1] though this is disputed by Johnson.[2] These subsequently fuse to form a plasmodium, while it is stated that fresh cells are invaded by a passage being bored through their walls.[3] At the approach of spore formation the plasmodium becomes very vacuolar, and then, according to Massee, a hollow sphere is formed, in the walls of which lacunae appear, while later polygonal cells (spores) are cut off, arranged in a single layer. Johnson [4] has corrected this statement, pointing out that the spore mass is a 'sponge-like' body. He further states [5] that each spore contains a number of nuclei (4 or 8), comparing this with Jahn's [6] and Olive's [7] observations on *Ceratiomyxa.* Massee saw only a single amoeba, which escaped on the germination of the spore.

Life-history.

Vegetative phase. Actual infection of the potato tuber by *Spongospora* has not been seen, nor have infection experiments been successful. The earliest stage in the life-history that has been observed is that of a single uninucleate amoeba in a young potato cell near an eye (Pl. XXVII, Fig. 1). The amoeba is somewhat rounded in outline, and consists of finely granular protoplasm, which has different staining properties from that of the host cell, so that it can be clearly differentiated from it. The nucleus appears to conform to the well-known Mycetozoon type, described by Lister [8] and others.

It has a membrane and linin network bearing chromatin granules, as well as a deeply staining body occupying a central position (Fig. 17). This central body retains the safranin with triple stain, and acquires an intense black with iron haematoxylin. As at times it appears to contain all the chromatin of the nucleus, it is, perhaps, best referred to as the karyosome, rather than as the nucleolus.

The nucleus divides in a manner to be described later, and this is generally followed in the early stages of infection by a division of the amoeba

[1] Massee, G.: loc. cit., p. 596.
[2] Johnson, T.: loc. cit., 1909, p. 171.
[3] Massee, G.: loc. cit., p. 597.
[4] Johnson, T.: loc. cit., 1908, p. 455.
[5] loc. cit., p. 456.
[6] Jahn, E.: Ber. d. deutsch. bot. Gesell., vol. xxvi*a*, 1908.
[7] Olive, E. W.: Trans. Wiscon. Acad. Arts. Sci. Litt., vol. xv, pt. ii, 1907, p. 753.
[8] Lister, A.: Journal Linn. Soc. Bot., vol. xxix, 1893, p. 529.

itself. This process continues for some time, so that a number of myxamoebae are to be found in one cell (Pl. XXVII, Fig. 2).

The amoebae are to be found in the cambium of the tuber, generally in the outer layers, though, in advanced stages of the disease, apparently also in the medullary cambium.[1] On the division of the host cell (Fig. 3) it is a purely fortuitous circumstance whether each resulting cell shall contain an amoeba, and so be infected or not. As far as my observations go, the whole spread of the organism from cell to cell takes place in this way. I have never seen any signs of the migration of an amoeba to a neighbouring cell, nor any continuity of protoplasm, such as Massee has described. This passive infection of fresh cells, or rather, handing on of the parasite to daughter cells in a dividing tissue, is like that described by Nawaschin [2] for *Plasmodiophora*, and Bloomfield and Schwartz [3] for *Sorosphaera*.

The amoebae continue to divide as has been described, while the host cell increases in size, so that a late stage of infection will show many amoebae, now not infrequently multinucleate, occupying the greater part of its area (Fig. 4). The nuclei during this period divide in an amitotic manner much the same as characterizes the divisions in a similar stage in *Sorosphaera* [4] and *Plasmodiophora*.[5] The chromatin arranges itself in the form of a ring around the karyosome, giving an appearance that has been referred to as the 'Saturn stage'. This ring of chromatin now splits into halves which travel apart (Fig. 18, *b*), from which it will also be seen that the nuclear membrane has become drawn out into an elliptical shape. The karyosome divides by becoming elongate, then dumb-bell shape, the halves subsequently pulling apart; this does not occur until the chromatin ring has split, a slight point of difference from the occurrences recorded in the other genera (Fig. 18, *c*, *d*, &c.). As the chromatin approaches the poles of the much elongated nucleus its constituents, derived from the halves of the ring and of the karyosome, blend together, and in the concluding stages appear as single, rounded, deeply staining masses near the poles (Fig. 18, *f*). Nuclear membranes form around these daughter nuclei, part of the membrane being derived from that of the parent nucleus (Fig. 18, *g*). It will thus be seen that at this stage the karyosome apparently contains all the chromatin of the nucleus. The chromatin granules of the latter appear later, and are apparently given off from the karyosome (Fig. 18, *h*). The linin network is not distinguishable until the granules are formed. I have not been able to determine the presence of spindles or centrosomes during this type of division, nor have I seen polar radiations during this or any

[1] Read, T.: Annals of Botany, vol. xxiv, 1910, p. 537.

[2] Nawaschin, S.: Flora, vol. lxxxvi, p. 404.

[3] Bloomfield, J. E., and Schwartz, E. J.: loc. cit., p. 40.

[4] See also Maire, R., and Tison, A.: Ann. mycolog., vol. vii, 1909, p. 226.

[5] See also von Prowazek, S.: Arb. aus dem kaiserl. Gesundheitsamte, vol. xxii, 1905, p. 396.

other of the nuclear divisions. Maire and Tison[1] and Prowazek[2] record these in *Sorosphaera* and *Plasmodiophora,* and the former regard this type of nuclear division as an 'intranuclear karyokinesis combined with an amitosis', a statement which is in accordance with their advocacy of the 'dual hypothesis' of nuclear structure.

Reproductive phase—akaryote stage. When the amoebae have exhausted most of the food material in the cell (though the host nucleus and occasional starch grains are still to be seen at this stage), they coalesce to form a plasmodium (Fig. 5). The plasmodium is thus the product of the fusion of a number of vegetative amoebae, and this fusion is the first step to spore formation. It would seem to be usual for only one plasmodium to form in a cell, though exceptions are to be found, and in one case as many as eight mature spore balls were seen (Fig. 16).

The formation of the plasmodium is followed by an akaryote condition in which the nuclear matter appears to be scattered throughout the whole plasmodium. The chromatin granules on the network, hitherto a prominent feature, disappear in all the nuclei of a plasmodium at the same time, being possibly conducted to the nuclear membrane along the linin threads and there extruded. In the same way the karyosome diminishes in size and is gradually lost (Fig. 19), while the protoplasm of the plasmodium (now rounded in shape wherever conditions of space permit) becomes filled with deeply staining granules which may be termed chromidia. This appearance is shown in Fig. 7, which is a drawing of a plasmodium at this stage. It will be seen that the sites of the nuclei are not lost to view, but remain as circular areas free from any trace of chromidia and showing up in marked contrast to the surrounding protoplasm. This appearance might at first sight be thought to be suggestive of vacuolation; the nuclear areas, however, when examined in sections stained with iron haematoxylin and light green for instance, are perfectly distinct. A similar occurrence is recorded for both *Sorosphaera* and *Plasmodiophora*, while a chromidial state is well known for certain Protozoa.[3] It is impossible to give any idea of the time of duration of the akaryote condition, but to judge from my preparations I do not think it to be very long.

The nuclei as they are developed the second time are of a very different appearance from the previous vegetative ones. There is a membrane, network, and chromatin granules staining an intense black with haematoxylin, but no karyosome. It is not easy to trace the development of the new nuclei, but there is evidence that they are constructed *de novo*, rather than reconstituted on the sites of the old ones. The evidence for such a statement rests on such stages as are figured in Figs. 8 and 20. By the side of the clear spaces, representing the previous vegetative nuclei, there may be

[1] Maire, R., and Tison, A.: loc. cit., p. 230. [2] von Prowazek, S.: loc. cit., p. 398.
[3] See literature quoted by Dobell, C. C.: Q. J. Micro. Sci., vol. liii, N. S., 1909, p. 279.

seen rods and granules of deeply staining chromatin surrounded by a non-staining area. These gradually become more marked until in the new nucleus there may be seen a considerable mass of chromatin in a lump, often lying to one side of the membrane (Pl. XXVII, Fig. 21), while at the same time the protoplasm appears less granular.

The most satisfactory explanation of these facts would appear to be that on the reconstruction of the nuclei the chromidia are reduced in number, though they do not totally disappear, and thus there is a certain wastage of chromatin, which ultimately degenerates. The chromatin mass in the new nucleus gradually becomes less contracted, and a network arrangement is to be seen (Fig. 22), though there is no sign of a karyosome or nucleolus. Bloomfield and Schwartz, when describing a similar stage in *Sorosphaera Veronicae*, were unable to state where the fresh nuclei appeared in relation to the old ones, but in *S. Junci* Schwartz says he observed 'granules and irregular masses of chromatin forming fresh nuclei in the vacuoles'.[1]

Karyogamy and spore formation. The reproductive nuclei are to be seen at first irregularly scattered through the whole plasmodium. It is soon to be noticed, however, that there is a very definite association in pairs (Figs. 9 and 10). This in itself is suggestive of a fusion, and all stages of the occurrence have been observed in numerous plasmodia (Fig. 11). In a pair of fusing nuclei, the membrane at the point of contact breaks down, and their contents merge one into the other (Fig. 23). The union occurs at approximately the same time for all pairs of nuclei in a plasmodium, so that the various stages are by no means rarely to be seen (Fig. 24). Such nuclei as are unable to pair degenerate and are quickly lost to sight.

The fusion nucleus has an appreciably increased diameter (5 μ), while its chromatin matter appears in the form of threads (Fig. 25). It is at this period that there is the greatest difficulty in arranging the various stages in their proper sequence. The plasmodia are so small that no progressive series of changes can be seen in the single plasmodium, as has been recorded for various Mycetozoa, but careful comparative study of different plasmodia of *Spongospora* leads to the following account. The chromatin matter contracts to form a dense irregular mass lying within the enlarged membrane. The appearance at this stage is strongly suggestive of a synapsis (Fig. 26). On emerging from this state the chromatin is in the form of granular threads (Fig. 27) arranged along a diameter of the cell (Fig. 12), but unfortunately I cannot state with certainty the various stages that must intervene between this condition and the first karyokinesis, nor am I able to say, from my own observations, whether a condition of diakinesis occurs or not.

The first karyokinetic division is marked by a well-defined but very dense plate of chromatin lying equatorially on a spindle which is relatively long compared with the diameter of the plate (Fig. 13). The spindle, how-

[1] Schwartz, E. J.: loc. cit., p. 516.

ever, is very small (7 μ), as may be seen by a comparison with that of *Badhamia utricularis*, which is about 18 μ in length. The spindle in *Spongospora* shows two clearly-defined poles with centrosomes, but the actual threads of the spindle in this division are not easy of differentiation, nor can the individuality of the chromosomes be made out. It is, of course, possible that there are no definite chromosomes in this division, the chromatin existing in a granular state, as Blackman has described in *Coleosporium*. The nuclear area often persists as a clear space during this first division (Fig. 28, *a*), though this is not so in all cases (Fig. 28, *b*). It is surprising how infrequently the ana- and telophases have been observed. A series of sections showing many plasmodia with their nuclei in the metaphase will show but few in the later stages of division, and this is true, moreover, for material of widely differing dates and hours of fixation. It is, perhaps, to be accounted for by a rapid movement of the chromatin masses towards the poles. Harper[1] found it otherwise in *Fuligo varians*, for, he says, 'all stages in the separation of the daughter chromosomes and their migration to the poles of the spindle can be observed in the greatest abundance.' All that can be said of the later stages of the first karyokinesis in *Spongospora* is that the chromatin splits into two apparently equal portions, which travel to the poles, retaining for some distance their plate-like character (Fig. 29). As they near the poles this appearance is lost, while the spindle fibres in the middle of the spindle disappear, though the apices and centrosomes are well marked (Fig. 30). The chromatin rounds itself off, while a new nuclear membrane appears. The second division is characterized by a shorter spindle (5 μ) and by the absence of any sign of the nuclear area at the metaphase (Fig. 14). The later stages of this division resemble the preceding one, the chromosomes travelling to the poles in a mass (Figs. 33 and 34). In the plate stage, however, the spindle fibres can be differentiated with less difficulty, while sections transverse to the long axis of the spindle show a number of chromosomes—eight in those cases in which it has been possible to count them (Fig. 32).

By the time that this division is completed the protoplasm has become rounded about each nucleus, so that there is a condition in which the organism consists of a number of uninucleate masses of protoplasm, the young spores, about which the spore wall then forms. The mature spores are spherical bodies about 4 μ in diameter, with a cell-wall, single nucleus, and a certain amount of oil (Fig. 35). The spore wall does not give a cellulose reaction with chlor-zinc iodine.

The individual spore thus bears a strong resemblance to that of *Plasmodiophora*, as described by Nawaschin. The spores are loosely aggregated to form the structures referred to as 'spore balls'. These are of a shape varying from spherical to ovoid, while the diameter also varies, though 50 μ

[1] Harper, R. A.: Bot. Gaz., vol. xxx, 1900, p. 233.

may be taken as an average size. The 'spore balls' are marked by numerous depressions and fissures (Pl. XXVII, Fig. 16), as has been described, arising from the formation of clefts in the plasmodium. Harper has described minutely the progressive development of the cleavages that characterize the development of the spores in *Fuligo*. The whole plasmodium is so small in *Spongospora* that no such full description can be given, but since cleavages are to be noticed in the plasmodium at any time after its formation (Figs. 7 and 10), though not generally till the time of the second mitosis (Fig. 14), it is obvious that in *Spongospora*, as in the Mycetozoa, the segmentation of the protoplasm is independent to a great extent of nuclear division.

The spore in all cases that I have observed is uninucleate, the nucleus having a karyosome and other chromatin matter.

Unfortunately all my cultures of the spore balls have proved intractable, so that I have not been able to observe the germination of the spores. Professor Johnson has not seen this either, Massee's account [1] being the only one published. He records that the contents of the spores escape intact, 'and are irregularly globose in form, with a few small projections. They show a very sluggish movement for some time, after which they become stationary. The diameter of the amoeboid body after its escape from the cell is about 3 μ.'

Effect upon the host plant. The effect of *Spongospora* upon its host has to some extent been described already. Under dry conditions of the soil the external appearance is limited to small circular patches about 5 mm. across. Under wet conditions the damage is more serious, and the scabs may be as large as 3–4 cm. in diameter and as much as 2 cm. in depth. This is the only external appearance; there is no sign of hypertrophy nor any distortion other than that caused by the pitting.

A definite cork cambium is formed below the seat of injury, though amoebae are to be observed in the deeper layers of the tuber under the cork.

In the host cells it is apparently the starch that is especially attacked, and starch grains of any size are generally absent at the time of spore formation. This is certainly not so in the initial stages, nor even up to the time of plasmodium formation. It is, of course, possible that starch does not develop in those cells that are attacked when young, the soluble carbo-hydrate material being absorbed as it enters the cell by the parasite. The subject, however, needs further investigation, which it is hoped to carry out.

About the time that the plasmodium is formed, the host cell appears to be exhausted, and most of its cytoplasm has disappeared. This is not the case with the nucleus, which has become much enlarged and unhealthy in appearance as the attack has proceeded. Many of the nuclei show remarkable lobing and indentations (Figs. 5 and 12), and they are often closely applied to the plasmodium of the invader (Figs. 7 and 12). The nuclei

[1] Massee, G.: loc. cit., p. 598.

may possess more than one nucleolus, and have densely granular nucleoplasm. By the time of the first karyokinesis, the host cell nucleus would appear to have degenerated or to have been absorbed by the parasite; it is not generally to be observed later than the akaryote stage. No multinucleate host cells have been observed, nor do the host cells appear to be much enlarged, which is an interesting point of difference from the cells attacked by *Sorosphaera Veronicae* and *Plasmodiophora*, though in *S. Juncí* their behaviour is similar.

In the last few years a number of papers have appeared adding to our knowledge of the Plasmodiophoraceae and the allied groups. These have been summarized recently in the 'Progressus Rei Botanicae',[1] but it is necessary to review some of the salient points here.

Nawaschin's[2] account of *Plasmodiophora* in 1899 described a method by which the amoebae infected new host cells, which is similar to that given here. The nuclei of the amoebae were found to divide in a special manner, differing markedly from indirect nuclear division. The ring of chromatin matter around the karyosome is described, also something in the nature of an achromatic spindle, but neither centrosomes nor polar radiations are mentioned. Spore formation was found to be preceded by plasmodium formation, followed by a reconstitution of the nuclei and their subsequent karyokinetic division.

In 1905 Prowazek's[3] description gave still further details of the divisions in the vegetative phase. Centrosomes and asters were described, and a distinction drawn between the tropho- and idiochromatin stated to be present in the divisions. The chromidial stage was described in great detail, also the presence of two karyokinetic divisions subsequent to it, which are referred to as generative divisions. Following these divisions the protoplasm is stated to round itself off about the nuclei. These uninucleate bodies unite in pairs before encystment. The spore membrane then forms, and the nuclei within the cyst divide; one nucleus from each pair then degenerates, while the remaining two nuclei fuse. Prowazek regards this division in the cyst as the reduction division, the two karyokineses in the plasmodium having no such significance.

Maire and Tison,[4] in their memoir on the group, state that they are unable to agree with Nawaschin that a plasmodium formation occurs, since they have several times observed in the same cell an amoeba in the metaphase and another in the anaphase of the sporogenous division. They agree with Prowazek on the matter of a double karyokinesis, but are quite unable to accept his statement as to the autogamy succeeding encystment, which they consider simply as an abnormal occurrence.

In addition to *Plasmodiophora*, Maire and Tison have in the same

[1] Pavillard, J.: Prog. Rei Bot., vol. iii, 1910, p. 474. [2] Nawaschin, S.: loc. cit., 1899.
[3] Prowazek, S.: loc. cit., 1905. [4] Maire, R, and Tison, A.: loc. cit., 1909, p. 239.

paper described *Sorosphaera Veronicae* in detail. The myxamoebae found in the leaves and stems of *Veronica* spp. contain, at first, a single nucleus with a karyosome, and chromatin on a linin network. The division of this nucleus is 'une mitose d'idiochromatine combinée avec une amitose de trophochromatine'. In this division an intranuclear spindle is described with centrosomes and polar radiations more or less visible. This stage is regarded as a 'schizont' condition of the organism, since small amoebae are constricted off from the larger ones. At the conclusion of this stage the nuclei are reconstituted, the chromatin passing out into the protoplasm, where it is found as chromidia. Later the contents of the nuclei reappear, synapsis follows, the nucleoli disappear, and chromosomes form. There is a double karyokinesis, the divisions of which are regarded as heterotypic and homotypic, eight double chromosomes being visible in the former. During the second division the protoplasm becomes rounded about each nucleus, forming a spore, a number of which are arranged in a hollow sphere.

Very similar results were obtained by Bloomfield and Schwartz,[1] only recorded in less detail. The point of infection was found to be at the growing apices, and the infection of fresh host cells was produced, not by the penetration of the cells by the amoebae, but by the subsequent divisions of the cell originally infected. In *S. Veronicae* there is much hypertrophy of the tissues, and the infected cells of the host become multinucleate.

Schwartz [2] has more recently described a new species, *S. Junci*, the life-history of which is essentially the same, but no hypertrophy of the tissues of the host is produced.

Before entering upon a discussion of the points of difference between *Spongospora* and the other members of the Plasmodiophoraceae, it will be useful to consider the recent work on the Mycetozoa. Following the early work of Strasburger and Lister [3] (who suggested an amitosis of the vegetative nuclei in *Badhamia utricularis*), Harper [4] gave a detailed account of the cell and nuclear divisions in *Fuligo*. In regard to the cell-divisions, his work was of importance in showing that they are independent of those of the nuclei, and result from a progressive cleavage of the plasmodium.

In 1907 Fräulein Kränzlin [5] described the development of the sporangia in *Arcyria* and *Trichia*. Previous to spore formation the nuclei associate in pairs and a fusion occurs; any nuclei that do not fuse quickly degenerate and disappear. The fusion is interpreted as karyogamy, and is followed by a temporary enlargement of the nucleus and a synapsis. The nuclei, on regaining their normal size, show an arrangement of eight double chromosomes that is described as diakinesis. At the conclusion of this stage

[1] Bloomfield, J. E., and Schwartz, E. J.: loc. cit., 1910.
[2] Schwartz, E. J.: loc. cit., 1910.
[3] Lister, A.: loc. cit., 1893.
[4] Harper, R. A.: loc. cit., 1900.
[5] Kränzlin, Helene: Archiv f. Protistenkunde, vol. ix, 1907, p. 170.

a simultaneous division of all the nuclei occurs, which, on the ground of the prophases, is regarded as heterotypic. This first division is immediately followed by spore formation. 'Le processus de réduction se trouve ainsi interrompu, pendant toute la période de la vie ralentie de la spore mûre. La réduction s'achève à la germination,'[1] the succeeding homotypic division being the one described by Jahn[2] in the swarm spore.

In the exosporous genus, *Ceratiomyxa*, Olive[3] and Jahn[4] have described a karyogamy. This occurs, according to Olive, towards the close of the segmentation of the protoplasm, and is followed by synapsis, each potential spore mass receiving a nucleus in that state. Two divisions follow, giving the typical four-nucleate spore.

Jahn's account is quite different. Following nuclear fusion, which he places at an earlier stage, there are stages regarded by him as synapsis and diakinesis, though it is to be regretted that his figures of the latter are not more convincing. There is, then, one (heterotypic) division of the nucleus, followed by a degeneration of half the daughter nuclei. The remaining nuclei form the spores, in which a double karyokinesis occurs. Yet another nuclear division takes place on germination, so that eight swarm spores are freed. It is not easy to homologize this account with what is known of the endosporous genera. Jahn regards the nuclear reduction as completed by the heterotype division and the subsequent nuclear degeneration, the two divisions in the spore, and that on its germination as of no special significance. In his review of the work, Pavillard[5] suggests that probably the division on the germination of the spore is a true homotypical mitosis (comparable to that in the *Endosporae*), the two preceding divisions being ordinary mitoses interpolated between the heterotype and the homotype divisions, and of no special significance.

Discussion and conclusions. The formation of a definite plasmodium has been described in the life-history of *Spongospora*. Prior to spore formation the host cell is found to contain a large multinucleate mass of protoplasm in the place of separate amoebae. All the nuclei in each mass divide at the same time, and the spores resulting from these divisions are united as a rule in a single spore ball.

The question of the formation of a true plasmodium in *Plasmodiophora* is the subject of a disagreement among the various workers on the organism. In *Sorosphaera* there is stated to be no plasmodium formation.

If these three genera are to be united, either in the Plasmodiophoraceae according to Maire and Tison, or in the Sorophoreae of Schröter, the defini-

[1] Pavillard, J.: loc. cit., p. 510.
[2] Jahn, E.: Ber. d. deutsch. bot. Gesell., vol. xxii, 1904, p. 84.
[3] Olive, E. W.: Trans. Wiscon. Acad. of Arts, Sci. and Litt., vol. xv, pt. ii, 1907, p. 753.
[4] Jahn, E.: loc. cit., vol. xxvi *a*, 1908, p. 342.
[5] Pavillard, J.: loc. cit., p. 511.

tion of these groups as one in which a plasmodium does not form must be suitably modified.

It is hardly safe to express any definite opinion on the significance of the dissolution of the nuclei and on the occurrence of chromidia, in view of the small amount of evidence at present in our possession. It may well be that the nuclei rid themselves of a portion of their trophochromatin before entering upon a reproductive phase. However, in the present state of our knowledge, such a deduction, involving, as it does, the 'binuclearity hypothesis', is unjustifiable as anything more than the merest speculation until further facts give the theory a firmer basis. As far as the present observations on *Spongospora* go, this loss of chromatin at the time of the formation of the reproductive nuclei is the only one to be observed. No constant stream of chromatin leaving the karyosome has been seen as described for *Sorosphaera.*

The karyogamy observed in *Spongospora* shows a striking similarity to that described by Fräulein Kränzlin in *Arcyria* and *Trichia*; the comparison with *Ceratiomyxa* is less easy. The nuclear fusion cannot be confused with a direct division, since, apart from the direct evidence as to its nature, it has none of the features of the very definite amitosis that occurs in the vegetative amoebae.

The observations on the peculiar method of karyogamy described for *Plasmodiophora* have not been confirmed by more recent workers, while as yet no fusion of nuclei has been observed in the life-history of *Sorosphaera.* It is not impossible that a karyogamy has been overlooked in these two organisms, and that further work on them will complete this gap in the knowledge of their life-histories.

It is unfortunate that a more definite account cannot be given of the prophases of the two karyokineses preceding spore formation in *Spongospora*; since, except for the enlargement of the nuclei and a contraction of the chromatin contents, no further details are known. Maire and Tison figure a synapsis in *Sorosphaera Veronicae*, and they are further of the opinion that the two mitoses are those of a reduction. Fräulein Kränzlin has described synapsis and diakinesis in the *Mycetozoa*, so has Jahn in *Ceratiomyxa.*

Personally I incline to the view that there is a synapsis in *Spongospora*, and that the two following mitoses are the heterotype and homotype of a reduction division. However, in default of further evidence, this must be stated as an opinion rather than as a fact, but as an opinion that receives considerable support by comparison with the occurrences in *Sorosphaera* and *Trichia* and *Arcyria.*

Accepting, then, the validity of this assumption, it is seen that the life-history of *Spongospora* resembles in the main a Mycetozoon as regards its nuclear constitution. The nuclei throughout the whole vegetative period are of the haploid form (x generation); the diploid state ($2x$) being only

attained for a short time just previous to spore formation. The reduction in *Spongospora* is completed before this occurrence; in the Mycetozoa the homotype division does not take place till the spore has germinated.

Should the observations of Prowazek on *Plasmodiophora* be confirmed, the case of that organism is wholly different from the Mycetozoa or *Spongospora*. It is diploid from spore to spore, the x generation being limited to a short period within the cyst. This may be more in accordance with 'all Protozoa', as Hartmann[1] has observed, but it does make it more difficult to trace the homologies between the plasmodia of *Plasmodiophora*, on the one hand, and those of *Spongospora* and the Mycetozoa on the other. Thus, though the evidence regarding *Plasmodiophora* is to some extent conflicting, it may be assumed that karyogamy in the plasmodium preceding the karyokinesis is the normal occurrence in the Plasmodiophoraceae; it is interesting to note that Pavillard[2] has already forecast this in his summary of the work upon the group.

Summary.

1. *Spongospora subterranea* is an intracellular parasite of the potato tuber, living in the cells in an amoeboid condition, and invading the daughter cells as they form in the process of cell-division.

2. The nuclei of the amoebae divide in an amitotic manner during the vegetative phase; on its conclusion the amoebae fuse to form a plasmodium.

3. Plasmodium formation is followed by a degeneration and disappearance of the vegetative nuclei, chromidia appearing in the protoplasm. This is the akaryote stage.

4. On the conclusion of the akaryote stage the nuclei are formed on different sites to the previous ones, some of the chromidia being used in the process while the remainder degenerate.

5. Karyogamy occurs between pairs of the nuclei.

6. Karyogamy is succeeded by a temporary enlargement of the nuclei and a contraction of the chromatin, which is possibly a condition of synapsis.

7. Two karyokinetic divisions of the nuclei follow each other rapidly; the first is marked by its length of spindle; the spindle of the second is shorter, with more sharply defined fibres, and has eight chromosomes.

8. The spores are uninucleate, and are aggregated in rounded masses traversed by fissures and marked by irregular depressions, but remaining attached in structures known as 'spore balls'.

9. *Spongospora* is a member of the Plasmodiophoraceae, which group has many points of relationship to the Mycetozoa, differing chiefly in the parasitic habit, the method of division of the vegetative nuclei, and by the less constant presence of a flagellum on spore germination.

[1] Hartmann, M.: Archiv f. Protistenkunde, vol. xiv, 1909, p. 284.

[2] Pavillard, J.: loc. cit., 1910, p. 506.

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EXPLANATION OF PLATE XXVII.

Illustrating Mr. Osborn's paper on *Spongospora*.

All figures were drawn in outline with a Zeiss camera lucida at the table level, with a tube length 160 mm. Except where stated, a Zeiss 3 mm. apochromatic oil-immersion objective (1·30 aperture) was used with (Figs. 1–15 inclusive) a × 6 compensating ocular and (Fig. 17 ad. fin.) with a × 18 comps. occ. The magnification was thus roughly 900 and 2,750 diameters respectively.

Fig. 1. Single uninucleate amoeba in young host cell.

Fig. 2. Three amoebae in a cell, one binucleate; note the young starch grains which are also present.

Fig. 3. Host cell which has recently divided, showing cell-plate formation with amoebae in each daughter cell.

Fig. 4. Several large amoebae in a cell. Starch grains and host cytoplasm are still present; the host nucleus is becoming enlarged.

Fig. 5. Plasmodium formation. The host nucleus is here considerably enlarged and shows marked indentations.

Fig. 6. Plasmodium showing the vegetative nuclei in process of degeneration.

Fig. 7. The akaryote and chromidial stage.

Fig. 8. Conclusion of akaryote condition, the new nuclei forming apart from the old nuclear sites. Cleavages in the protoplasm are shown in this plasmodium.

Fig. 9. Nuclei approximating. The plasmodium still contains a number of densely staining particles.

Fig. 10. Nuclei pairing.

Fig. 11. Fusion of nuclei in pairs (Figs. 9 and 11 are from contiguous host cells).

Fig. 12. Nuclei after pairing in synapsis state. The host nucleus is still visible, much lobed, and closely applied to the plasmodium.

Fig. 13. First karyokinesis.

Fig. 14. Second karyokinesis; the cleavages are more apparent and the segmentation of the protoplasm is also marked.

Fig. 15. Section through a mature spore ball, showing the rounded spores, and the cleavages between them which give the spongy appearance when seen in surface view.

Fig. 16. Host cell with eight mature spore balls, and a starch grain. Under this power the spaces between the individual spores are not clearly visible, the cysts appearing polygonal, not rounded. (Reichert objective 7 *a*.)

Fig. 17. Nucleus of a myxamoeba in a resting condition.

Fig. 18. Division of nucleus in myxamoebae. *a*. Ring of chromatin around karyosome. *b*. Splitting of chromatin ring before the karyosome has elongated; the nuclear membrane is becoming elliptical. *c* Karyosome dumb-bell shaped. *d*. Later stage. *e*. Halves of karyosome and the chromatin of the plate nearing the poles. *f*. Karyosome and plate chromatin blending to form the fresh karyosomes. *g*. Nuclear membrane disappearing between the two chromatin masses. *h*. Two daughter nuclei. The extra karyosome chromatin is appearing in the form of granules.

Fig. 19. Degeneration of the vegetative nuclei, showing the diminished karyosome and the extrusion of chromatin granules.

Fig. 20. Nuclei in process of reconstruction, showing the dense chromidia-containing protoplasm and the sites of the vegetative nuclei.

Fig. 21. Dense mass of chromatin matter in reconstructed nuclei.

Fig. 22. Chromatin network in pre-fusion nuclei, showing the absence of a karyosome.

Fig. 23. Fusion of nuclei (Fig. 11 enlarged).

Fig. 24. Late stage in fusion of the nuclei.

Fig. 25. Post-fusion nuclei showing increased diameter.

Fig. 26. Synapsis.

Fig. 27. Spireme emerging from synapsis.

Fig. 28. *a*. First division, metaphase showing nuclear area. *b*. First division, metaphase nuclear area not visible.

Fig. 29. First division, anaphase.

Fig. 30. First division, telophase.

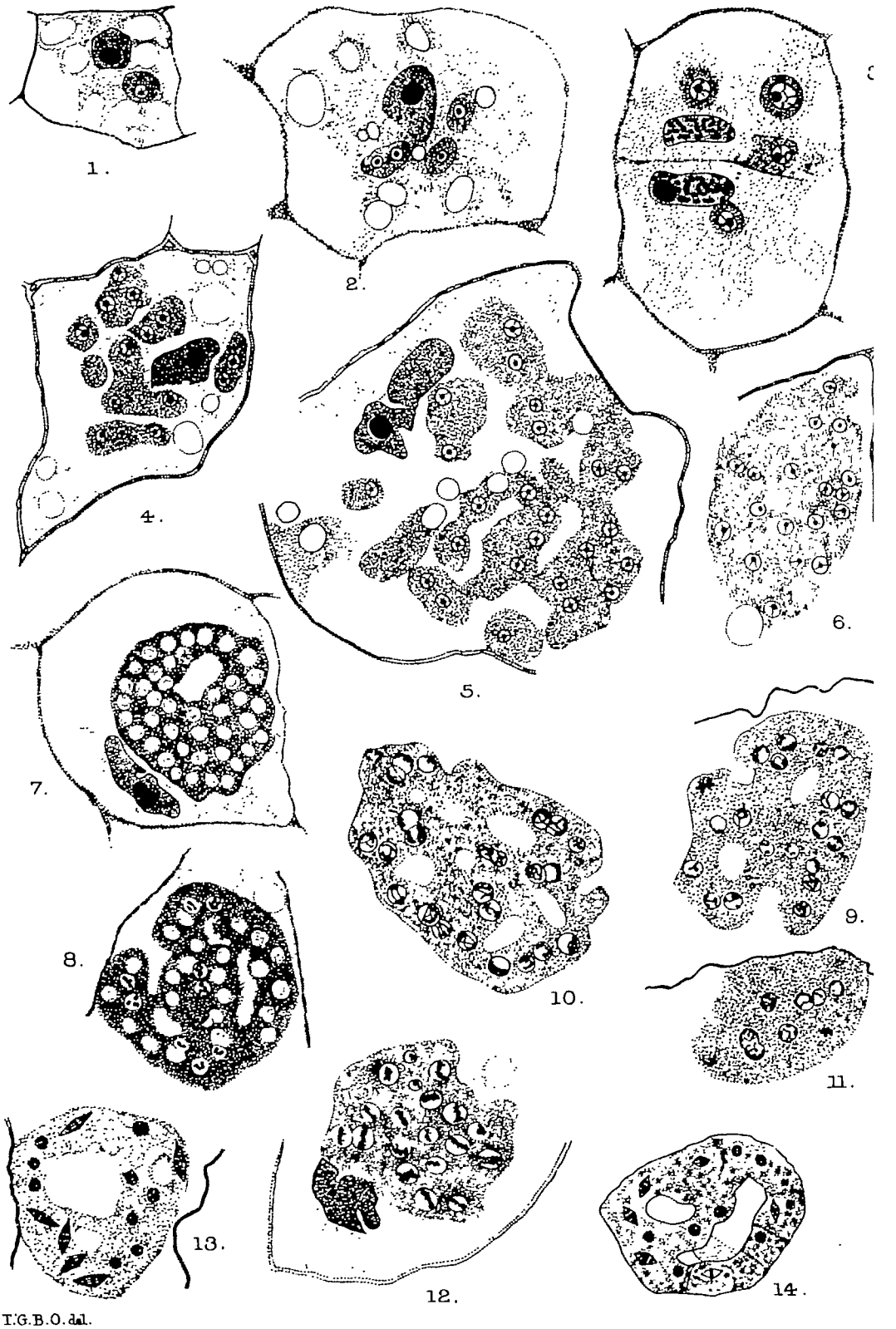
Fig. 31. Conclusion of first mitosis.

Fig. 32. Second division, showing eight chromosomes in plates cut transversely to the long axis of the spindle.

Fig. 33. Second division, metaphase.

Fig. 34. Second division, anaphase; the proplasm at this stage shows marked segmentation.

Fig. 35. Mature spores showing their rounded shape and single nucleus.



T.G.B.O. del.

OSBORN — SPONGOSPORA SUBTERRANEA.