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The Life-History, Cytology and Method of Infection of Plasmodiophora brassicae Woron., the Cause of Finger-and-Toe Disease of Cabbages and Other Crucifers

W. R. Ivimey Cook and E. J. Schwartz

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VI. *The Life-History, Cytology and Method of Infection of* Plasmodiophora brassicæ *Woron., the Cause of Finger-and-Toe Disease of Cabbages and other Crucifers.*

By W. R. Ivimey Cook, *B.Sc., Ph.D., and* E. J. Schwartz, *M.A., D.Sc.*

Communicated by E. J. Butler, *F.R.S.*

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(Plates 19–21.)

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1. *Historical Survey.*

The study of the organism responsible for Finger-and-Toe Disease began in 1873. For several years the fungus had been widespread in Russia, where it had caused considerable damage, especially in the neighbourhood of Leningrad. In 1872 the Russian Gardeners' Association established a prize for its scientific study. However, no papers on the subject were received in 1873, and the offer was extended to 1875, and again to 1877.

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In 1873, WORONIN began his study of the fungus. He finished it in 1877 (67). The results first appeared in a series of Russian papers, which have received but little attention; but a complete account of his work was published in German in 1878, and it was this paper which is generally considered to mark the discovery of *Plasmodiophora brassicæ*. It must be admitted, however, that WORONIN gives CASPARY the credit for the first investigation of the swollen roots.

In 1892, EYCLESHYMER (14) published his paper on a study of the disease in the United States. This paper consists largely of a confirmation of WORONIN'S results and contributes little more to our knowledge of the fungus. In 1892, WAKKER (62) also studied the effect of the parasite on the host tissues, but he was interested in the pathological rather than the morphological aspect of the subject.

With the exception of minor contributions by EYCLESHYMER and WAKKER no further work was done on the fungus until 1893, when NAWASCHIN worked out its cytology in material sent him by WORONIN. In the main his results, which were published in 1899 (45), still remain essentially correct, although he failed to observe the second of the nuclear divisions which occur before spore formation. In a later paper on the same subject, NAWASCHIN (46) elaborated some of his observations on the character of the two types of nuclear division. The results so obtained caused considerable general interest, since this was the first case discovered where two distinct types of nuclear division regularly appeared in a life cycle. In 1901, PROWAZEK (53) undertook a further study of the organism, and he was able to confirm all NAWASCHIN'S observations, except in the divisions prior to spore formation, where he was able to show that there were two mitoses, instead of one, as NAWASCHIN had thought.

From 1901 onwards a very large amount of literature accumulated upon the subject, both from the cytological and the pathological aspects. Many papers have appeared, particularly in America, describing in general terms the appearance of the fungus, and the resulting injury to its host. The majority of these papers have not contributed much to our knowledge regarding its life-history, and are often little more than a summary of the work previously published.

In 1909, MAIRE and TISON (33) compared their observations on *Plasmodiophora brassicæ* with those on *Sorosphæra veronicæ*, and came to the conclusion that the two life cycles were remarkably similar. In the same year FAVORSKI (15) also studied the cytology. The same result was shown to be true of *Spongospora subterranea* by OSBORNE (48) and others (24) in 1912. In 1913, LUTMAN (32) gave a further account of the life-history and cytology, though his observations only served to confirm previous work.

Meanwhile, various other organisms had been described which were referred to the genus *Plasmodiophora*. The inadequate description of many of these makes it impossible to decide whether their claims of affinity are of any significance or not, but there are others which certainly should not be included among the Plasmodiophorales.

In 1917, CHUPP (6) published an account of *Plasmodiophora brassicæ*, in which

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considerable interest centred round the method of infection and the position in the life-cycle of a nuclear fusion. This latter problem has received no satisfactory answer from a study either of the genus *Plasmodiophora* or of the other genera which are considered to be closely allied to it. In CHUPP's paper a particularly interesting stage is figured on p. 436, fig. 104, *h*. The conclusions which he drew from this stage were that the amœba, which had been formed in the root hair, was dividing up to form spores. As will be shown later, there is another interpretation which can be placed on this phase, and this view is supported by recent work on other members of the group. The stage figured by CHUPP is important, however, in being the first case figured of the subsequent development of an amœba which was restricted to the infection of a root hair.

Another aspect of Club root was investigated by KUNKEL (30), who in 1918 published a paper on the effect of the fungus on the host and the hypertrophy which it promoted in the invaded tissue. By means of a large number of excellent photographs, KUNKEL shows the origin and subsequent development of the galls normally associated with an attack of *P. brassicæ*. He studied for the most part plants which had become fairly heavily infected, and consequently failed to attach much importance to the very early infection stages which occur in the root hair. In the main, the results obtained on the mechanical effect upon the invaded tissue and the nature of the response made by the host to an attack of *P. brassicæ* are fully supported by the present investigation in so far as that aspect of the problem has been studied.

In the years which followed the publication of KUNKEL's paper very little work on the organism was done. TERBY (59) studied the structure of certain granules formed during the meiotic divisions which he considered to indicate the early stage of a blepharoplast (37), and various authors (2) (16) (25) (26) (28) (39) (40) (47) (60) (61) published accounts of fungi which, in their opinion, resembled *P. brassicæ*, and which were therefore added to the genus under other specific names. Some of these occur in the tropics, where important crop diseases have been ascribed to them.

A review of the species which definitely belong to the genus *Plasmodiophora* will be given, and reasons will be put forward why it is considered impossible to include some of these species without unnecessarily expanding the original diagnosis of the genus.

In 1927 a paper (27) was read by P. M. JONES on the behaviour of *P. brassicæ* in culture. This account was given at the Science meeting at Philadelphia, where it met with strong opposition at the hands of a number of American pathologists.* The author studied the behaviour of the amœbæ of *P. brassicæ* in various culture media, and under these conditions was able to study the complete life-history of the organism. The most important result of the investigation on *P. brassicæ* lay in the discovery of a conjugation process. Most workers since the time of PROWAZEK are agreed that there is a reduction division in the formation of the spores, but none is certain of the position of the conjugation. If the formation of a zygote could be artificially induced in a

* In a letter from Prof. Charles Chupp.

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culture, it seemed reasonable to hope that a similar stage could be found in the organism when living in the tissues of its natural host.

2. *Methods used in the Preparation of Material for examination.*

The material used for this investigation originated in a number of different ways. Some of the slides have been in the possession of the authors for many years. They were stained with Heidenhain's hæmatoxylin after being mordanted with iron alum, and despite the fact that a few are over twenty years old, they still show the cytological structures in perfect detail. The majority of the slides, however, were freshly prepared from material fixed in Bouin's fluid. With early infections in young roots, complete sections of the root, cut transversely into pieces about $\frac{1}{2}$ cm. long, were used. In instances where the hypertrophy was greater and the disease more advanced, small cubes about 0·4 cm. in length were cut out. All the material was embedded after fixation and stored in paraffin wax.

For making slides the paraffin blocks were microtomed at a thickness of from 3–5μ. Both iron alum with Heidenhain's hæmatoxylin and Flemming's triple stain methods were employed. The latter, however, was found to be more difficult to manipulate where large numbers of slides were involved, and the results obtained were in no way superior to those of hæmatoxylin, except in slides showing the Akaryote stage and in the meiotic divisions before spore formation. In this stage, and particularly in the metaphase of the first division, it was more possible to distinguish individual chromosomes in slides stained with safranin than in those in which hæmatoxylin had been employed.

The infection stages were worked out on very young cabbage seedlings. Seed was sown in small pots full of heavily-infected soil, and the young seedlings were kept rather abnormally moist. The roots of these seedlings were examined daily, and as soon as any of the root hairs appeared to have some infection in them the complete root was cut off from the hypocotyl and fixed in Bouin's fluid. The first infection appeared when the seedlings were about an inch high and when the cotyledons were just commencing to open out. A number of seedlings were fixed in this way daily. Great care was needed in dehydrating the delicate root hairs without causing shrinkage, and it was found necessary to grade them through an alcohol series, each of which was only $2\frac{1}{2}$ per cent. stronger than the previous solution. After allowing the roots about an hour in absolute alcohol they were placed in a long tube containing half cedar-wood oil and half absolute alcohol, and the roots were then allowed to sink slowly into the cedar oil under their own weight. The upper layers of alcohol were then pipetted off, and the roots, after washing in fresh cedar oil, were mounted in Canada balsam. Roots prepared in this way were used for all the infection stages and showed complete unshrunken root-hairs in which the young amœbæ could be easily made out.

The photographs reproduced in Plates 19–21 were taken with a special Zeiss photographic camera, under a 1/12″ oil-immersion objective, giving an initial magnification

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on the plate of 550 diameters. The drawings were made at table level, with a Zeiss 1/12″ oil-immersion objective and compensating ocular × 20 or × 30 diameters. These gave magnifications on the paper of 3,200 and 4,500 diameters respectively. With these very high powers the use of suitable light filters materially assisted in obtaining the maximum amount of detail. In photography panchromatic plates were always used, and as a rule either a direct green filter, tricolour green, or a combination of orange and blue. Wratten filters Nos. 25 and 78 together were also successfully used.

Although the photographs so obtained were of great interest, they are but a poor representation of what was actually present under the microscope. Instinctively when examining an object under high power one moves the fine adjustment. With a photograph this is not possible ; one can only photograph in one single focus, consequently only a small number of nuclei in an amœba will be sharply in focus at any one time, and therefore photographs cannot in themselves bring out the whole of the detail present.

3. *The Life-history of Plasmodiophora brassicæ.*

The general life-history of *P. brassicæ* has been described by various authors, and for the most part they are in agreement as to the essential facts. The life-history may conveniently be divided into three stages : (*a*) the infection of the host by the parasite, (*b*) the growth of the organism at the expense of the host, and the resultant hypertrophy of the invaded tissues, (*c*) the multiplication of the fungus by the production of masses of spores within the diseased cells.

(*a*) *The growth of the fungus within the host tissues.*—If an examination be made from sections cut from a very young diseased root, numerous uninucleated bodies will be found lying in the cortical tissues. These bodies represent the first easily recognisable stage in the disease of the host plant. As yet, the cells which have been invaded exhibit little or no ill-effects from the parasite. The fungal elements consist of small amœbæ which are endowed with the power of slow, sluggish movements, motion being effected in a typically amœboid way by means of pseudopodia. These amœbæ have the power of penetrating the walls of the host cells, and in this way can travel through the cortical tissue of the host. Many of them remain, however, in the outer layers of the cortex, but a few migrate to the cells of the cambium, and by virtue of the meristematic nature of this tissue are enabled to infect those cells which are cut off by the cambium as they are formed. It is found that the medullary rays are the structures most favoured for attack, the xylem being but seldom, if ever, attacked.

Soon after the amœba has become established in a suitable cell for development the amœboid movement stops, and it begins to grow, at the expense of the food reserves in the cell. In the early stages of the infection starch grains are frequently found in those cells containing the fungus, but in cells containing full-grown plasmodia these are seldom, if ever, present, and it is concluded that they are broken down by the fungus

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for food. As the amœba enlarges, the nucleus divides. This division is not a normal mitosis, but a special primitive form, generally known as protomitosis. Various other names have been given to it; it is frequently referred to as "the Cruciform division" on account of the appearance of the chromatin mass at this particular stage. The details of this type of nuclear division will be considered later.

In their early stages separate amœbæ have the power of combining together to form large plasmodia. This coalescence of separate individuals is in no way a conjugation, but a simple joining up of separate protoplasts in a way similar to that which regularly occurs in the Mycetozoa during the formation of a plasmodium. With increase in size of the vegetative soma, the daughter nuclei divide again by protomitosis. All the nuclei divide simultaneously, whether they are all the product of the one nucleus of the uninucleate amœba, or the nuclei derived from the aggregation of several distinct individuals. In the association of separate amœbæ to form what may be termed a plasmodium, they lose their individuality and continue to develop as a single organism. This joining up of separate amœba within the host cell, however, does not always occur. In time these may become enveloped in each other by mutual growth, but in such instances no fusion of the protoplasts occur; each retains its individuality. This fact may be clearly seen by the nuclei of the two organisms dividing separately, and not simultaneously, as is characteristic of all the nuclei of a single plasmodium.

The size of the mature plasmodium depends partly upon the quantity of available food, and partly upon the size of the infected host cell. Large plasmodia, having lost their power of movement, are unable to pass from cell to cell of the host. As a general rule the plasmodium, before spore formation, almost completely fills the host cell. In some cases two or more smaller plasmodia are found lying within the same cell. It is possible to distinguish these by the absence of co-ordination in the stages of nuclear division, since the nuclei of two distinct plasmodia do not necessarily divide at the same time. Sometimes there appears to be an almost complete coalescence of the protoplasts of distinct large plasmodia, but in such cases a definite line can be seen between the two which gives the impression of a thin wall bounding the outer limits of the protoplasm. This wall is not always to be observed, and its occasional presence has suggested that where it is present the plasmodia were in a resting, and possibly in an encysted, condition, as in such instances the nuclei have never been observed in active division.

The final stage of the growth of the vegetative soma is marked by a change in the appearance of the nuclei. When the amœba is mature, the karyosomes disappear, and the nuclei appear as vacuoles lying in the cytoplasm. It is, however, still possible to make out the nuclear membrane at this stage. The chromatin thus lost from the nuclei is recognizable as darkly staining granules lying in the cytoplasm. For this reason the amœbæ during this condition have a generally darker and denser appearance than those which have not yet reached full maturity.

(*b*) *Multiplication by the formation of spores.*—After the loss of chromatin at the end of the growth of the plasmodium, the nuclei again become supplied with fresh

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stainable chromatin, and then proceed to divide. Each nucleus divides twice, but the divisions are mitotic, distinct chromosomes being differentiated. Although an estimation of the chromosome number is impossible, by comparison of the sizes of the metaphase plate in the first and second division it is considered evident that there is a reduction division, and that the first represents the heterotypic, and the second the homotypic, phase of such a meiosis. At the close of the second of these two divisions of the nucleus, the protoplasm of the plasmodium becomes furrowed and the whole organism is thus divided into small fragments, each consisting of a nucleus and a small quantity of cytoplasm in which it is embedded. Each of these masses constitutes a spore. At first the spore is naked, but very soon a wall is laid down around it. The spore wall is thin and shows no characteristic marking.

The spores vary considerably in size ; this variation may be more marked in different host plants than in the individual spores derived from a single plasmodium, though even within the same spore mass there is frequently a distinct difference in size. The actual sizes vary from 2 μ to 3 μ in diameter, though oval spores were occasionally measured up to 4·6 μ × 6·0 μ. All the spores of the same plasmodium were found to agree closely in size and shape. This variability may be due to differences in the amount of available food obtainable from the host. The variation in spore size has been noted by previous investigators and has led to some confusion, and may in part be responsible for the splitting of *Plasmodiophora brassicæ* into several species which are in all probability only host varieties. WORONIN in 1878 gave 1·6 μ as the average spore size. MOLLIARD (44) in 1909 found the spores 1·8 μ—2·2 μ, while CHUPP in 1917 found 3·3 μ to be the average size, although he recorded individual mature spores up to 4·3 μ.

The mature spores are spherical, with a smooth wall, which is neither very thick, nor shows any characteristic markings upon it. The spores are not arranged in any particular system, but lie scattered, or frequently closely packed together, in the host cells. The whole spore mass is not enclosed in a common membrane, as is found in the genus *Sorosphæra*. The nuclei of these mature spores, when they can be observed through the deeply staining wall, do not exhibit any features which differ from those found in the nuclei during the differentiation of the spore coat, and it is considered that they remain in a resting condition until the germination of the spore takes place.

The mature spores remain within the host tissues until these decay, and they can retain their vitality for several years. Eventually, however, the host cell decays, and the cell wall is ruptured. The spores in this way become scattered in the soil. It is improbable that the dissemination of the fungus through the soil is effected after the germination of the spores. CHUPP has shown that if healthy seedlings were planted in boxes in which decaying roots infected with *Plasmodiophora brassicæ* were buried, seedlings only five inches away from the source of infection remained healthy almost to maturity. This result agrees with our own observations, that seedlings must be planted in close association with the disease for infection to take place. CARRUTHERS and others

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have suggested that wind may be an important factor in distributing the spores. This is probably true in light, dry, loose soils which are subjected to strong winds. The chief method, however, is undoubtedly by actual transference of the infected soil, particularly in damp weather, by means of the boots of labourers, the wheels of carts passing from field to field, and the tools used in cultivating the soil. In this country far too little attention is paid to this method of distributing disease in the case of diseases which pass the winter in the land. In some instances small clods of soil picked up in this way also become exposed to sun and wind and may then be blown over the surrounding fields and the spores of *Plasmodiophora brassicæ* distributed to even greater distances.

Although the spore coats are relatively thin, they are able to resist desiccation to a great extent. To test this, some diseased roots of cabbages were dried and stored in a tin box until the whole mass of roots and spores became powdery. After about nine months some of this mixture was introduced into a pot of sterilized soil free from *Plasmodiophora brassicæ* in which some cabbage seed had been sown. Within fourteen days these seedlings showed signs of having become infected by *Plasmodiophora brassicæ*.

All workers are agreed that it is very difficult to induce the spores of *Plasmodiophora brassicæ* to germinate under artificial conditions. The authors of the present paper have experienced similar difficulties. Various solutions have been tried, but the most satisfactory results have been obtained with Knop solution and with 1 per cent. and 2 per cent. glucose. In these three solutions swarm spores have been found, though the actual emergence of the amœba from the spore has not been observed. It is, therefore, not possible to say definitely that the amœboid bodies met with in the solution have originated from the germination of the spores of *Plasmodiophora brassicæ*. These amœboid bodies appear from 36 to 48 hours after the spores have been introduced into the solutions, which were then kept at 24° C. in the dark. Since it was impossible to obtain completely pure material of *Plasmodiophora brassicæ* there was always the possibility that they might have been introduced with the spores, although they were never observed till 36 hours after the cultures had been set up.

These structures are small bodies, resembling in general the swarm spores of *Stemonitis* and other Mycetozoa. They are about 3 μ to 3·5 μ in length, and possess a single apical flagellum about the same length as the swarm spore. There is a single nucleus situated at the apical end, which resembles in size and general appearance that present in the mature spore. Similar structures have been found in slides made from very young cabbage seedlings which were being examined for stages of infection. There seems, therefore, little doubt that these organisms really are the swarm spores of *P. brassicæ*.

Eycleshymer (14) found that if swarm spores were left in culture for several days they apparently fused into larger bodies. A similar feature has been observed by Kunkel (29) in *Spongospora*. These observations are not in accordance with what we

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have found in *P. brassicæ*, nor with those we have made previously in *Sorosphæra veronicæ* (3), *S. radicale* (12), or *Ligniera junci* (7) (8) (9). In our cultures of the spores of *P. brassicæ* no further development of the swarm spores was observed, and after a culture had been kept for four or five days all the swarm spores were found to be dead.

(*c*) *Infection of a fresh host plant by the swarm spores.*—In order to investigate the very early stages in the infection of Crucifers by the swarm spores of *P. brassicæ*, cabbage seed was sown in pots of heavily-infected soil. These were kept at room temperature under bell-jars, and the seedlings examined daily from the time they appeared above the soil. The first stage observed in living preparations was that small spherical bodies were found lying in the tips of some of the root hairs in seedlings six or seven days old. These times were reckoned from when the seed was sown ; the plants were then about an inch above the soil. These bodies were more or less spherical and they possessed no flagellum, but in the centre of each was a nucleus with a well-marked central karyosome (see Plate 21, fig. 5). The nucleus was, in fact, identical with that found in plasmodia. These bodies had the power of slow amœboid movement, and were able in this way to travel up the root hair. Root hairs were not, as a rule, attacked until they had attained about a third of their mature length. The actual penetration was not seen, but many actively motile swarm spores were found in association with the root hairs.

These uninucleate swarm spores measure from 2·5 μ to 3·5 μ in diameter. That is to say, they are approximately the same size as the spores from which they were derived. For this reason we are definitely of the opinion that no fusion has taken place between the time they emerged from the spores in the soil and their appearance in the root hairs of the first host plant. The young swarm spore now migrates further down the root hair, and there the nucleus divides, and a small plasmodium is formed (see Plate 21, fig. 6). A mature plasmodium may possess up to thirty nuclei at this stage. Usually as a result of this development the root hair swells out, though as a general rule it is the lower half only which becomes enlarged. These plasmodia grow very rapidly, and their life, as judged by the age of the seedlings, does not exceed two or three days.

When mature, each nucleus becomes surrounded by a mass of cytoplasm, and walls are laid down between them, cutting up the plasmodium into a number of separate bodies. CHUPP, among others, has observed these, and considers that they are spores. This view is, however, incorrect. They are, in fact, zoosporangia. The existence of a second method of reproduction by means of zoosporangia was first described by one of the present writers (9) in 1928. In the genus *Ligniera* the zoosporangia represent a second method of reproduction which all plasmodia were potentially able to adopt. In *P. brassicæ* such is not the case, as zoosporangia only develop in the root hairs.

After the separation of the plasmodia into zoosporangia, the nucleus of each divides up to give four, or sometimes six, nuclei, and around each a small mass of protoplasm collects. The wall of the zoosporangium, which is quite thin, collapses, and the zoospores make their way to the exterior. The zoosporangia vary in size from 6 μ to about 6·5 μ in diameter. The zoospores are small, spindle-shaped bodies about 1·5 μ in

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length and 0·5 μ to 0·7 μ in diameter. Owing to their very small size, and the difficulty of seeing them at all in fresh material, observations had to be restricted to stained material, and consequently no information could be obtained as to their method of motility, nor could a flagellum be always made out. In the few instances when one appeared to be present, it was about equal in length to the zoospore. Plate 21, fig. 16, shows a number of zoosporangia, from most of which the zoospores have already escaped, leaving the empty zoosporangia, which are only recognized by their thin walls. Two have not divided up, and will probably die subsequently.

Very critical examination of seedlings from five to ten days old showed that these zoospores had migrated from the root hairs to the epidermal and cortical cells of the root, and had passed down into the root tip. In a few cases slightly larger objects were found, containing two nuclei, while in a few other cells two zoospores were found lying alongside one another. From these observations we are confident that this really represents a fusion stage in the life cycle of *P. brassicæ*. After fusion of the zoospores in the very young cortical cells, the zygote becomes more spherical, and more closely resembles in general appearance the swarm spore just after entering a root hair. It is on account of this, we believe, that the differences between the swarm spores in the root hairs and the conjugated swarm spores in the cells of the root have not been recognized before. Moreover, seedlings more than seven or eight days old do not generally show any stages of infection. Very careful examination of densely stained root hairs may occasionally show the remains of the zoosporangia, but in other respects the only infection observable is the presence of young uninucleated amœbæ in the cortex.

The question as to whether the plasmodia have the power of penetrating cell walls has been raised by many workers previously. We do not believe that they have. In the large number of slides we have examined no clear case of a plasmodium in process of migrating from one cell to another has been found, and we are inclined to think that the multiplication is due rather to a stimulus imparted to the diseased cells to divide, thereby enabling the fungus to infect both daughter cells, than to any power on its part to pass through the cell wall. Very frequently young plasmodia are found in the cells of the cambium, where they are able to infect the new cells as they are cut off.

4. *Cytology.*

The nuclei of *Plasmodiophora brassicæ* are very minute and consequently a study of their divisions was not made by Woronin. Nawaschin, in 1893, working with material sent to him by Woronin, first described the remarkable process gone through each time a nucleus divided. His first paper was published in 1899 (45) and gives a very detailed account, illustrated by very accurate figures, of the two types of nuclear division as he observed them in his material. He distinguished between chromatin and karyosome, and was able, with the aid of the improved technique then available, to stain

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them differently, using safranin and Gentian violet. These differences are shown in the coloured plate which illustrates his paper.

During the whole of the growth of the plasmodium from the zygote condition all the nuclei divide by a simple method, which is generally termed protomitosis. In the divisions which accompany spore formation there is a meiosis or reduction division, and in the development of the zoospores in the root hairs the nuclei also divide by mitosis. It would appear, therefore, that a protomitotic nuclear division is invariably associated with a diploid nucleus. Whether there be any significance in this or not is not known, but intervening between the two methods there is a stage, generally termed the Akaryote, in which the greater part of the nuclear content is extruded into the cytoplasm.

(*a*) *Protomitosis*.—The resting nucleus, see Plate 19, fig. 1, and Plate 20, figs. 1–3, of the amœba is easily recognised; it consists of a central karyosome surrounded by a nuclear vacuole which is bounded externally by a layer of chromatin granules bordering upon the nuclear membrane. The nuclei are usually spherical, from 1 μ to 2 μ in diameter, though a few oval nuclei, measuring up to 4 μ by 2·5 μ, have been observed occasionally. Their size is generally constant for any one plasmodium. The first stage in the nuclear division is the appearance of a thin ring around the karyosome and in close association with it (see Plate 19, fig. 2). This ring gradually increases in size and becomes drawn away from the karyosome. The stage is frequently called the "Saturn Stage," the karyosome being compared with the planet and the chromatin with its ring. Plate 20, figs. 4–6, shows the condition viewed in different planes. Meanwhile, a spindle has developed around the ring in such a way that the latter comes to lie across the equator at right angles to the spindle fibres. Although asters have been described by previous workers on *P. brassicæ*, the present authors have never observed any indications of them. In a few instances (Plate 20, fig. 4) the nuclear membrane becomes drawn out just over the two poles of the spindle, but this condition is by no means constant.

The chromatin ring now splits into two similar rings, and begins to be drawn to the two poles (Plate 19, fig. 3, and Plate 20, fig. 7). As the two halves draw further apart the diameters of the two rings decrease and concurrently become thicker. At the same time the karyosome becomes drawn out, its distal ends keeping pace with the chromatin rings. Such a stage is figured in Plate 20, figs. 8, 9, which shows the drawn-out karyosome with the two chromatin rings, which have also become slightly curved. This stage is sometimes referred to as the Double Anchor or Dumb-bell stage. It is sometimes possible, both at this point and also just prior to the split, to see the complete ring in nuclei which are lying slightly obliquely (Plate 20, fig. 5). As the two rings pass further towards the poles they tend to lose their individuality and become more completely merged into the daughter karyosomes, the whole appearing as spherical chromatic masses connected together by an ever-decreasing thread (Plate 20, figs. 7–10). Finally, the two chromatic masses become separated. The spindle disappears

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and the nuclear membrane, which persists throughout the whole process of division, constricts and closes around the two daughter nuclei (Plate 20, fig. 10). These two nuclei gradually move away from one another and are then ready for a further division.

In young plasmodia it is invariably observed that all the nuclei divide together, and this rule is generally followed even in those containing a large number, though in these latter one may sometimes observe a slight lag in the nuclei at one side, suggesting that the stimulus for division has passed as a wave across the amœba. In every case where apparently only some of the nuclei of a large plasmodium were dividing, careful examination has revealed that two separate plasmodia were involved, which, owing to their size and age, had not coalesced to form a single plasmodium.

The view has been previously put forward that the scarcity of nuclei in division in the plasmodia indicates that the whole process is very rapidly completed. The present investigation has fully substantiated such a conclusion, nuclear divisions being only very rarely found. We were unable to correlate any particular conditions, such as temperature, humidity, time of day or night when the material was fixed, with any special tendency towards nuclear division. Recently GURWITSCH (22) has suggested that "mitogenetic rays" induce mitosis; the theory which he puts forward might easily explain the fact that all the nuclei in a plasmodium divide together, but such an idea is not supported by two associated but distinct plasmodia being found, one with resting nuclei, while in the other the nuclei are dividing.

(*b*) *Akaryote Stage*.—When the supply of food material fails, or when the plasmodium completely fills the host cell, growth ceases, and the organism is stimulated to produce spores. Before this can take place, however, it appears to be necessary for the chromatin in the nuclei to be completely rearranged. Accordingly, the nuclei pass through a special phase called the Akaryote stage. Many investigators only very incompletely describe the changes which occur between protomitosis and meiosis. This is probably due to the very chaotic appearance of the nuclei, which frequently give the appearance of having been imperfectly fixed. A critical study of many different slides, and a comparison of several genera, has convinced us that the series of stages which we are about to describe invariably occur, and that they have a very important significance in the life-history.

The series of changes collectively spoken of as the Akaryote stage consists firstly in the gradual reduction in the size of the karyosome. Plate 19, fig. 4, and Plate 20, figs. 12–14, show this. Occasionally it fragments and two or more minute independent karyosomes can be made out. Finally the karyosome completely disappears and the cytoplasm surrounding such nuclei becomes increasingly filled up with granules of chromatin (see Plate 20, fig. 15). In preparations stained with safranin and Gentian violet there were indications that the extruded chromatin in the cytoplasm consisted not only of that derived from the karyosome but also that normally lying around the nuclear membrane.

It is difficult to decide how completely the nuclear content is extruded into the

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cytoplasm, since the latter becomes so thickly impregnated with chromatin that it renders the observation of the later stages very difficult to follow. OSBORNE (48) considered that the nuclei were completely disintegrated and that subsequently fresh nuclei were formed on fresh sites. We have never been convinced of this in any of our slides of *Plasmodiophora brassicæ*; moreover, the frequent appearance of vacuoles about this stage increases the difficulty; but in all the plasmodia which we have examined in this stage we have been able to distinguish clearly the nuclear membrane in a considerable number of the Akaryote nuclei. We consider, therefore, that the nuclear membrane, together with a small proportion of residual chromatin, does remain throughout the whole of the Akaryote stage.

(*c*) *Meiosis or Reduction Division.*—The stages which follow are often very difficult to observe owing to the great similarity to those prior to the extrusion of the chromatin. Plate 20, fig. 16, shows the first definite stage. The chromatin is arranged in a rather thick thread, scattered along the length of which are several spherical bodies, which may correspond to the nucleoli of higher plants. This stage is considered comparable with synapsis, though it may be slightly modified in function by the previous chromatin extrusion. It is of short duration, since almost immediately the nuclear membrane disappears, the chromatin thread fragments, and the chromosomes arrange themselves in the equator of a spindle. The plate formed in this way is comparable with the typical metaphase in the meiotic division of higher plants. Although it is not possible to count the individual fragments, it is possible to observe that the plate is not homogeneous, but that it is composed of separate entities, chromosomes, whose number is relatively small (see Plate 19, fig. 6, and Plate 20, figs. 17–19. Such a conception is fully confirmed by the polar view of this stage (Plate 20, fig. 20). During the anaphase of the division the two plates separate and are drawn towards the poles of the spindle (Plate 19, fig. 7, and Plate 20, fig. 21). On reaching the poles the two masses of chromatin become completely disorganised (Plate 20, fig. 22), and the spindle disappears. Asters have never been observed attached to the spindle during this or the subsequent division.

It is due to the work of PROWAZEK (53) that a second nuclear division was first demonstrated. NAWASCHIN considered that there was a single nuclear division before spore formation. Since, however, it is quite impossible to count the number of the chromosomes with any accuracy, we have no direct evidence that the first division is a reduction division in which the chromosome number is halved. We have, therefore, to rely upon indirect information. A number of metaphase plates from the first division were measured and their diameters compared with a similar measurement of those of the second. The average diameter of the metaphase plate in the first division was $1 \cdot 6\ \mu$, while that of the second division was $0 \cdot 8\ \mu$. This fact was also clearly brought out by a comparison of Plate 19, fig. 6, with Plate 19, fig. 8. We feel, therefore, justified in maintaining that a meiosis does occur in the nuclear divisions prior to the formation of spores, which have, therefore, the haploid chromosome number.

The stages in the second division are essentially similar to those of the first division,

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except that, owing to the smaller size, the details cannot be made out so well. The prophase cannot be satisfactorily demonstrated in the majority of our preparations, though in some instances we have observed a loosening up of the chromatin which has passed the telophase of the previous division. The metaphase is always very clear (Plate 19, fig. 8, and Plate 20, figs. 23, 24), the chromatin appearing as a thin rod lying across the equator of the spindle. It generally gives the appearance of being beaded, though this is difficult to observe, except in polar view (Plate 20, fig. 25). The spindle is generally elongated and sometimes curved, though its size and shape vary in different plasmodia. In the anaphase the chromatin is seen as two rods lying across the spindle fibres, and the individuality of the chromosomes appears completely lost (see Plate 19, fig. 9, and Plate 20, figs. 26–28). It is only in polar view that the chromosomes can be observed, and this is particularly clear in the metaphase (see Plate 20, fig. 25), but sometimes it can be made out in the anaphase as well. Finally, the two masses of chromatin migrate to the poles and the nuclear membrane reappears. The spindle may persist for a short while, but finally it becomes absorbed into the cytoplasm.

As soon as the daughter nuclei have become constituted the cytoplasm becomes cut up into areas by vacuolation and it is seen to lie around the nuclei. In the vacuolar areas between the cytoplasmic masses the walls are formed, dividing the whole plasmodium up into spores. The spores may vary considerably in size, but before the structure of the spore wall becomes opaque it is possible to see that the chromatin of the nuclei has loosened, and structures which may be nucleoli have made their appearance. The wall of the spores is thin and smooth, without any characteristic markings, and is similar to that found in the genus *Ligniera*. In general character of the spore walls the Plasmodiophorales resemble the Mycetozoa. In the latter, however, characteristic markings are frequently present. The appearance of the spores of *Plasmodiophora brassicæ* is shown in Plate 19, figs. 10–11, and in Plate 21, figs. 1–3.

The spore wall shows considerable avidity for stains and this consequently renders it very difficult to study the nuclei within the fully-matured spores. Plate 20, fig. 29, shows the appearance of the nuclei shortly after the walls have been laid down. Careful observations made on these nuclei have convinced us that no further change occurs.

As has already been mentioned, no observations could be made upon the actual process of the germination of the spore, but swarm spores have been observed in culture solutions and their nuclei agree both in size and structure with those found in the spores. It is concluded, therefore, that no nuclear division takes place between the formation of the spores and their germination into a uniflagellated swarm spore. These swarm spores vary somewhat in size, but they are quite distinct from those developed subsequently in the root hairs, and for this reason we prefer to use the name swarm spore rather than zoospore, retaining the latter term for the products of the zoosporangia. Plate 21, fig. 4, shows one of the swarm spores as they appear in culture solution.

(*d*) *Mitosis during the Formation of Zoospores.*—The nuclear divisions in the formation of the zoospores within the zoosporangium have been carefully examined. The resting

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nucleus resembles in all essential features the resting nucleus of the plasmodium, except that it is considerably smaller. Plate 21, fig. 7, shows the nucleus of the zoosporangium in the prophase of its first division. The chromatin is seen lying around the nuclear membrane, and there is a definite karyosome which is separated from the chromatin by a nuclear vacuole. Plate 21, fig. 9, represents the metaphase of this division; the chromatin is arranged in a mass lying across the equator of the spindle. It is almost impossible to make out more than an indication of beading, but we are convinced that separate chromosomes, whose number is small, are actually formed. The spindle is rather short and the fibres radiate out from it in such a way as to make the breadth almost as great as the length. No indication of either centrosomes or asters was observed. The chromatin mass now splits into two groups (Plate 21, fig. 10), which migrate to the poles. When they reach them each mass becomes rounded up to form the chromatin of the two daughter nuclei (see Plate 21, figs. 12, 13).

The nuclear division is similar to that of the second division prior to spore formation and differs completely from that of protomitosis. In the first place, both the karyosome and the nuclear membrane disappear, and there is no indication of the chromatin being arranged in a ring. It differs from the division in spore formation in the size of the component parts. It is uncertain how many times the original nucleus of the zoosporangium divides, but since generally either four or eight zoospores are produced (see Plate 19, figs. 13–15, and Plate 21, figs. 8, 14, 15), it is obvious that there must be several divisions. As far as our observations go, all these divisions of the nuclei are similar in character, but the nuclei become progressively smaller.

The nucleus of the zoospore does not show any characteristic structure (Plate 21, fig. 17); there is a clearly-marked nuclear membrane, but it is difficult to distinguish any karyosome in our preparations. The whole of the nuclear content stains uniformly and appears more or less granular. It has not been found possible to observe the fusion of the two nuclei in the production of the zygote, but it is not until after this has occurred that the typical nucleus of the amœba is seen, and that the karyosome becomes clearly differentiated as a central structure, separated from the peripheral chromatin by a nuclear vacuole.

5. *Relation of the Parasite to the Host tissue.*

It is not intended in this paper to do more than touch briefly on this aspect of the disease. In an excellent paper, published in 1918, Kunkel (30) gives an account of the effect of *P. brassicæ* on the cabbage plant. During the early stages of infection the fungus does not produce any marked influence upon the host tissue; it appears mostly in the cortex, where it induces increase in the size of the cells. Either as a result of this increase in size, or as a direct response to the fungus, the parasitised cells are stimulated to divide; frequently in this process the organism is found to have become enclosed in each of the divided cells, then the division is repeated, and in a short time a small meristematic area of tissue heavily diseased by the fungus is formed.

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The immediate external result of this activity depends upon where the meristematic zone is situated. If it is in the outer layers of the cortex a small gall will develop at once.

In the majority of cases it is the medullary ray tissue which becomes most seriously attacked. The fungus spreads originally from the cortex into the cambial cells, often near the protoxylem; from here it travels down into the deeper layers of the medullary ray. Here fresh activity is set up which promotes growth usually at right angles to the normal division of medullary ray cells, resulting in the widening out of the whole ray. As this process continues, the original primary xylem becomes broken down and the secondary xylem much dissipated, so that in slightly older roots the xylem vessels appear as small isolated areas surrounded by diseased tissues.

P. brassicæ, in our experience, never attacks the xylem vessels, though the cambial cells adjoining them may be diseased. Kunkel states that the plasmodia have the power of penetrating the cell wall and of travelling from cell to cell in the root of the host. This we consider to be improbable, and careful examination of our slides has failed to show any indications at all of plasmodia either in process of migration, or any place through which such an infection could have occurred.

6. *Relationship between Bacteria and P. brassicæ.*

The question of bacteria playing an important part in the development of certain Mycetozoa was first discussed by Pinoy, in a series of papers (49) (50) (51) (52) published between 1902 and 1915. It was shown by Lister in 1890 (31) that the swarm spores ingest bacteria, and his observations have recently been expanded by Gilbert (18) (19) (20) (21), who found that not only bacteria, but other organisms and fungal spores, provided they were small enough, could be eaten. Chupp (6) carried out further experiments on the lines of Pinoy's work but came to the conclusion that bacteria were not necessary for the nutrition of the parasite, since he found that they do not enter the host with the swarm spores of *P. brassicæ*, but follow later after the tissue has been ruptured. Maire and Tison (33) stated that bacteria could be ingested by members of the Plasmodiophorales, and Kunkel (29) found that cultures of *Spongospora subterranea* grown on agar died in the absence of bacteria.

Our own observations on the influence of bacteria have been restricted to the early infection stages, where we have found that bacteria are usually associated with the swarm spores of *P. brassicæ*. It seems quite likely that the bacteria assist, or in some cases precede, the penetration of the root hairs by the fungus. We have observed several root hairs which were so far free from swarm spores, but full of active bacteria. These have also generally been observed in root hairs containing very young uninucleate amœbæ. With the growth of the amœba they disappear, and it seems likely that they are ingested by the growing amœbæ. Whether they also serve as food for the plasmodia growing in the tissues of the root is uncertain, though occasionally their presence has been observed, but they were only found in large roots already heavily

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hypertrophied. This agrees with CHUPP's observations, that he was unable to find bacteria in sections of younger roots, though he observed them in older ones.

It seems probable that *P. brassicæ* is not dependent upon bacteria at any stage in its life-history, but when they are present the plasmodium possesses the power to ingest them, whether they appear in the young amœboid stage in the root hairs or during the development of the plasmodium in the roots.

Bacteria have also been considered to play an important part in the germination of the spores of Mycetozoa by PINOY (49) (50) (51) (52), but his conclusions are opposed by both SKUPIENSKI (57) and WILSON and CADMAN (63). Although bacteria invariably appear in cultures of spores which take many hours to develop, we never found (11) any indication that the rate or percentage germination was affected by their presence, and as WILSON and CADMAN point out, in species such as *Reticularia Lycoperdon* (which may germinate in thirty minutes in water) it is inconceivable that bacteria can play any part in the process.

7. *Discussion.*

The life-history of *P. brassicæ* up to spore formation agrees essentially with what has been found in all the other species. The growth of the plasmodia is similar. The method of migration from cell to cell in the host has already been discussed, and it has been shown to be controlled, at any rate partly, by attacking meristematic tissue, or in some instances possibly exciting the cells in which it is present to become meristematic. The direct result of this is rapid proliferation of plasmodia in certain tissues, such, for example, as the medullary rays of the roots, or in the outer layers of the cortex. The widening of the medullary rays, or the cortex, as the case may be, being induced by great increase in cell division brought about by the presence of the fungus and by the enlargement of individual cells. In general, the same hypertrophy occurs in other genera, and the process by which it is brought about is similar in *P. brassicæ* to that in *Sorosphæra veronicæ* and *Spongospora subterranea*.

The stages in protomitosis in *P. brassicæ* most closely resemble those of *Sorosphæra veronicæ*. The ring is generally thicker than in *Ligniera junci*, but similar to *Spongospora subterranea*. The stages during the division are comparable with *Sorosphæra*, a more typical "dumb-bell" being formed than is generally found in the other two genera. The resting nucleus, after careful staining with safranin and gentian violet, reveals a considerable mass of chromatin lying around the nuclear membrane. This is not usually found either in *Spongospora* or *Sorosphæra*, although we have it illustrated in *Ligniera*. It would seem likely that there is generally more chromatin in the resting nucleus of *P. brassicæ* than in any of the other genera.

The significance and nature of the Akaryote stage has been discussed previously (9). In *P. brassicæ* there is no doubt at all that the nuclear membrane persists throughout, and that the chromatin alone passes into the cytoplasm. There is no marked difference in the process in any of the genera we have studied, and we have been unable to find

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stages in slides of potato tubers showing the complete disappearance of the nuclei, such as OSBORNE has suggested. In both *Spongospora subterranea* and *P. brassicæ*, however, great vacuolation of the cytoplasm frequently occurs about this time, which might be mistaken for an indication of nuclear disintegration.

We have also found in certain instances a tendency for the cytoplasm, prior to the formation of spores, to become delimited by dark-staining membranes, which cut up the plasmodium into cysts. These cysts are large, and usually not more than three or four are found in any one plasmodium. These cysts, which are shown in Plate 19, fig. 5, and Plate 20, fig. 11, are all multinucleate; they do not appear to indicate a special stage in the life-history, but merely serve as a protection to the plasmodium in unfavourable conditions for growth. After a short while, without any changes taking place in the nuclei, these cysts disappear and the normal life cycle is resumed.

The nuclear divisions prior to the formation of the spores follow exactly the same sequence of events as has been described in the other genera. The nuclei are, however, generally smaller than those of either *Ligniera junci* or *Sorosphæra veronicæ*. As a result, there was no opportunity to investigate the chromosome number.

It is in the subsequent fate of these spores that our observations differ from those of previous workers. JONES (27) by cultural methods obtained certain evidence as to the sequence of events, and although he advanced the work to a certain degree, yet we believe that the actual process is even more complicated. According to his observations, the swarm spores liberated from the spores fused in pairs, to produce a zygote, which ultimately developed into a fresh plasmodium. He found that there were two kinds of gametes produced, small ones and large ones, and thought that they both came from the same source. In this respect we believe him to be wrong.

The large swarm spores naturally correspond to the organisms we have observed coming from the spores. They are, as he records, pyriform and uniflagellate. The second kind of gamete, the very small one, we consider to be the product of the zoosporangia which develop in the root hairs. JONES thinks both types are true gametes, and therefore that both are capable of fusion to form zygotes. According to the life cycle which we believe takes place, such a conclusion would entail two fusions, one prior to infection of the root hairs, and another in the subsequent infection of the plant tissues as a whole. If this is true, at some stage in the life cycle two separate reduction divisions would be required.

It has previously been pointed out by MAIRE and TISON (33) (34) (35), OSBORNE (48), WINGE (64), and others (6) (32), that the young organism found in freshly-infected roots was not larger than the swarm spore which emerged from the spore, and this fact presented a grave difficulty against the conclusion that this structure was a zygote derived from the fusion of two swarm spores. We have never been able to satisfy ourselves that the swarm spores did fuse in pairs, though at one time we felt that this could be the only way in which a doubling of the chromosomes could occur. *P. brassicæ* presents a new aspect of the problem.

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We now believe that the swarm spore enters the root hair without fusion, forms a small plasmodium, and then develops into a comparatively small number of zoosporangia. These zoosporangia are larger than the spores, but superficially resemble them. The original nucleus of the zoosporangium divides mitotically and several zoospores are produced, which correspond in all respects to the small gametes described by JONES. We agree with him that these fuse in pairs, though we have not been so fortunate as he has in studying the actual process, having restricted our observations to the plant tissues. The zygote thus formed produces the plasmodia found in the root tissues.

There are several problems which as yet remain unsolved. Firstly, do the zoospores migrate into the host tissues before fusion, or does this take place invariably near the zoosporangium from which they were derived ? Secondly, are the gametes which fuse together always the product of different zoosporangia and different plasmodia ? Thirdly, has the zygote the power of penetrating from cell to cell of the host ? Regarding the first two of these questions, we do not feel we have sufficient evidence to bring forward. As far as the third is concerned, we believe that the gametes only have the faculty for passing from one cell to another ; once a fusion has occurred further migration only takes place as a result of division of the host cell itself. This, we know, frequently occurs, and our observations agree with those of KUNKEL (30) that the distribution of the parasite is closely associated with the development of the medullary rays. We found no evidence to support the view expressed by CHUPP (6) that plasmodia can pass from cell to cell of the host.

The life cycle of *P. brassicæ*, as we have interpreted it, is shown in a diagrammatic sketch in the accompanying figure (p. 302). It is interesting to compare it with that published by JONES (27), since it shows clearly how the results obtained in culture agree with our observations in the host tissues up to the infection of the root hair, and how by the interpolation of a plasmodium stage the life cycle may be simplified. Such a stage might very easily be overlooked in culture work.

8. *Systematic account of the Species included in the Genus Plasmodiophora.*

Since WORONIN described *P. brassicæ* as the type species of the Plasmodiophorales, various more recent workers have referred other organisms causing similar diseases to the same genus. As far as we have been able to discover, eleven species have, at one time or other, been placed in this genus.

Plasmodiophora alni (WORON.) MOELL.

The organism was first described by WORONIN (65) under the name of *Schinzia alni*, and placed in the Hyphomycetes. BRUNCHORST (5) gave it the name *Frankia subtilis*, but later it was placed in the genus *Plasmodiophora* by MOELLER (41) and SCHRÖTER (55). Later, MOELLER (42) (43) retracted his view and agreed with WORONIN. In 1909, MAIRE and TISON (33) re-examined the galls and showed that they consisted of

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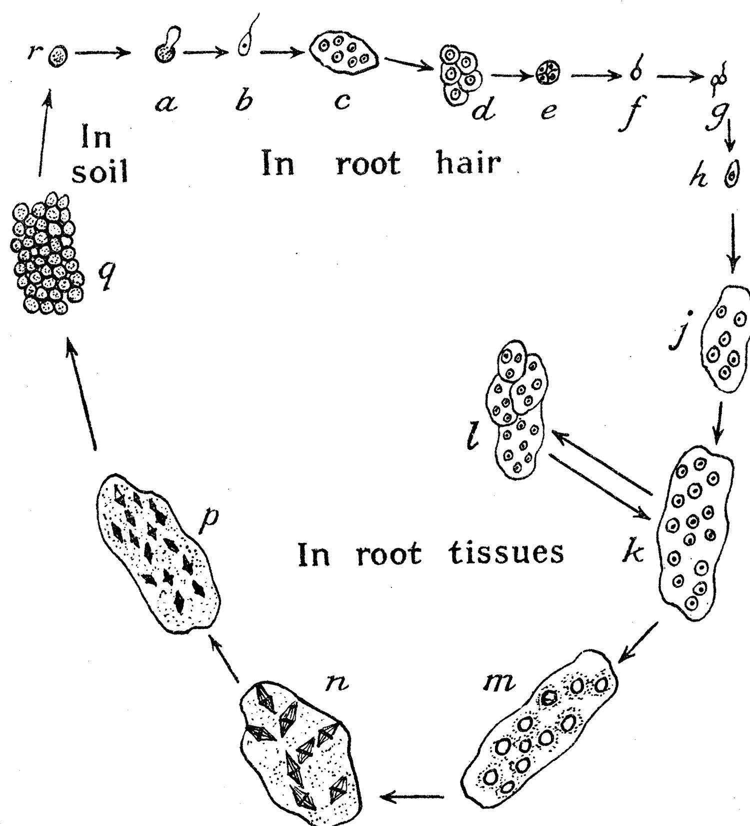

TEXT-FIG. 1.—Diagram illustrating the life cycle of *Plasmodiophora brassicæ*. *a*, germinating spore; *b*, swarm spore; *c*, plasmodium in root hair; *d*, zoosporangia in root hair; *e*, zoosporangium forming zoospores; *f*, zoospore; *g*, conjugation of zoospores; *h*, zygote; *j*, young plasmodium in host tissues; *k*, mature plasmodium; *l*, cyst stage; *m*, akaryote stage; *n*, heterotypic nuclear division; *p*, homotypic nuclear division; *q*, spore formation; *r*, single ripe spore.

very fine hyphæ which passed from cell to cell of the host, and gave it the name *Frankiella alni* (WORON.) MAIRE and TISON.

P. elæagni SCHRÖT.

BRUNCHORST (5) compared the structure of the tubercles on *Elæagnus* with those on the Alder and considered them alike. MAIRE and TISON examined similar galls on *Hippophae rhamnoides* and considered it another species of their genus *Frankiella*, calling it *F. elæagni* (SCHRÖT.) MAIRE and TISON.

P. orchidis MASSEE.

MASSEE (36) first thought that the common "spot" disease of the leaves of orchids was due to this fungus. Subsequently (37) he retracted this view and stated that the "spot" disease was a purely physiological effect.

P. humuli KIRK.

The roots of hop plants in New Zealand were found to develop galls similar to those caused by *Plasmodiophora brassicæ*, and KIRK (28) concluded that they were caused

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by another species, which he named *P. humuli*. NICHOLLS (47) observed similar swellings on hops in Tasmania, and concluded that *P. humuli* was responsible. STEVENS (58) refers to another species, *P. humili* KIRK, which appears to be a printer's error for *P. humuli* KIRK.

P. halophilæ FERDINANDSEN and WINGE.

The species was found by C. H. OSTENFELD attacking the petioles of *Halophila ovalis*. FERDINANDSEN and WINGE (16) thought that sections of the leaves showed an organism having the morphological characteristics of the genus *Plasmodiophora*.

P. californica VIALA and SAUVAGEAU.

The organism was described by VIALA and SAUVAGEAU (61) as a disease of the vine in California. MASSEE (38) showed that the disease was entirely due to physiological effects.

P. vitis VIALA and SAUVAGEAU.

This organism was described by VIALA and SAUVAGEAU (60) as responsible for the "brunissure" disease of the vine in Europe and the United States. MASSEE (38) considers them physiological. It seems likely that the so-called organism is due to the presence of large quantities of tannin, which in a fragmented state might give the impression of spores.

P. tomato ABBEY.

ABBEY (1) compares the attack to that of *P. vitis* and *P. orchidis*, and stated that the causal organism is *P. tomato*, a species which was apparently never described.

P. vascularum MATZ.

The organism was described by MATZ (39) (40) in 1921 as the cause of a sugar cane disease in Porto Rico. M. T. COOK re-examined the fungus in 1929 (13) and placed it in the genus *Ligniera* under the name *L. vascularum* (MATZ) COOK.

P. tabaci JONES.

This organism was tentatively named by JONES (25) (26) as responsible for the leaf mosaic of tobacco plants. The fungus differs from other Plasmodiophorales, as we have shown elsewhere (7).

P. fici-repentis ANDREUCCI.

This species causes tubercles on the stem of *Ficus repens*. The life-history has been studied by ANDREUCCI (2). It consists of spores, zoospores, amœbæ and fresh spores. The spores are hyaline and nucleate, with a thin enclosing membrane. They give rise to zoospores which are 2·7 μ long, possessing a single flagellum of about the same length. These zoospores give rise to amœbæ, which vary in size from 6 μ to 24 μ, and subsequently fuse together to form plasmodia. The plasmodium may either produce cysts, called macrocysts, which subsequently develop into fresh plasmodia, or they may give rise to fresh spores. No details are given of the structure or division of the nuclei.

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Of the " species " of the genus *Plasmodiophora* described above, both *P. alni* and *P. elæagni* have been conclusively shown to be caused by a hyphal fungus, *Frankiella*, and they therefore can be removed from the genus. *P. orchidis*, *P. californicæ*, and *P. vitis* have been shown by MASSEE and others to be purely physiological effects and not to be caused directly by the presence of a fungus. No description of *P. tomato* has ever been published.

Plasmodiophora humuli KIRK is apparently based upon an appearance of hypertrophy of certain hop roots in New Zealand. No description of the causal organism exists, except a statement of KIRK that it is due to an organism which is similar to *P. brassicæ*. We possess neither figures of the fungus nor a description of either its morphology or cytology. NICHOLLS observed similar swellings on hops in Tasmania, and concluded that they were the same as those recorded by KIRK, but he, too, does not describe any causal organism. A mere statement that hypertrophy of the roots of hops is " caused by a slime-fungus, and belongs to the same genus as the club-root disease," is, in our opinion, insufficient to justify *P. humuli* being recognized as a species of the genus.

P. halophilæ is based upon a statement that the organism has " all the morphological characteristics like *P. brassicæ*." The authors were in the possession of a single gall, which was obtained from a collection of specimens from Java. Very little more than the spores were apparently available, and many of these were shrunken when they were examined.

P. vascularum has been the subject of several papers. COOK in his study of the plasmodium was unable to find any nuclei until the commencement of spore formation. In all the species of the Plasmodiophoraceæ which we have examined we have never experienced the slightest difficulty in demonstrating nuclei in the plasmodia ; they can be recognized even in living material. COOK prefers to place the organism in the genus *Ligniera*, because it does not cause hypertrophy of the host tissues, but in our opinion (based upon the two accounts which we have read) there is very little ground on which to refer it to either genus.

P. tabaci has already been criticised (7) : there are certain points in its life-history which resemble those of *P. brassicæ*, and certainly it offers better grounds for inclusion in the genus than any of those we have so far reviewed. JONES himself, however, appeared doubtful whether his organism should be placed in the Plasmodiophorales, and it seems to us a pity that a definite specific name should have been given to it at that stage in the work.

P. fici-repentis has also been described without a very critical examination, no cytological details being available, nor are any figures given illustrating the species. So far as information is available, it would appear to be allied to *P. brassicæ*, and in view of the fact that it causes hypertrophy of the host cells the genus *Plasmodiophora* would be the correct place to which to refer this new species.

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WORONIN does not give a precise diagnosis of the species which he described. So far as we have been able to discover, the name *Plasmodiophora brassicæ* first appears in his paper (67) in 1877. In the paper in 'Pringsheims Jahrbücher' he speaks as if the diagnosis had already appeared. ZOPF in 1885 (69) diagnosed the genus and the described species from WORONIN'S paper of 1878, and points out that he considers that *P. brassicæ* should be taken as the type species of the genus. SACCARDO (54) subsequently made use of ZOPF'S diagnosis, which he translated into Latin for his 'Sylloge Fungorum.' SCHRÖTER (55) made use of the term Phytomyxinæ in the way in which Plasmodiophoraceæ is now generally employed. In 1909, MAIRE and TISON (33), while recognising the Phytomyxineæ, preferred to consider that the genus *Plasmodiophora* and the other genera which had been recently described should be placed in a separate family, and they recognised Plasmodiophoraceæ in the sense used by ZOPF.

For the purpose of the present paper we have recognised SACCARDO'S diagnoses both for the genus *Plasmodiophora* and for the species *P. brassicæ*. Although the diagnosis is not so complete as our present knowledge would allow, there seems but little advantage to amend it. The following Latin diagnoses are given of the only two species which we believe should be included in the genus *Plasmodiophora*.

GENUS PLASMODIOPHORA WORON. (After SACCARDO.)

Sporæ non quaternæ, liberæ, in soris dispositæ. Plasmodia maturitate in cellulas globosas, in cellulas matricis libere nidulantes, divisa. Sporæ zoosporas germinatione emittentes.

1. **P. brassicæ** WORON. (After SACCARDO.)

Sporis globosis, 1·6 μ diam., tenui-tunicatis, hyalinis, zoosporas germinatione emittentes, zoosporis antice cilio præditis ; plasmodio hyalino, granulis et oleosis instructo tandem in sorum zoosporarum mutato ; tuberculis forma variis, usque ad 10 cm. diam.

Hab.—In radicibus Cruciferarum nonnullarum (Brassica, rarius *Iberide umbellata*) in Germania.

2. **P. fici-repentis** ANDERUCCI.

Sporis globosis, 1·55 μ diam., membrana tenui præditis, hyalinis, nucleatis, liberis, non quaternis, zoosporas germinatione emittentibus ; Zoosporis piriformibus uniciliatis, vacuolo præditis, 2·7 μ long. ; corporibus zoosporarum cilio æquilongis. Amœbis forma variis, a μ 6·1 usque ad μ 24·1 long. Plasmodio ex amœbis aggregatis formato, granulis et guttulis oleosis instructo, primum integro, deinde in partes irregulares diviso, tandem in sporas globosas æquales mutato. Macrocystibus plus minusve rotundis, crassa membrana præditis, plasmodia emittentibus ; quibusdam geminatis unica membrana præditis, a μ 9·15 usque ad μ 42·7 diam. ; omnibus plasmodii prolongamenta germinatione emittentibus. Tuberculis forma variis, rotundis seu coralloideis, usque ad 5 cm. diam.

Hab.—In ramis ramulisve sterilibus *Fici repentis* Senis—In horto.

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9. *Summary.*

1. The life-history of *Plasmodiophora brassicæ* has been studied afresh in the tissues of Cruciferous plants. The results confirm the work of WORONIN, NAWASCHIN, and PROWAZEK in all essential features.

2. The cytology has also been studied and has been found to agree with previous work on this species. Critical comparisons are made between the stages in *P. brassicæ* and those in the other genera where the cytology has been worked out.

3. New information has been obtained regarding the method of infection. It is believed that the swarm spore only enters the root hairs, where each forms a small amœba which gives rise to a zoosporangium. Each zoosporangium produces several zoospores. These zoospores are much smaller in size than the swarm spores. They migrate into the host tissue, fuse in pairs, and give rise to the plasmodia which are found in diseased roots. The amœbæ which produce the zoospores develop very rapidly, and soon disappear as the root hair dies.

4. The requirements of a reduction division in spore formation are satisfied by this fusion of the zoospores. There is no fusion of the swarm spores, which simply transfer the fungus from one root, or one plant, to another.

5. A study of the histological changes which occur in diseased roots was not made, but sufficient work was done to show that the conclusions put forward by KUNKEL could be fully substantiated. The organism was found most commonly in the medullary ray tissue in young roots, where it tended to increase both their size and development.

6. No evidence was obtained that bacteria play any significant part in either the life-history or in the infection by the fungus. Bacteria were sometimes present in the plasmodia, but they appeared to be in process of ingestion rather than exhibiting any symbiotic relationship.

7. A survey is made of all the species which have been placed in the genus *Plasmodiophora*, and reasons are given for excluding most of them, on the grounds either that they have been insufficiently described or that they have subsequently been shown to belong to some other group.

Latin diagnoses of the genus and the recognised species are appended.

10. *Acknowledgments.*

We wish to thank the various mycologists who have assisted us during the progress of the work; in particular, Dr. G. H. PETHYBRIDGE, Dr. L. O. KUNKEL and Dr. C. CHUPP. We are indebted to the Imperial Bureau of Mycology, and to the United States Department of Agriculture, for supplying us with many of the earlier papers on the subject.

Grants from the Dixon Fund of the University of London enabled us to purchase the special microscopic apparatus and incubators used in the work.

Our thanks are also due to Dr. E. J. BUTLER, F.R.S., Mr. J. RAMSBOTTOM, and Prof. R. R. GATES, for much helpful criticism and advice. Mr. C. S. SEMMENS has rendered us much valuable assistance with the photomicrographs.

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12. DESCRIPTION OF PLATES.

The photomicrographs were taken with a Zeiss Seidentopf photographic eyepiece at tube length of 115 mm., using a Zeiss 2 mm. (N.A. 1·4) objective, Plate 19, figs. 1–15. The drawings were made with a camera lucida at table level with tube length 160 mm., and have been reproduced without reduction. Magnifications are given after the descriptions.

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PLATE 19.

FIG. 1. Photomicrograph showing the resting nuclei of the plasmodium. × 1650.

FIG. 2. Photomicrograph showing the "cruciform" stage of protomitosis. × 1650.

FIG. 3. Photomicrograph showing the "dumb-bell" stage of protomitosis. × 1650.

FIG. 4. Photomicrograph of the early akaryote stage showing the nuclei without a karyosome and the dispersal of the chromatin. × 1650.

FIG. 5. Photomicrograph showing the formation of cysts which may sometimes occur before spore formation. × 1650.

FIG. 6. Photomicrograph of the heterotypic metaphase in the division before spore formation; the spindle can be clearly seen. × 1650.

FIG. 7. Photomicrograph of the heterotypic anaphase before spore formation, showing the spindle and the chromosomes appearing as two rings. × 1650.

FIG. 8. Photomicrograph of the homotypic metaphase in the division before spore formation; compare the size of the plate with that of the heterotypic metaphase. × 1650.

FIG. 9. Photomicrograph of the homotypic anaphase prior to spore formation. × 1650.

FIG. 10. Photomicrograph showing the nuclei within the newly-formed spores before the wall has become thickened. × 1650.

FIG. 11. Photomicrograph of a group of fully-developed spores. × 1650.

FIG. 12. Photomicrograph of a swarm spore just after entrance into a root hair; observe the nucleus with a central karyosome. × 1650.

FIG. 13. Photomicrograph showing the formation of the zoosporangia from the plasmodium. × 1650.

FIG. 14. Photomicrograph showing the division of the zoosporangia into zoospores. × 1650.

FIG. 15. Photomicrograph showing several fully-developed zoospores lying inside the zoosporangia. ×1650.

PLATE 20.

FIG. 1. Uninucleate amœbæ lying in the host cells, showing the relative size. × 1500.

FIG. 2. A young plasmodium lying in the host cell. Note the way in which it lies against the wall of the cell. × 1100.

FIG. 3. Four resting nuclei of a larger plasmodium, showing the karyosome and the chromatin lying around the nuclear membrane. × 3200.

FIG. 4. An early stage in protomitotic nuclear division, showing the chromatin in a ring around the karyosome. × 3200.

FIG. 5. An oblique view of a nucleus in the same stage as the last, showing clearly that the chromatin is arranged in a ring. × 3200.

FIG. 6. The "cruciform" stage in protomitotic nuclear division; the nucleus at the top shows the same stage in polar view. × 3200.

FIG. 7. A later stage in protomitotic nuclear division, showing the two daughter masses of chromatin separated and the karyosome becoming drawn out between them. × 3200.

FIG. 8. A still later stage in which the chromatic rings have lost their individuality and are bunching around the karyosomes at the poles of the spindle. × 3200.

FIG. 9. The final stage in protomitosis, only a thin thread of the karyosome still connecting the two together. × 3200.

FIG. 10. The final separation of the two daughter nuclei and the re-formation of the nuclear membranes around both the nuclei. × 3200.

FIG. 11. A drawing of several cysts formed by the division of the plasmodium into separate masses, these subsequently reform into a plasmodium. × 1100.

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Fig. 12. A resting nucleus just prior to the akaryote stage. Note that the karyosome has already become reduced in size. × 3200.

Fig. 13. A later stage in the akaryote condition, in which the karyosomes have begun to fragment prior to disappearing. × 3200.

Fig. 14. The complete disappearance of the karyosomes and the commencement of the diffusion of the chromatin into the cytoplasm. × 3200.

Fig. 15. The final condition, in which all the stainable chromatin has passed into the cytoplasm, leaving only the nuclear membrane with a fragment of chromatin. × 3200.

Fig. 16. The first recognizable stage after the re-formation of the nuclei at the close of the akaryote stage, showing the chromatin as a thread separated here and there by small nucleoli. × 3200.

Fig. 17. Heterotypic metaphase on the formation of spores. Note the beaded character of the chromatin plate as it appears lying across the spindle. × 3200.

Fig. 18. Another view of the same stage, showing the chromosomes slightly more separated. × 3200.

Fig. 19. Another view of the same stage, showing a series of rather smaller nuclei. × 3200.

Fig. 20. Polar view of a nucleus in the heterotypic metaphase; in this view it is possible to see the individuality of the chromosomes. × 3200.

Fig. 21. Heterotypic anaphase, showing the two daughter beaded groups of chromatin migrating to the poles of the spindle. × 3200.

Fig. 22. Heterotypic telophase, showing the re-formation of the chromatin in a dense mass at the poles. × 3200.

Fig. 23. Homotypic metaphase, showing the general appearance of the chromatin as a plate lying across the spindle. Compare the size of the plate with figs. 17–19. × 3200.

Fig. 24. Homotypic metaphase, showing the same stage as in the last figure. × 3200.

Fig. 25. Homotypic metaphase in polar view, showing that the individuality of the chromosomes is still possible to make out. × 3200.

Fig. 26. Homotypic anaphase, showing the separation of the chromatin into two equal bands. × 3200.

Fig. 27. A similar stage to that in the last figure, but of slightly larger nuclei. × 3200.

Fig. 28. A later stage, just prior to the telophase, showing the chromatin masses already becoming collected up into solid groups. × 3200.

Fig. 29. The final stage in the division; the daughter nuclei have re-formed their nuclear membranes and a wall has been formed around the nuclei, cutting the plasmodium into spores. × 3200.

Plate 21.

Fig. 1. A slightly later stage than the last, showing the development of the spore wall. × 3200.

Fig. 2. Some loose spores, showing the nuclei in which the chromatin has formed into a peripheral ring associated with a mass comparable with a nucleolus. × 3200.

Fig. 3. A general view of some spores, showing the smooth wall. × 3200.

Fig. 4. A mature swarm spore, showing its general character after emergence from the spore. Note the single flagellum attached to a small granule lying a little way from the surface. × 3200.

Fig. 5. A root hair, showing a young swarm spore lying within it; the flagellum has already been retracted. × 2500.

Fig. 6. A plasmodium lying within a root hair, showing that the general appearance is similar to what is found in the cells of the root. × 1100.

Fig. 7. A young zoosporangium, showing the resting nucleus lying in the centre. × 4500.

Fig. 8. A general view of a number of zoosporangia formed from a plasmodium in a root hair. × 2500.

Fig. 9. The metaphase of the nuclear division of the zoosporangium, showing clearly by the beading that separate chromosomes are formed. × 4500.

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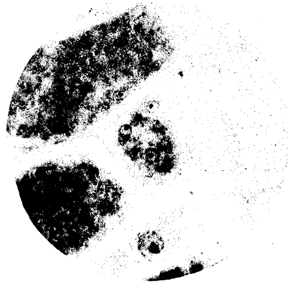
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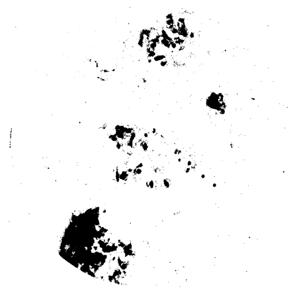
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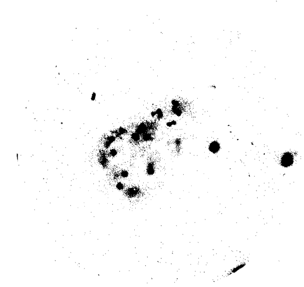
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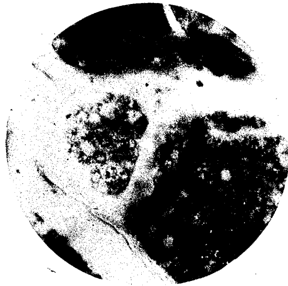
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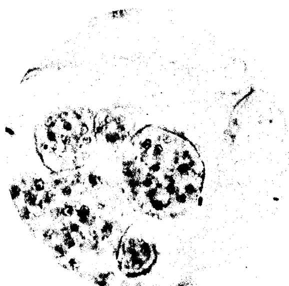
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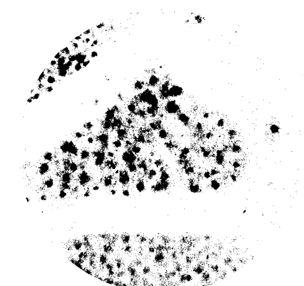
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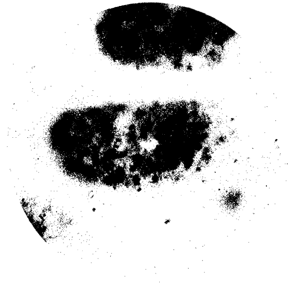
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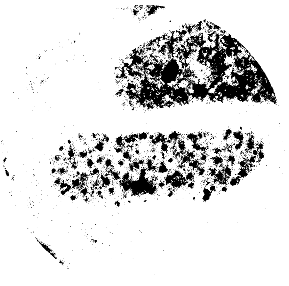
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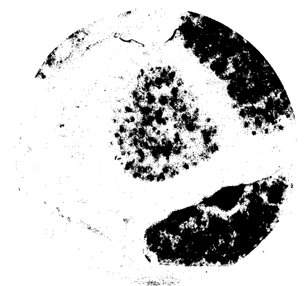
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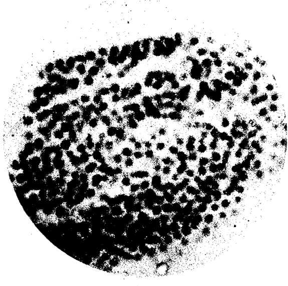
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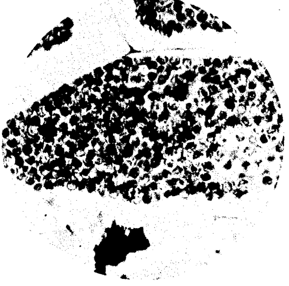
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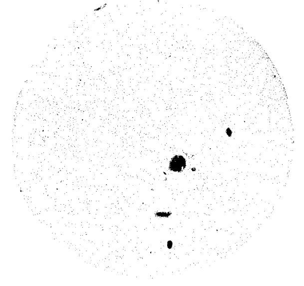
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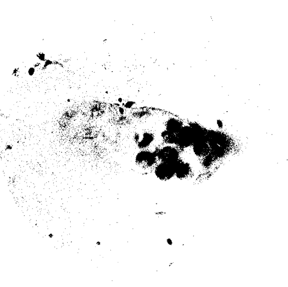
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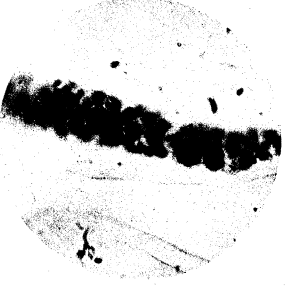
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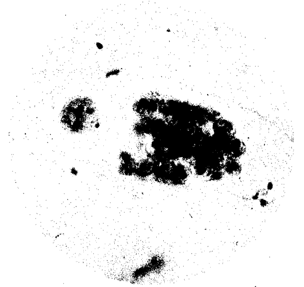
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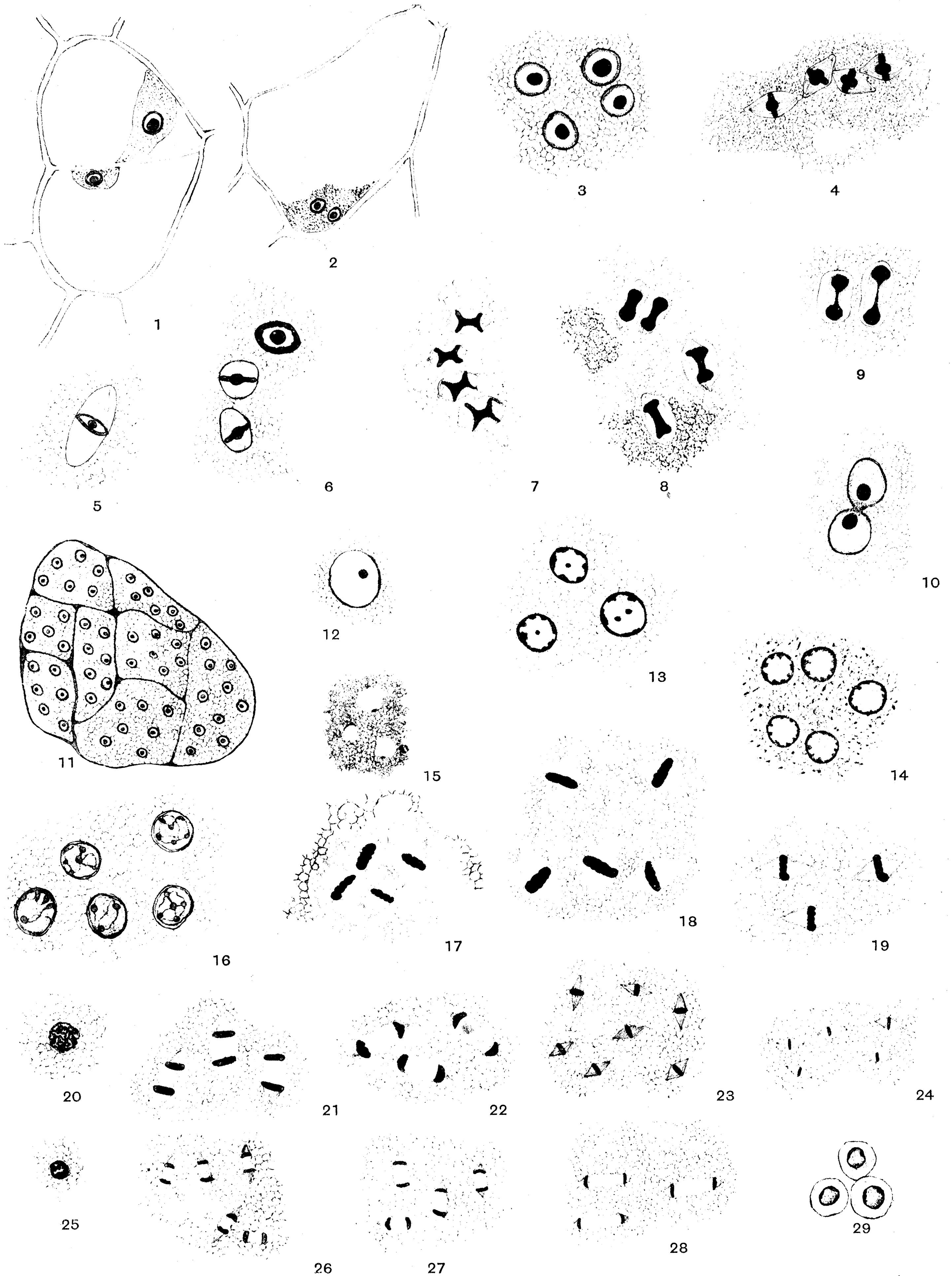

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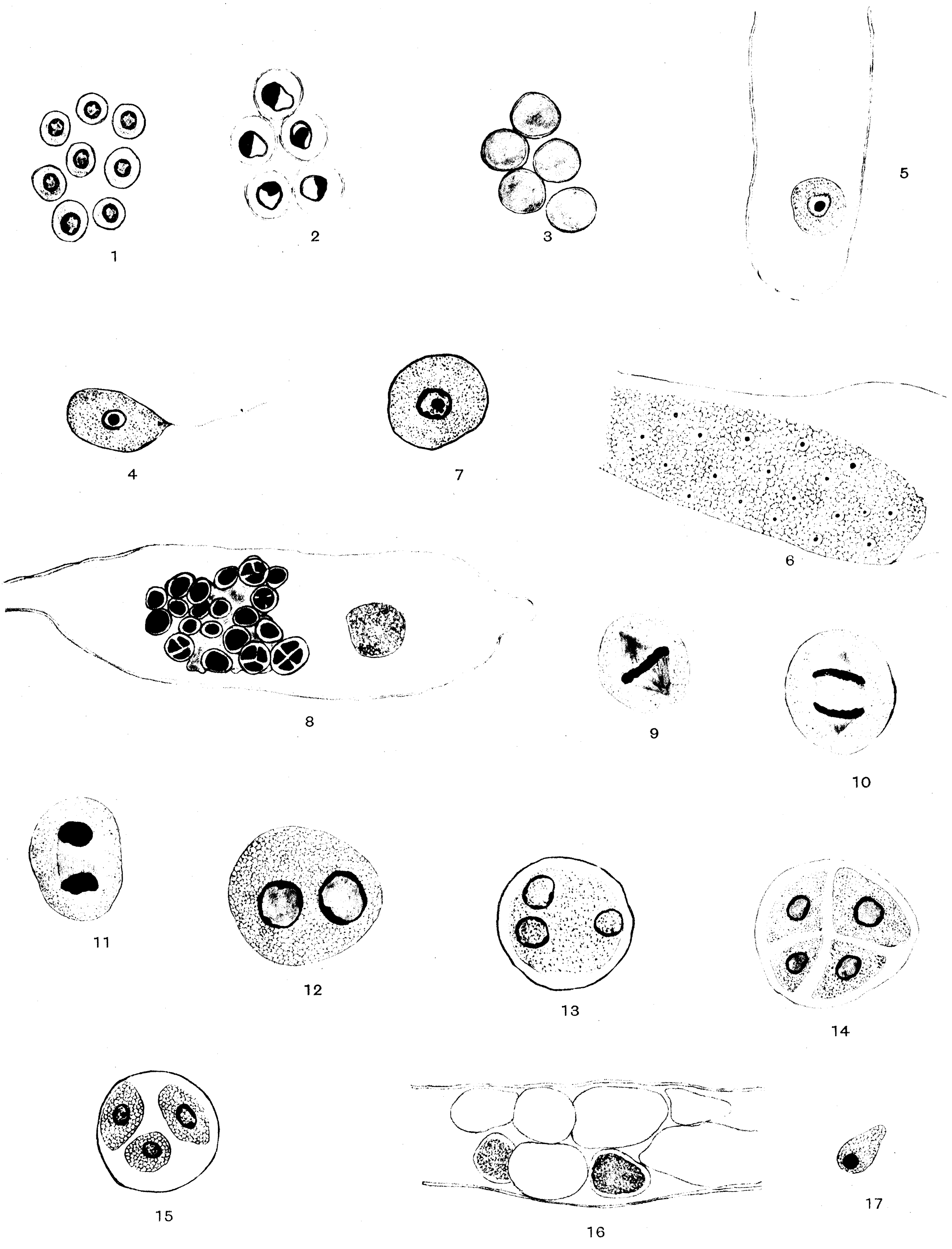

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Fig. 10. The anaphase of the same division, showing the separation of the chromatin into two beaded groups. × 4500.

Fig. 11. The telophase of the same division, showing the re-formation of the chromatin into two groups. × 4500.

Fig. 12. A zoosporangium, showing the two nuclei about to divide again prior to the formation of zoospores. × 4500.

Fig. 13. A zoosporangium with three resting nuclei, just prior to the furrowing of the cytoplasm. × 3200.

Fig. 14. A zoosporangium, showing the cytoplasm cut up into four zoospores, each with a single resting nucleus lying in the centre. × 4500.

Fig. 15. A zoosporangium with three zoospores lying free within the wall. Note that the zoospores have become spindle-shaped. × 3200.

Fig. 16. A group of zoosporangia lying in a root hair, showing some in process of development, while others have already discharged their zoospores. × 2500.

Fig. 17. A mature zoospore prior to fusion. This constituted a gamete, which will fuse with a similar one to form the zygote which infects the host tissues. × 4500.

Postscript—*February*, 1930.

When this paper was in proof, an interesting account by Dr. A. S. Horne of the nuclear division in the Plasmodiophorales appeared (' Annals of Botany,' vol. **44**, p. 199, 1930). Dr. Horne studied *Sorosphæra veronicæ*, *Spongospora subterranea* and *Plasmodiophora brassicæ*. He found in *Spongospora* that the somatic nuclear division was mitotic, and that the chromatin ring was composed of four separate chromosomes. In spore formation four chromosomes appear in the telophase of the heterotypic division. Dr. Horne considered that the homotypic division was equivalent with the somatic division, except that the nucleolus was less conspicuous. In order to satisfy his chromosome counts, he postulated a nuclear fusion during the akaryote stage. His observations on *Sorosphæra veronicæ* and *Plasmodiophora brassicæ* were apparently less fruitful, although they supported those on *Spongospora*.

From a study of the somatic nuclear divisions in *Sorosphæra veronicæ*, *Ligniera junci*, *Plasmodiophora brassicæ* and, to a less extent, in *Sorosphæra radicale*, *Ligniera verrucosa*, *Spongospora subterranea* and *Tetramyxa parasitica*, we have seen no instance in which the chromatin in the " cruciform stage " appeared to be arranged in anything but a uniform ring. As, however, we appreciated some time ago the possibility, we submitted slides of each species to other cytologists, some of whom were familiar with the nuclei of the protozoa, and they all confirmed our observations. We do not believe that any nuclear fusion occurs during the akaryote stage ; we have observed no approximation of nuclei, and the nuclei generally appear smaller rather than larger at the beginning of the reduction division. Further, such a fusion could not occur at this

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point, if our interpretation of the infection stages in *Plasmodiophora brassicæ* is correct, or if KUNKEL's account of infection in *Spongospora subterranea* is true. We have obtained no evidence of a third mitosis following the homotypic division, although we have found it commonly in the zoospore formation in *Ligniera junci*.

Dr. HORNE criticizes our suggestion that the sequence of nuclear stages in the Plasmodiophorales should be made a diagnostic feature of the group. In our opinion, since these phases appear very similar in all those species which have been carefully studied, it is very undesirable that fresh species should be included in this group, unless they show similar nuclear characteristics. We have brought out this point in this paper by a consideration of the invalid species of the genus *Plasmodiophora*.

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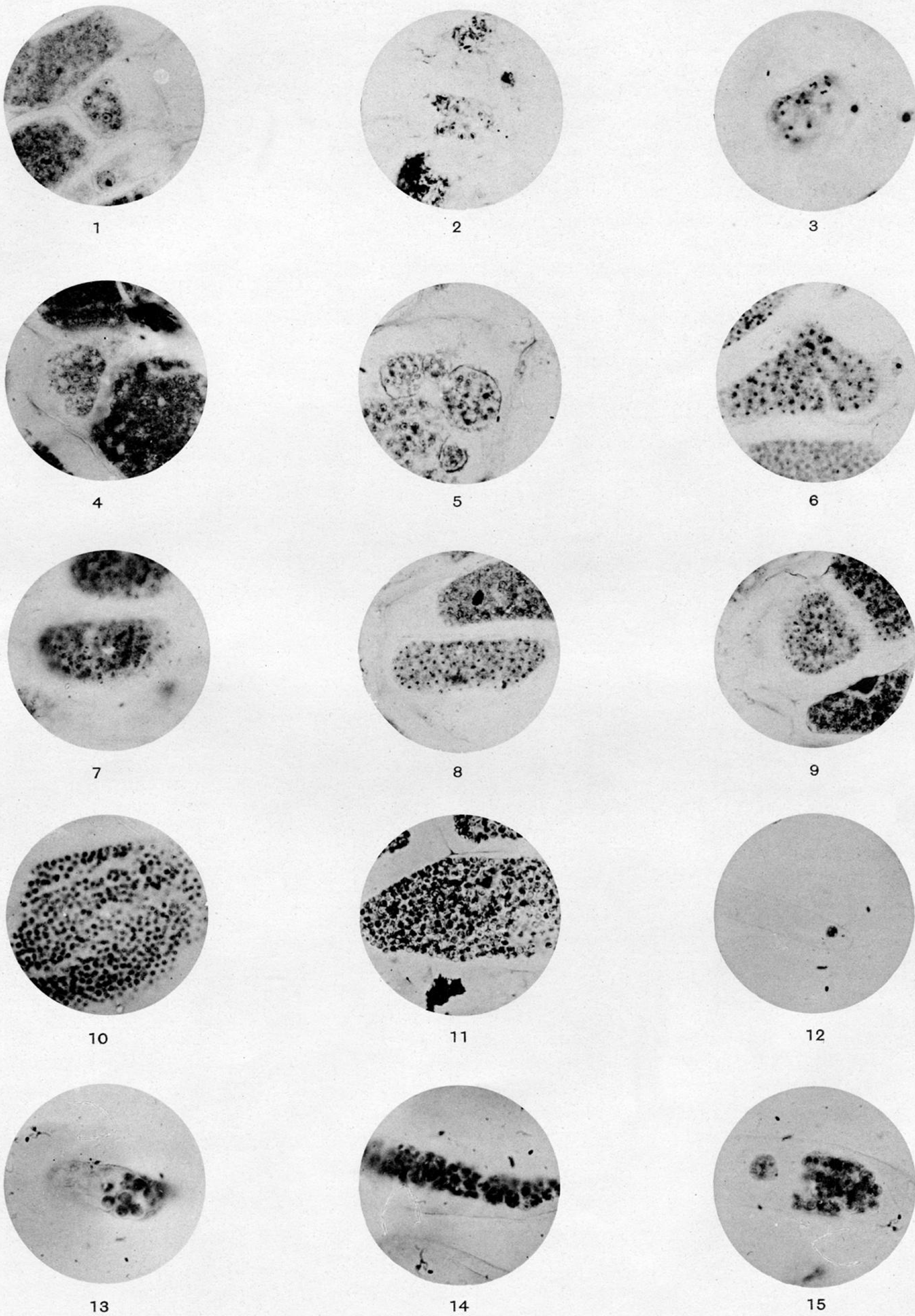

PLATE 19.

FIG. 1. Photomicrograph showing the resting nuclei of the plasmodium. × 1650.

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FIG. 2. Photomicrograph showing the "cruciform" stage of protomitosis. × 1650.

FIG. 3. Photomicrograph showing the "dumb-bell" stage of protomitosis. × 1650.

FIG. 4. Photomicrograph of the early akaryote stage showing the nuclei without a karyosome and the dispersal of the chromatin. × 1650.

FIG. 5. Photomicrograph showing the formation of cysts which may sometimes occur before spore formation. × 1650.

FIG. 6. Photomicrograph of the heterotypic metaphase in the division before spore formation; the spindle can be clearly seen. × 1650.

FIG. 7. Photomicrograph of the heterotypic anaphase before spore formation, showing the spindle and the chromosomes appearing as two rings. × 1650.

FIG. 8. Photomicrograph of the homotypic metaphase in the division before spore formation; compare the size of the plate with that of the heterotypic metaphase. × 1650.

FIG. 9. Photomicrograph of the homotypic anaphase prior to spore formation. × 1650.

FIG. 10. Photomicrograph showing the nuclei within the newly-formed spores before the wall has become thickened. × 1650.

FIG. 11. Photomicrograph of a group of fully-developed spores. × 1650.

FIG. 12. Photomicrograph of a swarm spore just after entrance into a root hair; observe the nucleus with a central karyosome. × 1650.

FIG. 13. Photomicrograph showing the formation of the zoosporangia from the plasmodium. × 1650.

FIG. 14. Photomicrograph showing the division of the zoosporangia into zoospores. × 1650.

FIG. 15. Photomicrograph showing several fully-developed zoospores lying inside the zoosporangia. ×1650.

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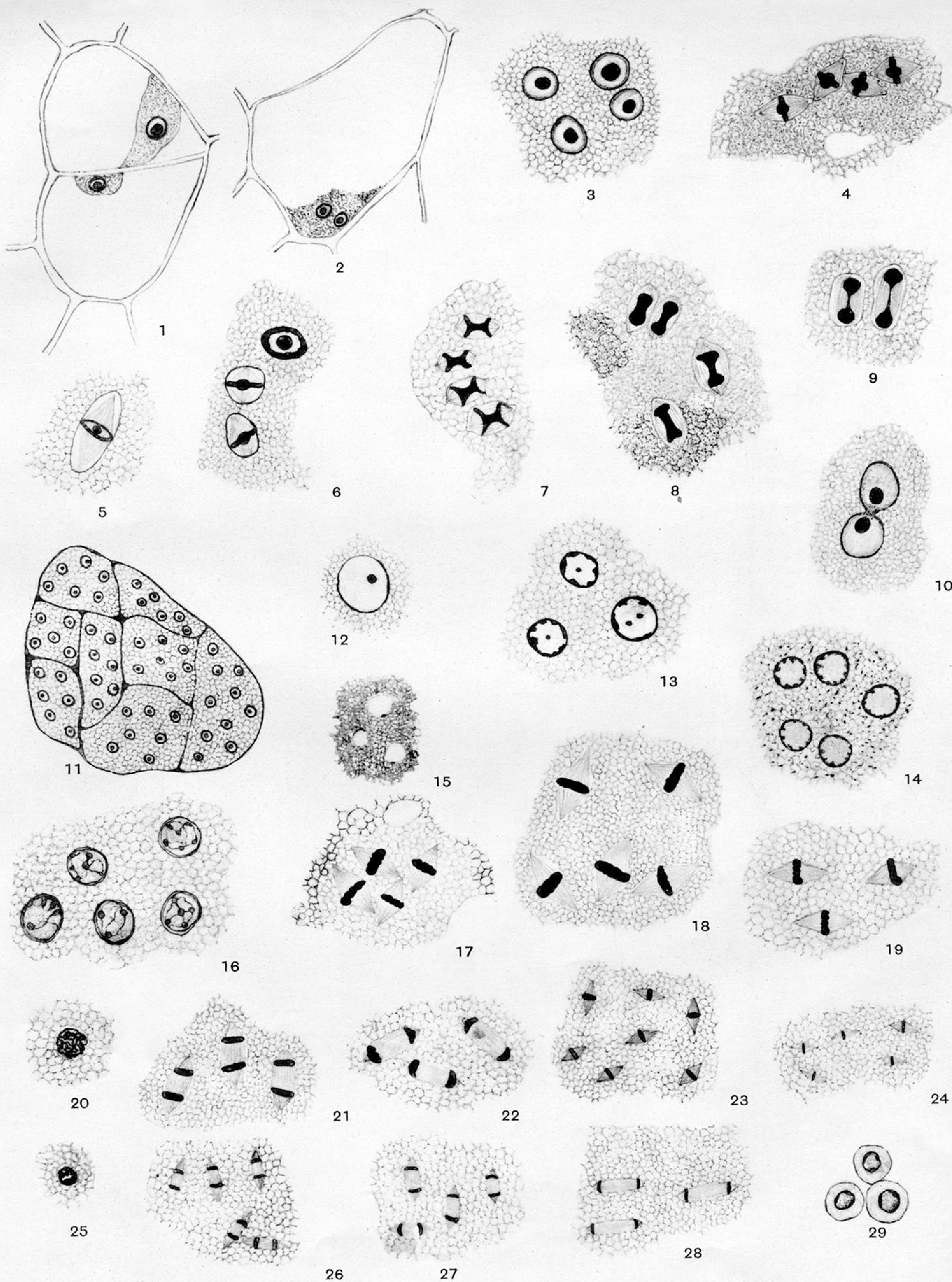

PLATE 20.

FIG. 1. Uninucleate amœbæ lying in the host cells, showing the relative size. × 1500.

FIG. 2. A young plasmodium lying in the host cell. Note the way in which it lies against the wall of the cell. × 1100.

FIG. 3. Four resting nuclei of a larger plasmodium, showing the karyosome and the chromatin lying around the nuclear membrane. × 3200.

FIG. 4. An early stage in protomitotic nuclear division, showing the chromatin in a ring around the karyosome. × 3200.

FIG. 5. An oblique view of a nucleus in the same stage as the last, showing clearly that the chromatin is arranged in a ring. × 3200.

FIG. 6. The "cruciform" stage in protomitotic nuclear division; the nucleus at the top shows the same stage in polar view. × 3200.

FIG. 7. A later stage in protomitotic nuclear division, showing the two daughter masses of chromatin separated and the karyosome becoming drawn out between them. × 3200.

FIG. 8. A still later stage in which the chromatic rings have lost their individuality and are bunching around the karyosomes at the poles of the spindle. × 3200.

FIG. 9. The final stage in protomitosis, only a thin thread of the karyosome still connecting the two together. × 3200.

FIG. 10. The final separation of the two daughter nuclei and the re-formation of the nuclear membranes around both the nuclei. × 3200.

FIG. 11. A drawing of several cysts formed by the division of the plasmodium into separate masses, these subsequently reform into a plasmodium. × 1100.

FIG. 12. A resting nucleus just prior to the akaryote stage. Note that the karyosome has already become reduced in size. × 3200.

FIG. 13. A later stage in the akaryote condition, in which the karyosomes have begun to fragment prior to disappearing. × 3200.

FIG. 14. The complete disappearance of the karyosomes and the commencement of the diffusion of the chromatin into the cytoplasm. × 3200.

FIG. 15. The final condition, in which all the stainable chromatin has passed into the cytoplasm, leaving only the nuclear membrane with a fragment of chromatin. × 3200.

FIG. 16. The first recognizable stage after the re-formation of the nuclei at the close of the akaryote stage, showing the chromatin as a thread separated here and there by small nucleoli. × 3200.

FIG. 17. Heterotypic metaphase on the formation of spores. Note the beaded character of the chromatin plate as it appears lying across the spindle. × 3200.

FIG. 18. Another view of the same stage, showing the chromosomes slightly more separated. × 3200.

FIG. 19. Another view of the same stage, showing a series of rather smaller nuclei. × 3200.

FIG. 20. Polar view of a nucleus in the heterotypic metaphase; in this view it is possible to see the individuality of the chromosomes. × 3200.

FIG. 21. Heterotypic anaphase, showing the two daughter beaded groups of chromatin migrating to the poles of the spindle. × 3200.

FIG. 22. Heterotypic telophase, showing the re-formation of the chromatin in a dense mass at the poles. × 3200.

FIG. 23. Homotypic metaphase, showing the general appearance of the chromatin as a plate lying across the spindle. Compare the size of the plate with figs. 17–19. × 3200.

FIG. 24. Homotypic metaphase, showing the same stage as in the last figure. × 3200.

FIG. 25. Homotypic metaphase in polar view, showing that the individuality of the chromosomes is still possible to make out. × 3200.

FIG. 26. Homotypic anaphase, showing the separation of the chromatin into two equal bands. × 3200.

FIG. 27. A similar stage to that in the last figure, but of slightly larger nuclei. × 3200.

FIG. 28. A later stage, just prior to the telophase, showing the chromatin masses already becoming collected up into solid groups. × 3200.

FIG. 29. The final stage in the division; the daughter nuclei have re-formed their nuclear membranes and a wall has been formed around the nuclei, cutting the plasmodium into spores. × 3200.

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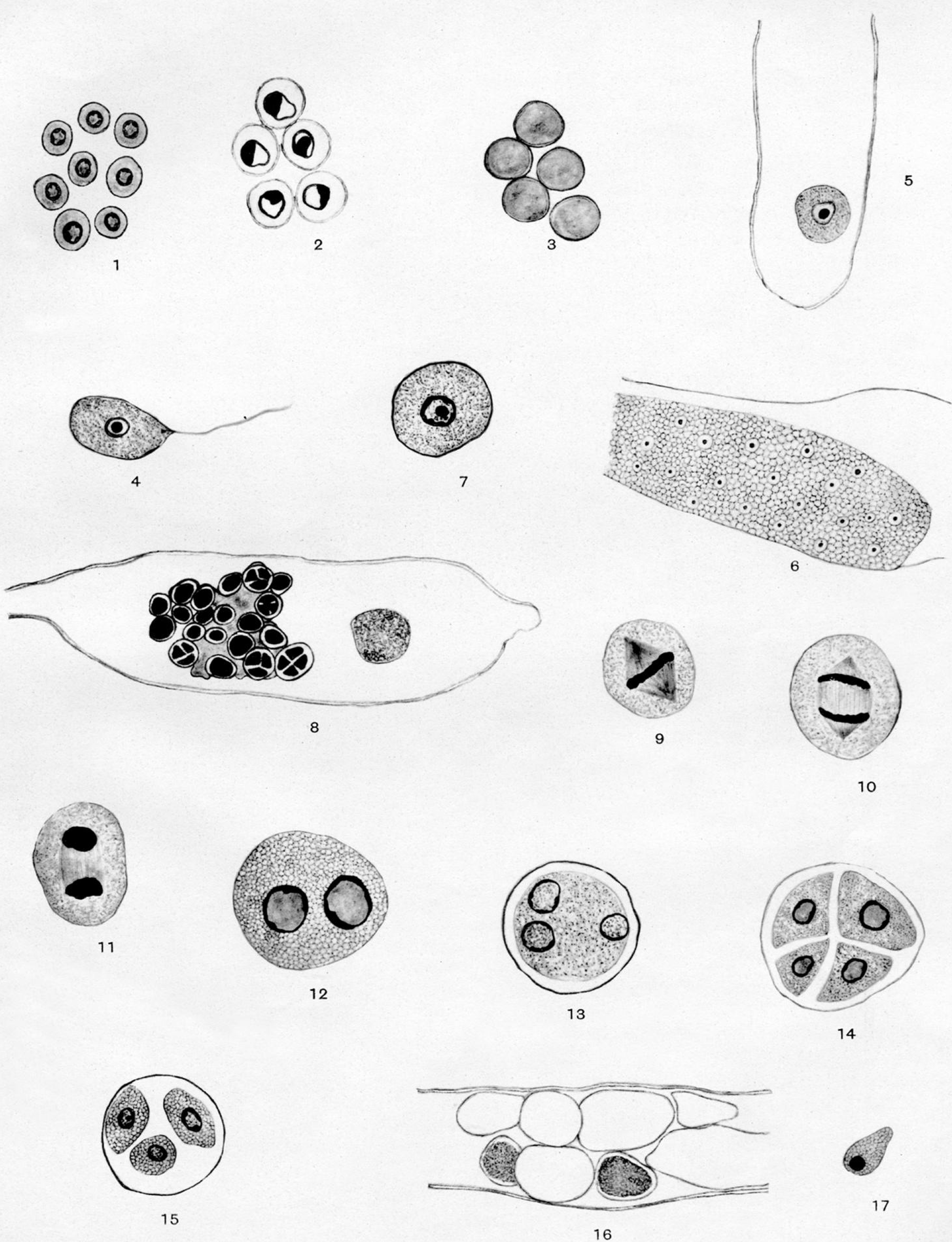

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