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Observations on the Protozoa in the Intestine of Mice.

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(With plates X—XII and 1 text figures.)

These observations were commenced on mice which I was using for experimental purposes at the Pasteur Institute, Paris, at the beginning of last year. The study of these Protozoa was continued in the laboratories of Prof. Richard Hertwig in the Zoological Institute of Munich. I should like to take this opportunity of acknowledging my great indebtedness to Prof. Hertwig for the help and advice he has so willingly given me.

In studying the Protozoa living in the intestine, one is struck by the varying degree to which they have become adapted to their host. All steps in the process of adaptation are fonnd from forms which only live occasionally in the intestine to forms, like the coccidia, which are very specially adapted to a particular form of existence.

There are forms like the amoebae described below, which live and multiply outside the body. Their cysts pass throngh the intestine of mice and occasionally the amoebae escape and multiply in the rectnm. This may be taken as the first step towards parasitism. In the case of the flagellate *Hexamitus*, it is found frequently in all parts of the intestine, bnt it can also live and multiply outside the body in decomposing material. *Trichomonas* exhibits a higher grade of adaptation. Its favourite habitat is the

caecnm, where it lives and reproduces. Large numbers of *Trichomonas* escape from the body and these may retain their vitality for many days in a contracted condition, though it is doubtful if they can live and multiply like the *Hexamitus*. In this contracted condition *Trichomonas* may be taken in by other animals and become active again in the mouth and find its way to the caecum. The *Amoeba muris* and *Lamblia* have lost the power of existing ontside the body of their host except in the encysted condition, and this leads up to highly specialised parasites like the coccidia, which live in the epithelial cells of the intestine.

In a series like this it is difficult to say where true parasitism begins. The flagellates and amoebae have, apparently, not the least ill effect upon their host and they live more as commensals than parasites. This applies more especially to the forms living in the large intestine, since their existence is probably dependent on the bacterial flora of this part of the alimentary canal. Forms living in the small intestine, as *Lamblia*, nourish themselves exclusively by absorbing the fluid constituents of the food, while those that live in the caecum, *Amoeba muris*, *Trichomonas*, *Hexamitus*, take in solid food also.

Under their respective headings below, will be found the observations upon these Protozoa. The *Amoeba* which is described as occurring sometimes in the rectum is left unnamed, as it may be already described in other associations. The same remark would apply to the form of *Hexamitus* inhabiting the caecum.

Amoeba muris Grassi.

This Amoeba was first described by Grassi as occurring in small nnmbers in the intestine of mice and rats. According to my observations it is present in about half the mice examined, and, though. as a rule, present in small nnmbers, this is not always the case. Rarely is there a very large infection. In two mice the amoebae were present to such an extent that 100 or more could be found in each cover-glass preparation of the contents of the caecum.

These amoebae live in greatest numbers in the caecum. They occnr to a less extent in the upper parts of the large intestine, and are never found above the caecum. In the ordinary course of events the free amoebae do not escape from the body of the mice, but, in diarrhoea, free forms may be fonnd in the faeces. In normal faeces only encysted forms occur.

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In the caecum the amoebae live free amongst the caecal contents and also upon the epithelial surface. They may even enter the glands and make their way to the remotest extensions of these. There is never any indication of their being able to penetrate the epithelium. The amoebae live in the company of *Trichomonas*, *Hexamitus*, numerous bacteria, yeast cells and spirochaetes.

Description of living amoebae.

When examined in the living condition this amoeba bears a very striking resemblance to *Entamoeba coli* (Amoeba coli), which lives in the human intestine. This resemblance was noted by Grassi, who found, however, the amoeba of the mouse to be much smaller. He gave 13,2 μ as the diameter of the largest forms. This is too low an estimate, as I have seen forms measuring from 30—40 μ.

There is a narrow ectoplasmic layer, clear and quite transparent and only distinctly visible in the formation of the pseudopodia. The ectoplasmic layer surrounds a more liquid and granular endoplasm, in which are situated the nucleus and food vacuoles. Within the vacuoles may be included anything that is present in the caecum — bacteria, bacilli and cocci, *Trichomonas*, *Lamblia*, *Hexamitus* and their cysts and yeast cells. Sometimes, in cases of coccidiosis where epithelial cells are cast off, these epithelial cells are taken in by the amoebae. A very striking picture is obtained where a large amoeba possesses a single vacuole containing actively swimming *Trichomonas*. The vacuole may be so large as to reduce the amoeba to a mere sac on one side of which is the nucleus. At first sight, these forms strike the observer as being cysts full of active flagellates (Pl. XII fig. 1). What is the fate of such an amoeba has not been determined. Similar large vacuoles are occasionally seen containing a large coccus (Pl. XII fig. 2). The presence of so many cocci of one kind in a single vacuole, and all apparently in a healthy condition without any sign of being digested, seems to suggest that the cocci have multiplied after having been taken in by the amoeba. The coccus in such a case would be a form of parasite and would lead ultimately to the death of the amoeba.

In the living animal the nucleus is distinctly visible. It lies in the endoplasm as a clear vesicle, over the surface of which are distributed bright refractile granules. In the interior of this nucleus very frequently can be distinguished a definite nucleolus.

The movements of *Amoeba muris* were stated by Grassi to be slow. This is, however, only correct when they are examined in the

cold. On the warm stage the amoebae are active and in their rate of movement and mode of forming pseudopodia resemble very strikingly *Entamoeba coli*. As a rule, only one pseudopodium is formed at one time. This consists at first only of ectoplasm (Pl. X fig. 3) into which the endoplasm suddenly streams, carrying the nucleus with it.

Cultivation.

All attempts at cultivating this amoeba outside the body have been met by failure. Both in aerobic and anaerobic culture the medium [1]) recommended by Musgrave and Clegg for the culture of *Entamoeba coli*, has given negative results. By smearing faeces on the surface of their agar in Petri dishes cultures of amoebae can occasionally be obtained, but these amoebae are never *Amoeba muris*, but a distinct amoeba which is described under another head below. I have also been able to cultivate amoebae from the intestine of a guinea pig and also from a human intestine in which *Entamoeba coli* was present. In this latter case, the amoebae resembled those I have cultivated from the faeces of mice and were not *Entamoeba coli*. Schaudinn has described the life cycle of *Chlamydophrys stercorea*, which lives outside the body but there forms cysts which have to pass through an intestine, human or animal, before the enclosed amoebae escape. It is probable that there are other forms of amoebae which pass through the intestine in the encysted condition and faeces containing such cysts would give a culture of amoebae, if brought upon a suitable medium. If contents of the caecum of the mouse in which *Amoeba muris* is present be sealed up from contact with air without admixture with any other liquid, it will be found that the amoebae live only a few hours, even when kept at the temperature of the body. In the light of these facts it must be very doubtful if it would be possible to cultivate an organism like *Amoeba muris*. The same remark would apply to *Entamoeba coli*, as in the experiments of Musgrave and Clegg no steps were taken to exclude the presence of other amoebic cysts. Further, the figures and descriptions of amoebae and cysts given by these workers suggest the amoebae I have cultivated and in no way the *Entamoeba coli*.

[1]) The medium is made as follows: — 20 grams Agar, 0,3—0,5 grams Sodium Chloride and 0,3—0,5 grams Extract of Beef (Liebig) are dissolved by heating in 1 litre of water. This solution is then titrated and made 1—5 per cent alkaline to phenolphthalein. The final reaction after autoclaving, distribution in tubes and sterilising will be about 1 per cent alkaline to phenolphthalein.

Description of fixed and stained amoebae.

For fixing, snhlimate alcohol (sat. aq. subl. 2 alcoh. 1) as recommended by SCHAUDINN was mostly used. Chromosmium fixative also gave good results. The preparations were stained in Iron Hematoxylin of HEIDENHAIN, DELAFIELD's Hematoxylin and Borax carmine.

In amoebae prepared in this way the same two layers of the body can be made out (Pl. X fig. 1—4). The ectoplasm is difficult to distinguish except in psendopodial formation. The endoplasm is grannlar and may contain vacuoles or not and, in forms with a psendopodium, is in marked contrast to the clear and transparent ectoplasm. The nucleus is spherical. It has a definite and fairly thick nuclear membrane. Within the nnclear membrane may be distinguished an achromatic network or alveolar structure. Over the snrface of the nnclear membrane the greater part of the somewhat scanty chromatin is scattered in grannles of varying size. Some finer granules are distrihuted over the network within the membrane and at one point of the network is the nucleolus in which, also chromatin is situated. There may be two nncleoli in the nucleus and this condition may be the first stage in nuclear division. Very frequentlý the chromatin is condensed into clnmps at one or two points of the nuclear membrane (Pl. X fig. 37 b). In specimens stained with Borax Carmine and differentiated in acid alcohol these clnmps of chromatin resemble certain darkly staining masses which lie around the nnclens in certain instances. It is probable that these clnmps of chromatin are thrown off from the nucleus and either disintegrate in the plasma or are thrown ont of the amoeba. This may be a preparation for encysting or may occur at any stage when there is a superfluity of chromatin in the nucleus. The nnclens of this amoeba at all stages is marked by its poorness in chromatin. Very often the reaction to chromatin stains is little, if at all, more intense than the protoplasm of the amoeba.

The type of nncleus here described for *Amoeba muris* corresponds exactly with the nnclens described by SCHAUDINN for *Entamoeba coli*.

Reproduction.

Mnltiplication of this amoeba is hy division and encysting. I have not been able to find any stages of schizogony as described by SCHAUDINN for *Entamoeba coli*, in which there is a division of the

nuclens into 8 smaller nuclei, followed by a division of the amoeba into 8 smaller amoebae.

Multiplication by division.

In simple division the nuclens divides by a form of mitosis. In the earliest stages there is seen within the nuclear membrane a small spindle (Pl. X fig. 37c). At either pole of the spindle is a more darkly staining area. Achromatic fibres extend between the two poles, and arranged npon these fibres in a longitudinal manner are the chromatin granules which have left their position upon the nnclear membrane. Surrounding the spindle at this stage can still be seen some of the achromatic nuclear network, while enclosing the whole is the nuclear membrane which is deprived of all its chromatin. There does not seem to be a formation of definite chromosomes or of an equatorial plate as occnrs in the amoeba described below.

At a later stage (Pl. X fig. 5 and 37d) the spindle is longer and is narrower at the middle. The same two darkly staining areas at either pole can be distinguished. The chromatin is becoming separated irregularly into two parts. The nuclear membrane is lying round the spindle. In later stages the constriction in the middle becomes more marked and the nucleus is divided into two smaller nnclei (Pl. X fig. 2). The division of the protoplasm does not follow immediately npon division of the nucleus. Free amoebae with two nnclei are frequently found and these may be watched npon the warm stage for some time without any signs of division. If this division of the protoplasm was longer delayed the nnclei might divide again and so produce a form of schizogony as described by SCHAUDINN for *Entamoeba coli*.

Multiplication by encysting.

Encysting of this amoeba for sexual reproduction and escape from the body of its host takes place in the caecnm. As a general rule it is possible to find only a few cysts at any one time in the voided faeces of infected mice. These cysts as they escape from the mice are spherical or slightly oval and contain eight nnclei (Pl. X figs. 33—35). By killing the mice and examining the contents of the caecnm and large intestine cysts in other stages of development can be found. Usually these cysts are scarce, but on two occasions they have been present in large numbers. It is prob-

able that in the normal course of events only a few of the amoebae are encysting at one time, but that when the contents of the caecum become unsuitable for the existence of the amoebae then large numbers of the amoebae encyst. On such occasions there is abundance of material and conditions are very favourable for the study of these stages.

Encysting as seen in living amoebae.

Amoebae about to encyst are distinguished by having an endoplasm cleared of all large inclusion products. Even at the beginning of encystment there may still be present granules of food material and bacteria. The cyst in its early stages is soft and gelatinous and the remains of the food material are thrown out of the body of the amoeba, apparently passing through the soft gelatinous wall. Only one amoeba is contained in each cyst. Three stages in the encysting of an amoeba kept under observation in the warm microscope chamber are shown in Pl. XII figs. 3, 4 and 5. In fig. 3 the animal is irregularly oval. It is surrounded by the soft gelatinous cyst and the protoplasm contains numerous food particles. Later on, the food particles were thrown out of the cyst (figs. 3 and 4) and at the same time the cyst becomes more spherical.

Fig. 5 is a later stage where the amoeba is within a spherical cyst. The protoplasm is cleared of all inclusions and lying on one side is the granular nucleus. The centre of the cyst is occupied by a large refractile body to be described below.

There are two types of cysts, one type in which there is present the refractile body just mentioned and a second type where this body is wanting. The subsequent development of the cyst is somewhat altered if this body is present. The centre of the cyst being occupied by this body, the result is that the nucleus is pushed to one side and the nuclear divisions have to take place in the limited space of the narrow layer of protoplasm. This also causes the development to proceed more slowly.

The presence of this refractile body seems to depend on the rate of encysting. If the amoebae encyst rapidly, probably owing to some sudden alteration in the intestinal contents, the large proportion of cysts contain this body. This seems to indicate that it is of the nature of food products which have not been thrown out of the animal. All intermediate forms exist between those which do not possess this refractile body and those which have it well

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developed. Later on in the development, this refractile body becomes irregular in shape and breaks up into separate fragments.

The cysts of the amoeba are spherical or slightly oval. When the refractile body is present there may be more irregularity and forms as in text fig. 6 are sometimes seen.

The diameter of the cysts is abont 12—14 μ but, exceptionally, smaller or longer cysts occur.

After the extrusion of food material and the formation of the cyst, the single nucleus divides by a process of simple division. The resnlt of this division is a cyst with two nuclei and the majority of cysts fonnd in the caecnm are in this stage.

These cysts may be examined on the warm stage or in the warm microscope chamber and, under favourable conditions, which nnfortunately are rare, the subsequent steps in their development may be followed.

Pl. XII figs. 7—17 are drawings of a cyst kept under observation during 4 hours in the warm microscope chamber. When this cyst first came under observation it had already undergone a part of its development. The single nucleus had divided and the process of matnration had taken place. These steps I have not followed in the living cyst but they will be described below in fixed and stained preparations. In the process of maturation each of the two nuclei gives up a great part of its chromatin to the protoplasm and also forms two reduction bodies. In Pl. XII fig. 7 is seen a cyst in which this has already taken place. There are two nuclei lying at opposite sides of the cyst, while the central portion of the cyst is occupied by the large refractile body. In one nucleus, the chromatin is evenly distributed, while, in the other, part of it is concentrated at one end. The refractile body was constantly changing in shape owing to the contractions of the surrounding protoplasm. The next stage in the development of this cyst was the migration of the nucleus with the irregularly distribnted chromatin towards the other (Pl. XII figs. 8, 9, 10). At the same time chromatin began to concentrate at one end of the stationary nncleus. Apart from the earlier concentration of the chromatin in one nucleus and its migration, the two nuclei are quite similar. It might be suggested that the moving nucleus represented the male element, while the stationary nucleus was the female. The two nuclei now remained side by side for about $1^1/_2$ honrs. During this time the chromatin which had concentrated at the ends of the nuclei was thrown out and collected in granules in the protoplasm (Pl. XII figs. 11, 12). The

nnclei at the same time became smaller in size and less distinct. There was no sign of the two nuclei fnsing. After the expiration of abont 1½ hours each nucleus began to elongate as a refractile clear band which finally reached from one side of the cyst to the other (Pl. XII figs. 13, 14). These two bands were parallel and slightly cnrved, owing to the presence of the refractile body round which they passed. These two bands were spindles for the division of the two nuclei. The result of this division was four nuclei which lay in pairs at opposite sides of the cyst. The two nuclei of each pair then apparently fused, prodncing again a cyst with two nuclei. These two nnclei then began to increase in size and almost immediately divided to form four nuclei. Pl. XII fig. 15 shows the cyst with one of the conjugated nuclei already divided while the other is in process of division. The grannles of chromatin which were thrown out of the nuclei are still seen in the protoplasm. The duration of the spindle formation and conjngation was at most only 10 minutes, and this explains the difficulty of finding these stages in fixed preparations. The fonr nuclei resulting from the first division after conjugation rapidly grow in size (Pl. XII fig. 16). At this stage the refractile body becomes irregular in shape and shows signs of breaking np. The development of this cyst was not followed any further, but the later stages were observed in other cysts.

In Pl. XII figs. 18—21 are represented four stages in the development of another cyst. In the first stage there are 4 nuclei with a refractile body. The nuclei finally divided to form 8, while the refractile body is becoming very irregular.

In Pl. XII figs. 22 and 23 are seen two stages in the development of a cyst which was left in the warm microscope chamber over night. In fig. 22 there is a spherieal cyst with two nuclei and a refractile body, while in fig. 23 the development is completed. There are now 8 nuclei and the refractile body has broken up and is represented by several shrivelled fragments.

Description of cysts in fixed and stained preparations.

For the stndy of the cysts the same methods of fixing and staining were used as for the free amoebae. The first stage in the process is shown in Pl. X figs. 6—9. In figs. 6 and 9 there is present the refractile body. The nucleus in these cases is large and contains a relatively large quantity of chromatin. This nuclens then divides by a process of simple division (Pl. X figs. 10, 11). The

first nuclear division takes place very soon after the formation of the cyst. The stage with two nuclei is one of long duration and in this stage the nuclei are reduced in size by a throwing out of chromatin. The chromatin passes out of the nuclei into the protoplasm causing the latter to stain very deeply, especially around the two nuclei, which themselves stain only faintly (Pl. X figs. 12 and 14). The chromatin is then either dissolved in the protoplasm or is thrown out of the cyst. Sometimes even at this stage remains of food products are still within the cyst. They are thrown out of the cyst also (Pl. X figs. 13, 20). This loss of chromatin reduces the nuclei to a much smaller size, while in some cases there appear to be no definite nuclei remaining, but only granules of chromatin in the protoplasm (Pl. X figs. 17—20). It may be that in these cases there is a complete destruction of the nuclei followed by their reformation from the chromatin in the protoplasm, as has been described by Schaudinn for *Entamoeba coli*. As these stages of *Amoeba muris* have not been followed in the living cyst and as a sufficient number of cysts showing this chromatin reduction have not been examined, a definite statement as to the dissolution and reformation of the nuclei cannot be made. It is, however, quite clear that a great part of the chromatin is thrown out of the nuclei. After this loss of chromatin the nuclei undergo a further reduction in the formation of reduction bodies. Each nucleus gives off two reduction bodies which are ultimately dissolved in the protoplasm or remain as darkly staining granules (Pl. X fig. 21).

The division of the one nucleus of the encysted amoeba and the following loss of chromatin and formation of reduction bodies I have unfortunately not been able to follow in the living cyst. All stages prior to the division of the one nucleus and stages after the formation of the reduction bodies I have followed in the living cyst as described above. There is considerable difficulty in keeping the cysts alive and as the stages I have failed to observe are of long duration this is easily explained. However, I have been able to examine a large number of fixed and stained cysts in this precise stage, so the steps in the development could be followed.

After the chromatin reduction, both by throwing out of chromatin from the nuclei and formation of reduction bodies, there remain two smaller nuclei in the cyst. The two nuclei then come together as described above for the living cyst and at the same time they give up more chromatin as a final preparation for spindle formation and conjugation. In Pl. X fig. 22 is shown such a cyst with two

nnclei lying close to one another and surrounded by a more darkly staining protoplasm due, probably, to the chromatin which has passed into the plasma. The next step is the formation of the spindles and division of the nuclei. This stage is seen in Pl. X fig. 23 a preparation stained with DELAFIELD's Hematoxylin. There are two spindles passing from the point where the two nnclei lay side by side, ronnd the refractile body. The darkly staining masses in the cyst are probably food material or broken off fragments of the refractile body. Some of these masses probably represent chromatin material. At either end of each spindle is a darkly staining cap. The granules of chromatin are arranged longitndinally along the fibres. As in the nuclear division, in the free amoebae there is no formation of chromosomes. The result of this nuclear division is two pairs of nuclei lying at opposite poles of the cyst. These nnclei then conjngate, giving a stage represented in Pl. X fig. 24. The nnclei resulting from conjugation have already increased in size and are preparing for the next division (Pl. X fig. 25, 26, 27, 28). The division of the four nnclei to form eight is in progress in Pl. X fig. 29 and 32, and is complete in Pl. X figs. 33—35. All these nnclear divisions are simple constrictions of the nuclei into two eqnal parts. The only spindles formed are those which give rise to the conjugating nuclei. The divisions of the nuclei take place at one time within the cyst. In the last division, for instance, all four nnclei divide together. In Pl. X fig. 30 is a cyst with only three nuclei where one nucleus has not divided, bnt snch an irregularity is the exception. After the conjugation of the nuclei the refractile body breaks up. This may take place soon after conjugation or it may be delayed till after the formation of the eight nnclei. The refractile body stains feebly and shows a course reticular structnre. When it breaks up, the separate parts shrink to form masses which stain deeply with Iron Hematoxylin and DELAFIELD's Hematoxylin. These masses can be distinguished from chromatin by not staining with borax carmine after differentiation in acid alcohol.

In the process of development the soft and gelatinons cyst wall becomes tough and resistent. At the same time there is formed within the cyst a second membrane which is well shown in Pl. X fig. 36, where the inner membrane has separated from the outer.

As stated above, it is the cysts which eight nuclei which escape from the intestine in the faeces. Such cysts remain without further development. The onter cyst wall becomes tough and irregular (Pl. XII fig. 24).

I have not been able to follow the division of the protoplasm within the cyst nor the escape of the amoebae which must presumably take place in the intestine of mice after their ingestion as is the case whith *Entamoeba coli*. One experiment is worth recording. though not absolutely conclusive. A mouse, which showed no amoeba cysts in its faeces after repeated examination, was fed upon cysts from another mouse. This mouse after 3—4 weeks was passing large numbers of cysts in its faeces.

It is probable that in the mouse there is a stage of active multiplication of the amoebae and that the formation of the sexual cysts does not occur till later in the infection, as is true in coccidiosis.

The whole of this cycle of development bears a marked resemblance to the development of *Entamoeba coli* (*Amoeba coli*) described by Schaudinn. Schaudinn, unfortunately, has given no figures and one has to rely on a verbal description. He describes the cysts of *Entamoeba coli* as containing a single amoeba with protoplasm divided into an outer and denser layer containing the nucleus and an inner more liqnid portion. The inner portion probably corresponds to what has been described as the refractile body in the cysts of *Amoeba muris*. After the division of the nucleus there ensues a throwing out of chromatin from the two nuclei. Schaudinn there says that the remains of the nuclei are finally thrown ont of the cyst, while another two nuclei are reconstructed from the chromatin in the protoplasm. As I have not followed these stages in the living cyst as Schaudinn did for *Entamoeba coli*, it is difficult to form an opinion on the resemblances or differences of these stages of the two amoebae. However, in *Entamoeba coli* this process it not invariable, as Schaudinn gives several alternative courses of development at this stage. The formation of reduction bodies and the development of eight nuclei correspond in the two cases. When we take into acconnt the striking similarity of these two amoebae. both in the free condition and in their encysting process, it is difficult to avoid the conclusion that they are identical. The *Entamoeba coli* of the hnman intestine is a harmless parasite as is the *Amoeba muris* in the mouse and rat. Schaudinn found *Entamoeba coli* present in a large percentage of normal and healthy individnals and it is quite conceivable, if not probable, that many of these intestinal Protozoa *Amoeba*, *Lamblia*, *Trichomonas* and *Hexamitus*, which are more commensals than parasites, may lead a harmless existence in the intestine of warm blooded animals of various kinds.

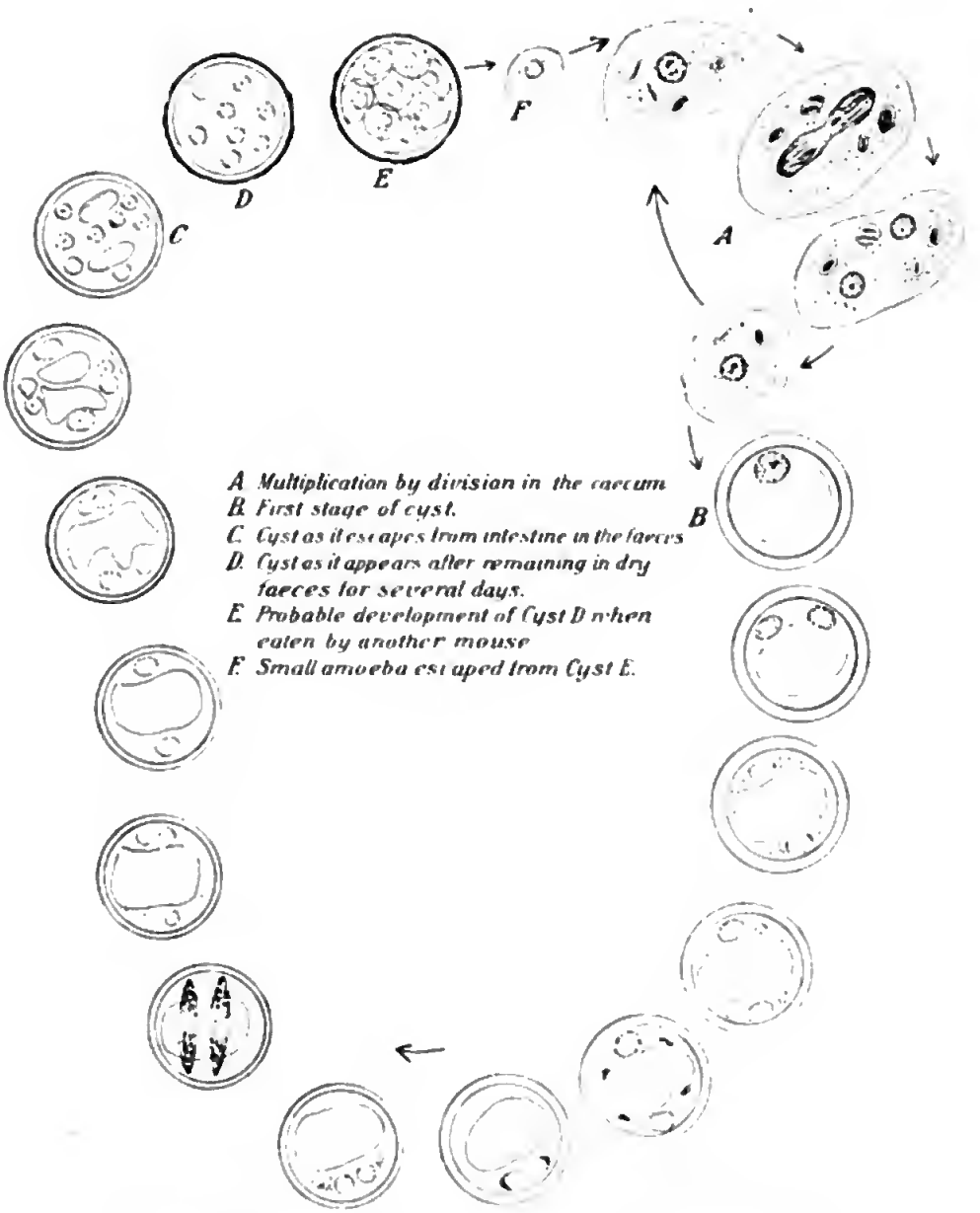

Diagram representing cycle of development of *Amoeba muris*.

Amoeba sp.

This amoeba, which is quite distinct from *Amoeba muris*, is found occasionally in the faeces of mice suffering from diarrhoea. In normal faeces the free amoebae are never found, but only their cysts. If faeces containing these cysts be kept moist for a few days, the free amoebae will escape from the cysts and commence multiplying

rapidly in the faeces. It is quite easy to cultivate these amoebae on the alkaline agar recommended by Musgrave and Clegg. A little of the faeces smeared on the surface of the agar in a Petri dish will give a rich cultnre in two or three days, even at the ordinary temperature of the laboratory. The reproduction is still more rapid at a temperature of 25°—30° C. The bearing of this amoeba on the supposed cultivation of *Amoeba coli* has been considered above.

In the free state (Pl. XII figs. 25—30) this amoeba is characterised by having a distinct ectoplasm, which is qnite clear and transparent and snrrounds the liqnid and granular endoplasm. The endoplasm contains the single nucleus and food vacuoles. There is no contractile vacuole. In some forms the endoplasm is full of small refractile grannles of uniform size (Pl. XII figs. 25, 27). The movements of the amoeba are slow. There may be several pseudopodia formed at one time or only a single one. The pseudopodia are lobose and may be branched and they appear to be formed only of ectoplasm. Single long pseudopodia are formed, giving the amoeba an appearance as in Pl. XII figs. 26, 27. At other times a broad pseudopodium extends ont from the body of the animal as a clear sheet of ectoplasm (Pl. XII fig. 28).

This amoeba multiplies by simple division, the nucleus first dividing by a form of mitosis. In the living animal little of the nuclear division can be seen, the spindle there appearing as a bright streak across the dividing animal. In fixed and stained preparations, the various steps in the nuclear division can easily be followed. The best pictures are given in specimens fixed in snblimate alcohol and stained with iron hematoxylin. Very good results are also obtained by fixing in chromosmium fixative and staining with borax carmine.

The resting nucleus is roughly spherical (Pl. X fig. 38). There is a definite nuclear membrane which is thin and devoid of chromatin. In the centre of the nucleus is a large deeply staining spherical mass. This is the nucleolus, over the snrface of which all the chromatiu of the nucleus is distributed. The space between the nuclear membrane and nucleolus is filled up by an achromatic network.

The first noticeable sign of division is a breaking up of the chromatin into smaller granules (Pl. X figs. 39, 40). Fonr is a very usual number for these granules, but more than this may occur. These granules arrange themselves at the equator of the nucleus

as an equatorial plate. In the side view, this plate appears as a dark line of grannles across one diagonal of the nucleus, while on each side of this line is a band of substance which stains a little more deeply than the rest of the nuclear contents (Pl. X figs. 41, 42). Fig. 41 represents the equatorial plate as seen from above. The equatorial plate then splits into two halves which move away from one another. There is probably here a splitting of the chromatin granules. A stage depicted in Pl. X figs. 43, 44, 45 is reached. There are two chromatin plates connected by fibres, while similar fibres extend from the two plates to the nuclear membrane. In fig. 45, the spindle is seen obliquely, while the four chromatin granules chromosomes in each plate are distinctly visible. At this stage the nuclear membrane is slightly elongated, while, stretched across its long axis, is the spindle, which is narrower than the transverse diameter of the nucleus. This leaves a considerable space around the spindle. As the spindle increases in length the two plates of chromatin separate and, at the same time, the transverse diameter across the nuclear membrane becomes reduced till it is about equal to that of the chromatin plates at the poles of the spindle (Pl. X figs. 46—49). During this elongation of the spindle the fibres stretching between the chromatin plates are replaced by a central spindle fibre, which is formed, as it were, by a fusion of these fibres. Towards its ends, the central spindle fibre opens out into a coneshaped structure which extends to the chromatin plates (Pl. X figs. 47, 49). At either extremity of the spindle is a hemispherical structure which fills up the cap-like ends of the elongated nuclear membrane. The whole spindle finally becomes much elongated and resembles the spindles of micronuclear division in infusoria. At this stage the transverse diameter at the middle of the spindle may be less than at either end. The amoeba then splits into two, the spindle dividing with it (Pl. X fig. 50). The nuclei of the resulting amoebae are formed by a fusion of the chromatin granules to the mass characteristic of the resting nucleus, while the remains of the spindle disappear.

The whole of this chromatin division and spindle formation takes place within the nuclear membrane, as is the case with the division of the nucleus in *Amoeba muris*, though in the two cases the spindles are different. The process resembles very closely the division of the micronuclei of infusoria, especially of *Paramaecium* as described by Richard Hertwig. Dangeard has described a somewhat similar process in *Amoeba hyalina*. In this latter case no

central spindle fibre is mentioned, bnt the formation of the chromosomes and their arrangement in the equatorial plate is similar in the two cases. In *Amoeba binucleata* there is also an intrannclear spindle formation as described by Schaudinn. In this case, however, there is a concentration of protoplasm around the poles of the nucleus as it occurs in the nuclear divisions of *Actinosphaerium eichhorni* (R. Hertwig).

The form of nuclear division found in this amoeba with its intranuclear spindle leads np to such forms as occur in *Amoeba binucleata* and *Actinosphaerium eichhorni* with their concentration of protoplasm round the poles of the nnclens.

The cysts of this amoeba are fonnd in the faeces of mice and are formed in large numbers in the cultnres. They have a diameter of from 7—14 μ, are spherical and of a light brownish colonr. The cyst wall is qnite smooth or very slightly irregular on its outer surface. Such a cyst is represented in Pl. X fig. 51. The cyst is completely filled by a single mass of protoplasm containing the nucleus, which resembles the nncleus of the free amoeba. Mice fed upon these cysts do not develop amoebae in their faeces. The cysts pass nnharmed through the intestine and, if bronght into suitable conditions, the amoebae will escape. Exceptionally, when the mice are suffering from diarrhoea, the amoebae may leave the cysts while still in the large intestine and there mnltiply. This resembles the passage of the cysts of *Chlamydophrys stercorea* through the intestine. In this case also the *Chlamydophrys* may leave its cyst and multiply in the rectum.

Trichomonas intestinalis.

This flagellate is often present in very large nnmbers in the caecum. It occurs above the caecum in the lower parts of the small intestine to a much smaller extent. It is also fonnd in the large intestine and large nnmbers of *Trichomonas* escape from the body in the faeces not contained in any cyst but contracted to a spherical form.

The characters of the living animal have been very well figured by Kunstler. Kunstler's figures often show more than three flagellae at the anterior end. This is never the case but the actual nnmber three is difficult to make out except in fixed and stained preparations. *Trichomonas intestinalis* was again described by Laveran and Mesnil, who figured most of the points in the anatomy of this complicated flagellate.

A marked feature of this flagellate is the ease with which it becomes deformed when removed from the caecum and examined on a slide. This consists in a breaking loose of the margin of the undulating membrane, which then lashes about as a long flagellum attached to the anterior end of the animal. The animal also changes its shape and performs amoeboid movements. This tendency to change of body form applies more especially to the larger forms of *Trichomonas*.

A point that has not hitherto been noticed is the great variation in size. Large forms 20 μ in length are found and all intermediate sizes down to 3 μ, so that differences in size are not sufficient to distinguish different species of *Trichomonas*. In Pl. XI figs. 15, 16. 17, 20 are represented some of the smaller forms of *Trichomonas* about 5 μ in length.

The general shape of the animal is well known (Pl. XII fig. 31). It is pear shaped with three flagellae springing from the blunt end and an undulating membrane with thickened border passing in a spiral manner round the body and terminating in a free flagellum. Projecting from the posterior end of the animal is a spine (Pl. XI fig. 1) which is the termination of a structure which passes through the body of the animal towards the nucleus. This is in all probability an organ of temporary fixation. Grassi compared this organ to the axial filament of spermatozoa. Laveran and Mesnil describe it as the "baguette interne". These last workers figure its continuation through the body up to the blepharoplast. It is connected in some way with this organ but even in fixed and stained preparations it is difficult to make out clearly this connection. In the region of the nucleus it becomes less distinct but a row of granules are often seen in continuous series along one or other side of this organ and they may be traced round the nucleus to the blepharoplast (Pl. XI figs. 1, 3). This organ is fairly firm, but bends slightly with the movements of the animal. It does not stain with nuclear stains like other parts of the flagellar apparatus presently to be described. In the living animal it appears as a refractile rod.

Running round the body on one side of the undulating membrane and following it in a spiral manner, is a shallow groove. This groove extends to the anterior or blunt end of the animal and often appears as a small fissure in this region (Pl. XI figs. 1, 9, 14, Pl. XII fig. 31).

The nuclear structure is best made out in specimens stained with Delafield's hematoxylin. The nucleus is oval and has a thin

nuclear membrane. In the resting condition the chromatin is distributed in the form of granules through the nucleus (Pl. XI fig. 8). Very frequently, lying against the nucleus is a small vacuole, while in forms in process of division and possessing two nuclei two such vacuoles may be present, one against each nucleus (Pl. XI figs. 11, 13, 14).

The blepharoplast consists of a darkly staining mass which can often be made out as two closely lying granules. From the anterior of the two granules arise the three flagellae and the thickened border of the undulating membrane. From the other granule arises the stiff rod like structure described by Laveran and Mesnil and which serves as a support for the undulating membrane. This rod like body is quite firm and rigid, and is the most resistant part of the animal. In deformed specimens it may be seen projecting from the body as a stiff rod with its shape still retained. When the animals die and break up, this rod remains for some time recognisable in its original form. Sometimes, other fibres may be seen in the undulating membrane. These have been figured by Laveran and Mesnil and they serve as additional supports. A marked feature in the structure of the animal is a row of granules which lie parallel to the stiff supporting structure of the undulating membrane. These granules, which are best demonstrated by staining with iron hematoxylin, commence in the neighbourhood of the blepharoplast. They are uniform in size and are lost at the posterior end of the animal (Pl. XI figs. 1, 3, 4, 21). The whole of the region around the nucleus is very granular. All these granules, together with the thickened border of the undulating membrane and its rod like support which are connected with the blepharoplast, stain very intensely with nuclear stains and are probably chromatin in nature. This chromatin has to do with the complicated flagellar apparatus, and is chromatin set apart to control the motor functions of the cell. In the division of the animal we shall see that the nucleus divides independently of the flagellar apparatus and there, thus, appears to be a fairly sharp distinction between the chromatin of the nucleus and that of the flagellar apparatus, the chromidium. Whether the chromatin of the flagellar apparatus is being constantly supplied with chromatin from the nucleus, or whether the chromatin of the nucleus represents the sexual chromatin which is distinct from the chromatin of the flagellar apparatus, the trophochromatin, as maintained by Schaudinn and Goldschmidt, cannot be definitely stated till more is known of the origin of the two forms of chromatin present in this complicated flagellate.

Occasionally within the body of the *Trichomonas* are large vacuoles containing a large coccus. Similar vacnoles have been described above in *Amoeba muris* and they may be so large as to reduce the *Trichomonas* to a mere sac. As suggested for the amoeba, this may be a form of parasitism (Pl. XII fig. 32).

Multiplication of *Trichomonas intestinalis*.

Trichomonas intestinalis divides by longitndinal division. There is a division of nncleus, blepharoplast and of the peculiar pointed organ which projects from the posterior end of the animal. The nndulating membrane and its snpport with the flagellae appear to be new formations.

The first step in the process is a division of nucleus and blepharoplast. The grannles of chromatin in the nnclens run together to form larger masses. The nnmber of these chromatin masses or chromosomes is usually six (Pl. XI fig. 10). The chromosomes, at first irregular, then become dumbbell shaped and each divides into two (Pl. XI figs. 2, 5, 6, 7, 12, 14). A constriction then appears in the nuclear membrane and the nucleus divides, each daughter nuclens apparently having one half of the divided chromosomes. In this process there is no indication of an intrannclear division centre as is found in *Euglena* and no definite spindle is formed. The chromosome formation is well developed, thongh other parts of the spindle apparatus are absent. After division of the nucleus, the large chromatin granules break up into the smaller grannles characteristic of the resting nnclens.

As a rnle the blepharoplast divides before the nucleus. It consists, as pointed ont above, of two closely related granules. In division, each of these granules divides and the two pairs of granules so formed move away from one another. A fibre can often be seen extending between the two pairs of granules even after considerable separation has taken place (Pl. XI figs. 2. 4, 10. 11). Soon after division of the blepharoplast and frequently before division is complete, the rod like body which is to serve for the support of the new undulating membrane can be seen attached to the divided-off half of the blepharoplast (Pl. XI figs. 5, 6, 7, 10). The first portion of this structure may be formed by a splitting off from the one already existing, but, however it may have originated, it increases in size as the division of the animal proceeds, probably by growing out from the blepharoplast. At first no second undulating membrane

can be distinguished, but this appears later and is probably a new formation like the flagellae. The undulating membrane and its supporting apparatus continue to increase in size till they equal the size of those already existing. The appearances suggest that, just as the rod like support increases in size by growing out from the posterior of the two granules which constitute the blepharoplast, so the thickened margin of the undulating membrane increases in a similar way by growing out from the anterior of the two granules. The staining reactions of the blepharoplast, the margin of the undulating membrane and its rod like support are identical, and it would appear that the two latter were prolongations, as it were, of the former.

After division of the nucleus and blepharoplast, there commences a division of the pointed organ. This divides by longitudinal division and is the last part of the animal to divide (Pl. XI fig. 3). In later stages, it is seen extending through the body of the long drawn out animal from the neighbourhood of one nucleus to that of the other (Pl. XI figs. 15, 21). In the final stage, two animals are attached simply by this organ, which finally gives way, leaving the characteristic pointed ends.

The multiplication of *Trichomonas* may take place very rapidly, with the result that increasingly small forms are produced. These small forms may be only 3 μ in length. At other times division proceeds less rapidly and only large forms of *Trichomonas* are present.

I have not been able to find any sexual stages of this parasite. Schaudinn mentions in a short note that *Trichomonas* becomes an amoeba and that two of these amoebae, after giving off each two reduction bodies, become encysted together and conjugate. Within the cyst there is then a division into several parts with the formation of a large residual body. Such stages I have not encountered in the mice.

In the normal way many *Trichomonas* escape from the intestine in the faeces. These forms are contracted and spherical. There usually appears to be no cyst enclosing them, but forms as in Pl. XI fig. 35 are met with which apparently have a cyst. In the faeces the spherical forms of *Trichomonas* will retain their vitality for a week or more, if prevented from drying. If a little of such faeces which have been kept moist at the ordinary laboratory temperature for a week be mixed with salt solution and examined on the warm stage, it will be noticed that in a quarter to half an hour the

spherical *Trichomonas* show signs of life. The undulating membrane moves very slowly and soon the whole animal begins to rotate. This movement increases till finally the *Trichomonas* commence to swim about as do the forms freshly taken from the caecum. This long survival of *Trichomonas* outside its host and the fact that no definite cysts are formed, as is the case with the amoebae and *Lamblia*, suggest the possibility of a direct infection taking place. To test this point some of the faeces containing *Trichomonas* which had been kept moist for several days was mixed with the juice from the stomach of a freshly killed mouse. On the warm stage the *Trichomonas* revived and remained alive for four or five hours, a space of time quite long enough to allow of the *Trichomonas* passing through the stomach of a living mouse. It is thus quite possible that the infection may be spread by the ingestion of *Trichomonas* in the unencysted condition. The peculiar resistance of *Trichomonas intestinalis* and its long survival outside the body, shows that it has not become very specially adapted to a life in the intestine. It is known that *Trichomonas* in the human subject can live in many other parts of the body. Prowazek has described them from the cavity of a tooth; they live in the vagina, and have been found in the lung in suppurative conditions and even in the stomach. It is exceedingly doubtful if these are distinct species. From the figures given, it is impossible to judge of any differences. Much more probable is it, that the normal habitat of this flagellate is the intestine and, that under certain conditions which give a good bacterial growth, it may find its may from the intestine to the vagina, month, lung and so forth. It is, also, not at all improbable that the *Trichomonas* which live in the intestine of mice and other animals are one and the same species.

It is unusual to find a mouse which is not infected with *Trichomonas*. In quite healthy mice, the caecum will harbour enormous numbers and the flagellates appear to have not the least ill effect on their host. In mice suffering from diarrhoea from coccidiosis or other cause, the *Trichomonas* escape in large numbers in the faeces. Such appearances in the human subject have given rise to the idea that diarrhoea may be caused by these flagellates. It is very probable that in the normal human intestine *Trichomonas* and other Protozoa are present much more frequently than has hitherto been imagined, and, in case of diarrhoea, escape in the free living form. Flagellates in the human faeces have been most frequently encountered in cholera and similar diseases, where no one would think of suggesting the

flagellates as the cause of the diarrhoea. In other cases where no definite cause for the diarrhoea can be found, the presence of the flagellates has erroneously led to their being taken as the cause in question.

Lamblia intestinalis.

This flagellate occurs sometimes in very large numbers in the upper part of the small intestine. As regards the general appearance of the animal and its movements there in nothing to add to the excellent description of Metzner. In his investigations into the structure of *Lamblia* as occnrring in the intestine of rabbits, Metzner did not use the iron hematoxylin method of staining which gives very good pictures of the nucleus aud flagellar apparatus. Very good results are obtained by fixing with sublimate alcohol and staining with iron hematoxylin and eosine.

As found in the small intestine of the mice, the Lamblia vary in size. There is little difference in the size of the peristome or sucking disc in different auimals, but the variation is due more to the thickness of the body. In the smaller forms the body is thin and leaf like (Pl. XI fig. 37), while in the large forms it is thick and approaches to an oval (Pl. XI fig. 38). The general structure of the animal is shown in Pl. XI figs. 36—38. There are two oval nuclei, each having a definite nuclear membrane. The greater part of the chromatin is concentrated to an irregular body at the centre of the nucleus, while smaller grannles are distributed over the nuclear membrane. There appears to be no connection between the two nuclei, as has been described by Metzner and other workers. The point to which the three pairs of posterior flagellae converge stains deeply in darkly stained individuals, and this region between the nuclei which lodges a large part of the flagellar apparatus might be taken as a link between the two nuclei. The individuality of the two nuclei is clearly brought ont in the encysting process.

Between the two nuclei are seen two darkly staining rods with expanded ends. Posteriorly, these rods are continuons with the prolongations into the body of the tail flagellae. Springing from the enlargements at the hinder end of the two rods, is the middle pair of flagellae; on each side of the anterior ends of the two rods is a small granule, from which arises the anterior pair of flagellae. These, before becoming free, cross one another and then pass up to the margin of the peristome, or snckiug-disc, which is slightly raised from the surface of the body. The two flagellae then run along the surface

of the rim of the peristome for a short distance. During this part of their course they are attached to the margin of the peristome and form, as it were, a narrow membrane. The flagellae finally leave the peristome rim and become free. From the pair of granules which gave origin to the anterior flagellae just described, there may be traced backwards two fine fibres which run parallel to the two darkly staining rods as far as their posterior ends, when they diverge and are continuous with the margin of the peristome and with the second pair of lateral flagellae. In some individuals there is present a group of granules which extends from the anterior end of the nucleus towards the anterior ends of the two rods, while, at the posterior end, there appears to be some sort of connection between the nuclear membrane and the peristome margin, at the point where it turns inwards round the posterior end of the nucleus.

The area of the body behind the two nuclei, the triangular area of Metzner is depressed to form a kind of groove in which the middle pair of flagellae lie. This groove runs towards the tail, on which it is lost. At the bottom of this groove may be seen two very darkly staining bodies one on each side of the middle line. They appear to lie on the continuations of the tail flagellae into the body (Pl. XI fig. 36), but in reality they are more dorsally situated (Pl. XI figs. 37, 38). These bodies were described by Metzner. Their function is unknown, unless they are connected with certain fibres which may be seen in some of the living animals. These are fibres (Pl. XII fig. 33), which arise from refractile granules situated in the anterior region of the animal. The granules are present in about equal number on each side of the middle line, while, running from them are fine fibres which, converging in a fan like manner as they approach the tail, terminate in a refractile body which probably corresponds with the darkly staining body described above. These fibres are not always distinguishable and have not been observed in fixed specimens. Their function is probably connected with the movements of the tail.

Though very large numbers of Lambliae may be present in the small intestine and these of different sizes, dividing forms are not to be found. There are, however, large numbers of encysted forms especially in the lower parts of the small intestine and large intestine. The cysts are oval and measure about 13 or 14 μ by 6 or 7 μ. The cyst wall is smooth and transparent. These cysts have been observed by several workers, but their contents have not been clearly described. Schaudinn, in a short foot note, mentions cysts, in each of which two Lambliae fixed together by their

suckers, are encysted. These are apparently sexual cysts. In cysts that I have observed only one animal is present. These cysts are formed by one of the larger forms of Lambliae described above. In the early stages, the several parts of the animal may be seen within the cyst. The details of the cyst contents may be readily brought ont by staining with iron hematoxylin (Pl. XI figs. 30—32). Soon after the formation of the cyst, the two nuclei move away from their central position and come to lie at the anterior end of the animal. Before this migration, each nucleus becomes spherical and in so doing gives up part of its chromatin. In many cases, it appears as if the posterior end of each nucleus is divided off from the rest and remains as a dark mass attached to the margin of the peristome at this spot.

In Pl. XI fig. 30 is fignred a cyst with two spherical nuclei at one end. The two darkly staining rods can be seen and also the crossing of the two anterior flagellae. The two darkly staining bodies are still present and are a striking feature in all the cysts. Other parts of the flagellar apparatus may be seen and the dark masses which represent the divided-off posterior ends of the nuclei. These latter gradually break up and pass to the posterior end of the cyst, where they become no longer distinguishable. The next stage in the development of the cyst is the division of the two nuclei. Each nucleus has a nucleolus. This becomes drawn out and dnmbbell shaped and finally divided into two. The division of the nnclei follows, giving fonr spherical nnclei. These four nuclei sometimes lie crowded together and suggest a possible conjugation, but this has not been observed. In this stage, the cysts escape in large nnmbers from the body. If kept outside the body in the faeces, the cysts become thick and opaque, so that little of their internal structure can be made out. In some cases, the cysts, before they escape from the body, appear to contain two animals, so that, in all probability, the encysting process is followed by a division of the *Lamblia* into two daughter individuals. These cysts, if swallowed by the mice, which must frequently happen, would give rise to two of the smaller forms of *Lamblia*. It is possible that these cysts are not sexual cysts and that the division of *Lamblia* can only take place in the encysted condition. No division of *Lamblia* in the free state has been observed and the large nnmber of cysts present would lend colour to this idea. I have not been able to observe escape of the Lambliae from the cyst which may take place in normal conditions, without it being necessary for the cysts to leave the host.

If such be the case, there may be another kind of cyst which would serve for the transmission of the infection to new hosts, or the one kind of cyst may serve both to maintain the infection in the host itself and, also, to spread the infection when they escape from the body.

Hexamitus muris (Grassi).

Syn. Dicercomonas muris.

This flagellate, first described by Grassi and later by Foa, is very commonly found in the small intestine of mice, where it lives in company with *Lamblia*. It is characterised by having six flagellae at the anterior end of its body and two tail flagellae. The body is very variable in shape, but, in active forms, it is broad anteriorly and tapers to a point posteriorly. Some of these forms have been described by Foa as having a dorsal and ventral surface. The forms present in the small intestine have, as a rule, a narrow body (Pl. XII fig. 34). In the caecum sometimes occur forms with a much thicker body and with large granules in the protoplasm (Pl. XII fig. 35). These latter may occur with or without the narrower forms, and they resemble very much *Hexamitus inflatus*, though they had no mouth clefts at the insertion of the tail flagellae as figured by Klebs for this form.

The narrower forms, which live mostly in the small intestine, but also, to a less extent, in the caecum, correspond with the *Dicercomonas muris* described by Foa. In these, the nucleus consists of two masses of chromatin lying one on each side of the anterior end of the body (Pl. XI figs. 24, 25, 29). Running through the body from the point at which the tail flagellae become free are two fibrous tracts, which stain darkly with nuclear stains. These tracts pass to the neighbourhood of the nuclei and there cross one another. They are then continued between the nuclei to end in certain granules, from which arise the six anterior flagellae. The arrangement of these granules is difficult to make out, owing to the minuteness of object. Foa figures one granule on each side, from each of which spring three of the six anterior flagellae. In the dorsal view of the animal figured by Foa each granule is connected by a darkly staining fibre to the nucleus of its side. After examining a large number of specimens it appears to me, that there are several granules, perhaps six, arranged on the anterior parts of the fibrous tracts which themselves unite at the extreme anterior end of the animal (Pl. XI figs. 24, 25, 29). The six flagellae are arranged in two sets of three, the

three flagellae of each side arising from granules situated closely together.

The two masses representing the nnclei are in intimate relation with the two fibrous tracts. Whether there is an actual union between the nucleus and the fibrous tract of each side cannot be definitely stated (Pl. XI figs. 25, 29).

The origin of the tail flagellae is variable. Sometimes they arise close together (Pl. XI fig. 24). At other times they arise from the sides of the body, while there is a prolongation of the body between them as a tail process (Pl. XI fig. 29). All intermediate forms between these two types may be found.

As mentioned above, a larger form of *Hexamitus* is found in the caecum. As this is sometimes fonnd when the form of *Hexamitus* jnst described is absent and as it is only found in the caecnm and never in the small intestine, it probably belongs to a distinct species. This is snpported by certain differences in the nuclear and flagellar apparatus. The tail flagellae arise close together and they are continned throngh the body towards the nuclens in what appears as a single darkly staining fibrous band (Pl. XI figs. 18, 19, 23). In specimens very much decoloured the two continnations of the tail flagellae may be seen extending through this band like strncture (Pl. XI fig. 22). In this form the nuclear and flagellar apparatus at the anterior end of the animal are mnch more compact, so that it is impossible to distinguish the separate parts. The nncleus consists of a mass of chromatin on each side and from out this mass arise the six flagellae.

In division of these larger forms the parts of the band-like structure corresponding to each tail flagellum become more distinct and separated from one another. There then follows a splitting of each part of the nucleus and along with this a division of the fibrous band-like structure associated with it. This process results in forms having four nuclear masses with four fibrons bands each ending in a flagellum (Pl. XI figs. 26, 27, 28). The body of the animal then divides so that each portion contains two chromatin masses and two fibrons bands which arrange themselves as characteristic of the free living forms. The division of the smaller form of *Hexamitus* takes place in a similar way. Some of the division forms of this *Hexamitus* have been figured by Foa.

In the caecnm certain oval cysts are to be found which contain *Hexamitus*. These cysts are about 6—7 μ in length and 3—4 μ in breadth. In stained preparations, the various parts of the animal

may be seen within the cyst (Pl. XI figs. 33, 34). Only one animal is contained in each cyst. In many of these cysts there appears to be a division of the nnclei, so that four chromatin masses result These cysts probably belong to the larger form of *Hexamitus*.

The larger form of *Hexamitus* may be simply the fully grown form of those that live in the small intestine, but the differences in body form, in nuclear structure and in habitat are sufficient to distinguish it from these.

If faeces of mice infected with *Hexamitus* be kept moist outside the body, it will be found that forms of *Hexamitus*, indistinguishable both in the living and in the fixed and stained conditions from those that live in the small intestine, begin to appear and multiply in the faeces. It is quite conceivable that this form of *Hexamitus*, five distinct species of which have been described by KLEBS as occurring in solutions of decomposing material, is capable of living as well in decomposing matter as in the intestine of mice.

Schizogony in Coccidium falciforme.

If one examines the intestines of mice in the early stages of the infection with this coccidinm, it will be fonnd that schizogony is proceeding very rapidly and enormons nnmbers of schizonts are present. Each epithelial cell may be attacked by many merozoites, often causing the epithelial cells to break down, thus liberating the schizonts, which, however, continue their development enclosed in a kind of cyst often with double wall (Pl. XI fig. 44). Large numbers of these schizonts may be found in the debris. To the wall of the cyst the protoplasmic body of the schizont is attached at one spot and at this spot the wall is thickened or slightly invaginated (Pl. XI figs. 44, 48, 52, 55). These appearances suggest that the cyst is formed by the schizont, perhaps by a hardening of its surface. In those cases where two layers are present (Pl. XI fig. 44), the outer one may represent part of the protoplasm of the broken down epithelial cell.

The interesting point about this schizogony is that the merozoites, after attacking new cells, commence the process of schizogony before they have attained the size of the schizont from which they were derived. In this way, there is a continnal diminution in the size of the schizonts in the stage of schizogony. The largest forms give rise to merozoites about 12 μ in length, while the smallest schizonts have a diameter of not more than 3 μ and give rise to

merozoites about 3 μ in length (Pl. XI figs. 41, 50, 53). The smallest merozoites have the same structure as the largest forms. They are sickle shaped and have a nucleus in which the chromatin is concentrated at the centre to form a karyosome. All intermediate sizes are met with. Later on in the infection, these small forms of schizont are absent. The rapid schizogony with the production of the increasingly small schizonts is comparable with the rapid division of *Trichomonas* which occurs sometimes and which results in the production of very small forms. In the case of the coccidium the early stages of the infection give an abundant food supply and conditions favourable for rapid multiplication.

The smaller forms of schizonts have smaller nuclei in proportion to their size than do the larger forms; this difference in size is quite ont of proportion to the difference in size of the schizonts (Pl. XI figs. 42, 47, 49, 50). When the larger schizonts undergo schizogony, the nucleus breaks up and the chromatin is scattered in the cell (Pl. XI figs. 39—42, 47). The greater part of this chromatin is either thrown ont of the schizont or is dissolved, while only a small part arranges itself, as the nuclei of the merozoites, over the snrface of the schizont. In these large schizonts there is thus a superfluity of chromatin present in the nncleus. In the smaller schizonts the nuclei are much smaller and the formation of the nuclei of the merozoites takes place by a process of binary fission, the whole of the chromatin of the nucleus being used up in the process. In the later stages of the infection only the large schizonts are present and at this time begin to appear the gametocytes.

The method of this schizogony is interesting in the light of facts brought forward by Richard Hertwig to show that cell division is dependent on the existence of a certain relation between the quantity of chromatin in the nucleus and the protoplasm of the cell. When the right relation exists between these two cell constituents, the cell will divide, but when this relation is disturbed in any way the cell division cannot take place till the relation is re-established. In the coccidium under consideration the relation existing between the nucleus and protoplasm of the small schizonts may be that one favourable to division. This relation is maintained and the rapid schizogony ensues. As the infection advances the condition of life of the coccidia is less favourable, many epithelial cells are destroyed and the mice may be acquiring some form of resistance. Under these conditions, the nutrition of the coccidinm

cell is disturbed and the relation existing between nucleus and protoplasm is changed. The relation is no longer one which stimulates division and the large schizonts with their large nuclei result. These large schizonts, before they can divide, discard a large part of their chromatin and so, re-establishing the relation, undergo schizogony. Later on in the infection the gametocytes appear and the production of these may be the result of those changes in nutrition which give rise to the large schizonts. The continued disproportion existing between nucleus and protoplasm may lead to a condition which can no longer be remedied by a throwing out of chromatin, but only by the conjugation of differentiated gametes.

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Description of Plates.

All the preparations from which the drawings were made were fixed in Sublimate-Alcohol (2:1) and stained with Ironhematoxylin except Pl. X figs. 5, 23, 37 and Pl. XI figs. 5 to 4, and 35 which were stained with very dilute Delafield's hematoxylin.

Plate X.

Figs. 1—37. *Amoeba muris.*

Figs. 1—5. Free amoebae. 1. Large form with single nucleus and many food vacuoles including bacteria and *Trichomonas*. 2. *Amoeba* with two nuclei. 3. *Amoeba* with pseudopodium. 4. *Amoeba* with clear endoplasm. 5. Form showing dividing nucleus in spindle stage.

Figs. 6—9. First stage of cyst formation in which the single nucleus is present. 6 and 9 show the large refractile body.

Figs. 10—11. Division of single nucleus.

Fig. 12. Cyst with two nuclei surrounded by darkly staining protoplasm due to chromatin which has passed out of nuclei.

Fig. 13. Cyst with two nuclei and remains of food material being thrown out through gelatinous cyst wall.

Fig. 14. Cyst with two nuclei and very darkly staining protoplasm.

Figs. 15, 16. Cysts with two nuclei and refractile body occupying centre of cyst.

Fig. 17. Cyst with two nuclei and two refractile bodies. From one nucleus chromatin is passing out of cyst.

Figs. 18, 19. Cysts showing chromatin which is being thrown out. In these cysts no definite nuclei are left.

Fig. 20. Cyst with two nuclei much reduced in size. Chromatin and remains of food material passing out.

Fig. 21. Cyst with two nuclei and reduction bodies.

Fig. 22. Cyst with two nuclei after formation of reduction bodies. The two nuclei are lying together and chromatin is passing from the nuclei into the protoplasm which is staining darkly round the nuclei.

Fig. 23. Cyst with refractile body and two spindles. This is a stage a few minutes later than the stage represented in fig. 22. The darkly staining bodies present are partly food material and partly chromatin. The spindles will give rise to four nuclei which will conjugate in pairs.

Fig. 24. After conjugation of the nuclei, the two resulting nuclei increasing in size.

Fig. 25. A stage a little later than fig. 24 in which one nucleus has divided and one is almost divided.

Figs. 26, 27. Stages showing division of the two nuclei.

Fig. 28. Cyst with four nuclei and darkly staining food material.

Fig. 29. Cyst with four nuclei preparing for the next division.

Fig. 30. Cyst with refractile body and three nuclei. — Irregular division of nuclei.

Fig. 31. Cyst with refractile body and four nuclei.

Fig. 32. Cyst with four dividing nuclei.

Figs. 33—35. Cysts with eight nuclei. In 35 there is still a mass of the refractile body present.

Fig. 36. Cyst with somewhat shrunken walls to show the double nature of the cyst.

Fig. 37. a. Resting nucleus with greater part of the chromatin on the nuclear membrane. b. Nucleus with chromatin clumps which will be thrown off and ultimately disappear in the protoplasm. c. Stage in nuclear division. Within the nuclear membrane is the small spindle. At either pole is a more darkly staining region and between these the spindle fibres run. The granules of chromatin have left the nuclear membrane and now lie along the spindle fibres. d. Later stage of the spindle. The nuclear membrane now fits closely round the spindle while the chromatin is separating irregularly into two parts. All these nuclei from unencysted amoebae.

Figs. 38—41. Amoebae cultivated from faeces of mice. These amoebae are found occasionally in the rectum.

Fig. 38. *Amoeba* with resting nucleus.

Figs. 39—40. *Amoeba* with nucleus preparing for division with chromatin breaking up into smaller particles.

Figs. 41—42. Two views of equatorial plate stage.

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Figs. 43—44. Amoebae with nnclei in process of division. The equatorial plate has divided. In 44 the spindle fibres can be seen extending between the poles of the nnclens.

Fig. 45. Obliqne view of spindle at a stage a little later than in fig. 44. The two halves of the eqnatorial plate seen in surface view. In each plate four chromosomes.

Figs. 46—49. Different views of later stages of the splndle. In 47 and 49 the pole caps can be easily seen and also the central spindle fibre.

Fig. 50. Spindle drawn ont to its ntmost extent and division of amoeba almost complete.

Fig. 51. Encysted amoeba. These cysts are fonnd in the faeces of mice and also in old cnltnres of the amoeba.

Plate XI.

Figs. 1—17, 20—21. *Trichomonas intestinalis.*

Fig. 1. General view of animal.

Fig. 2. Showing divided blepbaroplast with connecting fibre and the dividing chromosomes in the nnclens. The supporting rod for the new nndnlating membrane is present thongh smaller than the original one.

Fig. 3. Division almost complete. The pointed organ only partially divided.

Figs. 4—7, 9—14. Various stages of division.

Figs. 15—17, 20. The smaller forms of *Trichomonas*, drawn under higher magnification. Figs. 15, 17 are forms in division. Fig. 16 form measnring about 4 μ in longest diameter.

Fig. 21. Form in last stage of division.

Figs. 18, 19, 22—29, 33, 34. *Hexamitus muris.*

Figs. 18, 19, 22, 23. Larger form of *Hexamitus* only found in caecum.

Figs. 24, 25, 29. Smaller form fonnd in small intestine.

Figs. 26—28. *Hexamitus* in division.

Figs. 33—34. Cysts of *Hexamitus.*

Figs. 30—32, 36—38. *Lamblia intestinalis.*

Figs. 30—32. Cysts of *Lamblia.* 30 with two nnclei, 31 with nnclei in division and 32 with fonr nnclei.

Fig. 36. View of *Lamblia* from ventral surface.

Fig. 37. Side view of small form of *Lamblia.*

Fig. 38. Side view of larger form of *Lamblia.*

Figs. 39—55. *Coccidium falciforme* in stages of schizogony.

All the drawings in Pl. X were made with Zeiss drawing apparatus under Zeiss $^1/_{12}$' achromatic and oc. 4. In Pl. XI the same magnification was used for all except the fignres of *Hexamitus* and figs. 15—17, 20 which were drawn under Zeiss apochromatic 2 mm and 18 comp. oc., figs. 30—32 made with $^1/_{12}$' achromatic and oc. 5, and figs. 36—38 which were drawn in ontline with $^1/_{12}$' achromatic and 18 comp. oc. while the details were filled in under oc. 4.

Plate XII.

All figures are taken from the living objekt.

Fig. 1. Amoeba with large vacnole containing *Trichomonas.*

Fig. 2. Amoeba with vacnoles containing cocci.

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Figs. 3—5. Drawings of Amoeba dnring process of encysting.

Fig. 6. Oval cyst of Amoeba.

Figs. 7—17. Stages in the development of a cyst as observed in a preparation kept warm in warm microscope chamber for four hours.

Figs. 18—21. Final stages of development of a cyst.

Figs. 22, 23. Two stages of a cyst left in warm chamber throngh the night.

Fig. 24. Cyst of Amoeba kept dry for two weeks.

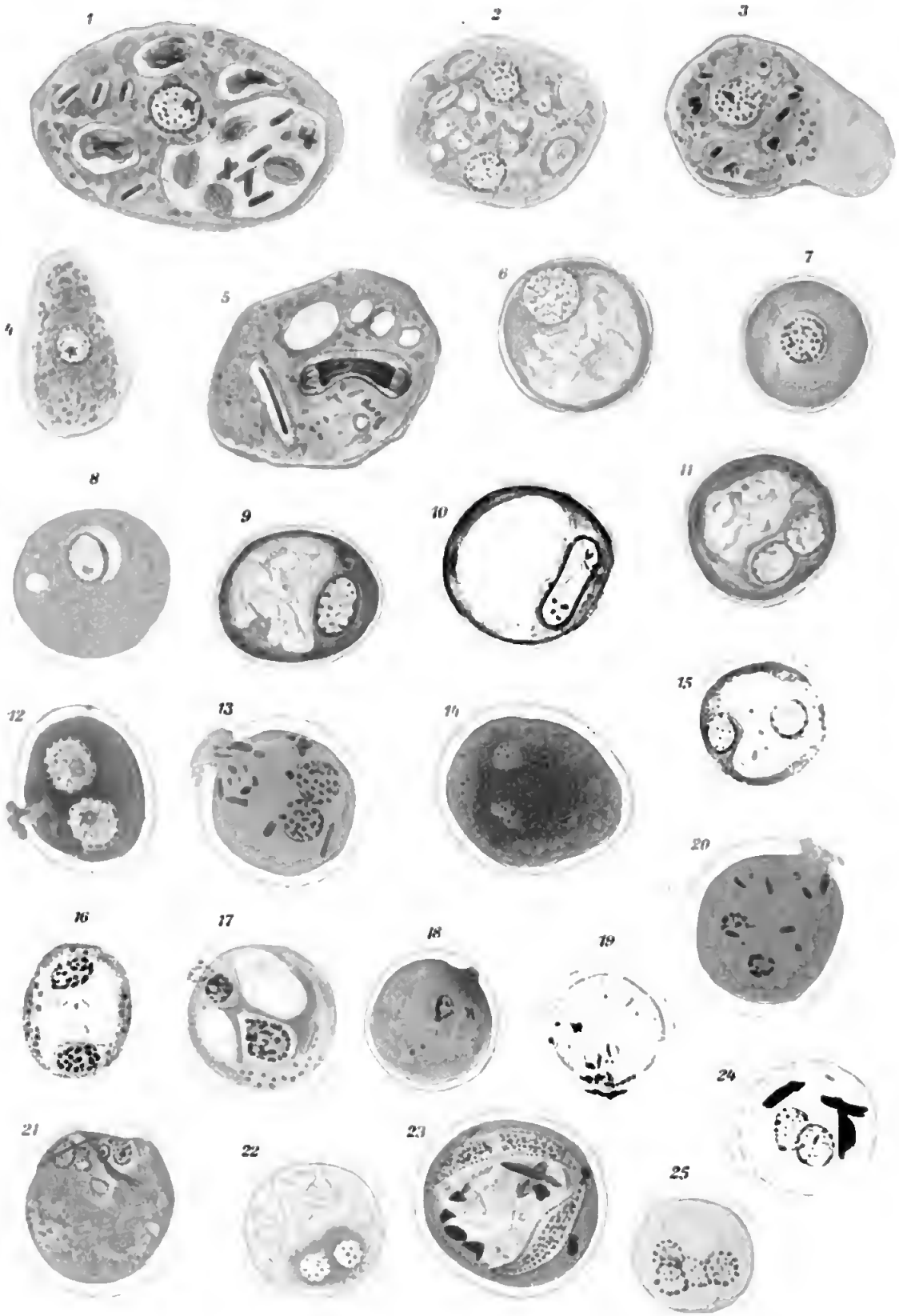
Figs. 25—30. Various forms of the Amoeba cultivated from faeces of mice.

Fig. 31. Semi diagramtaic representation of structure of *Trichomonas intestinalis*.

Fig. 32. *Trichomonas* with large vacuole full of cocci.

Fig. 33. Showing fibres in living *Lamblia*.

Figs. 34—35. Two forms of *Hexamitus*.



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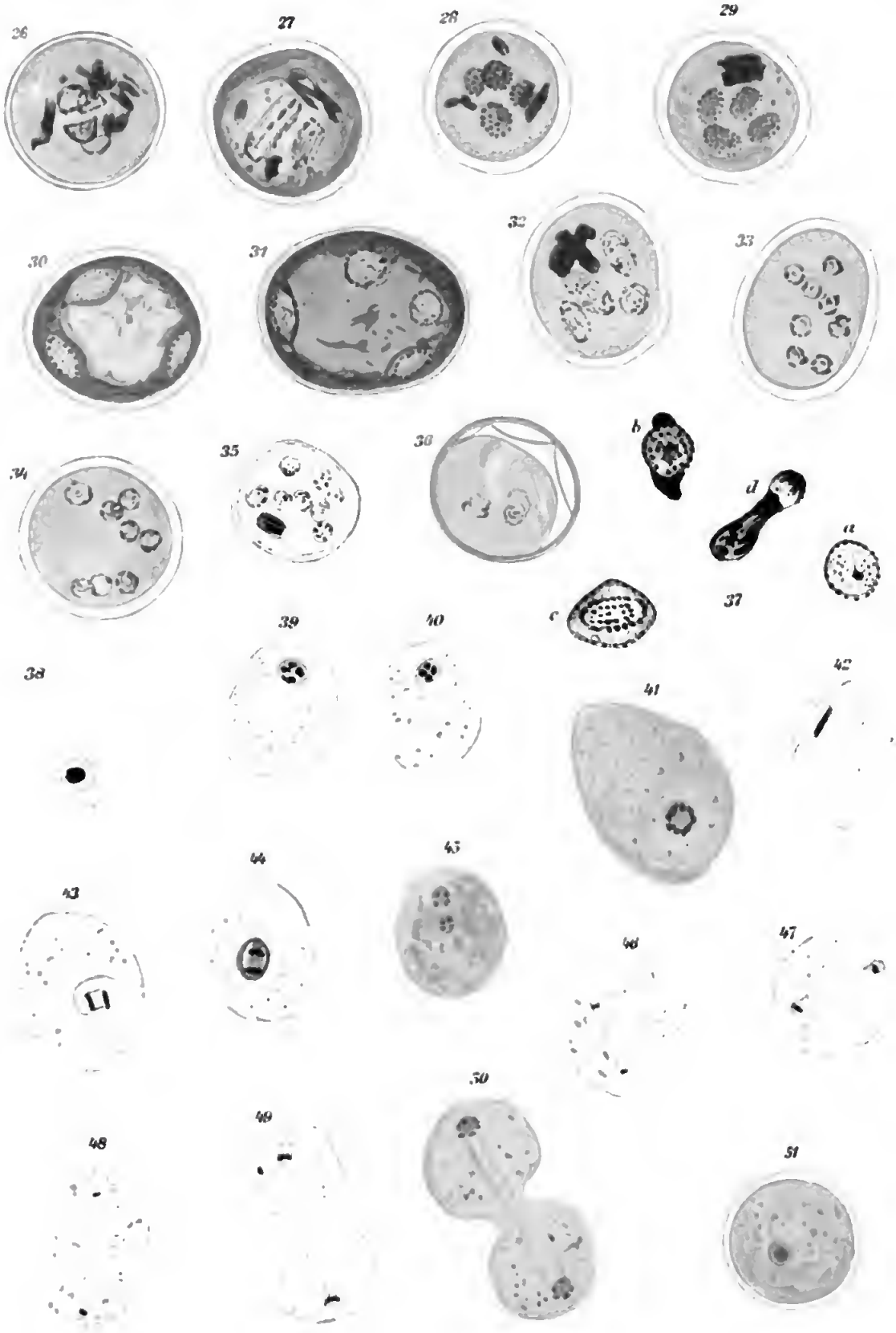

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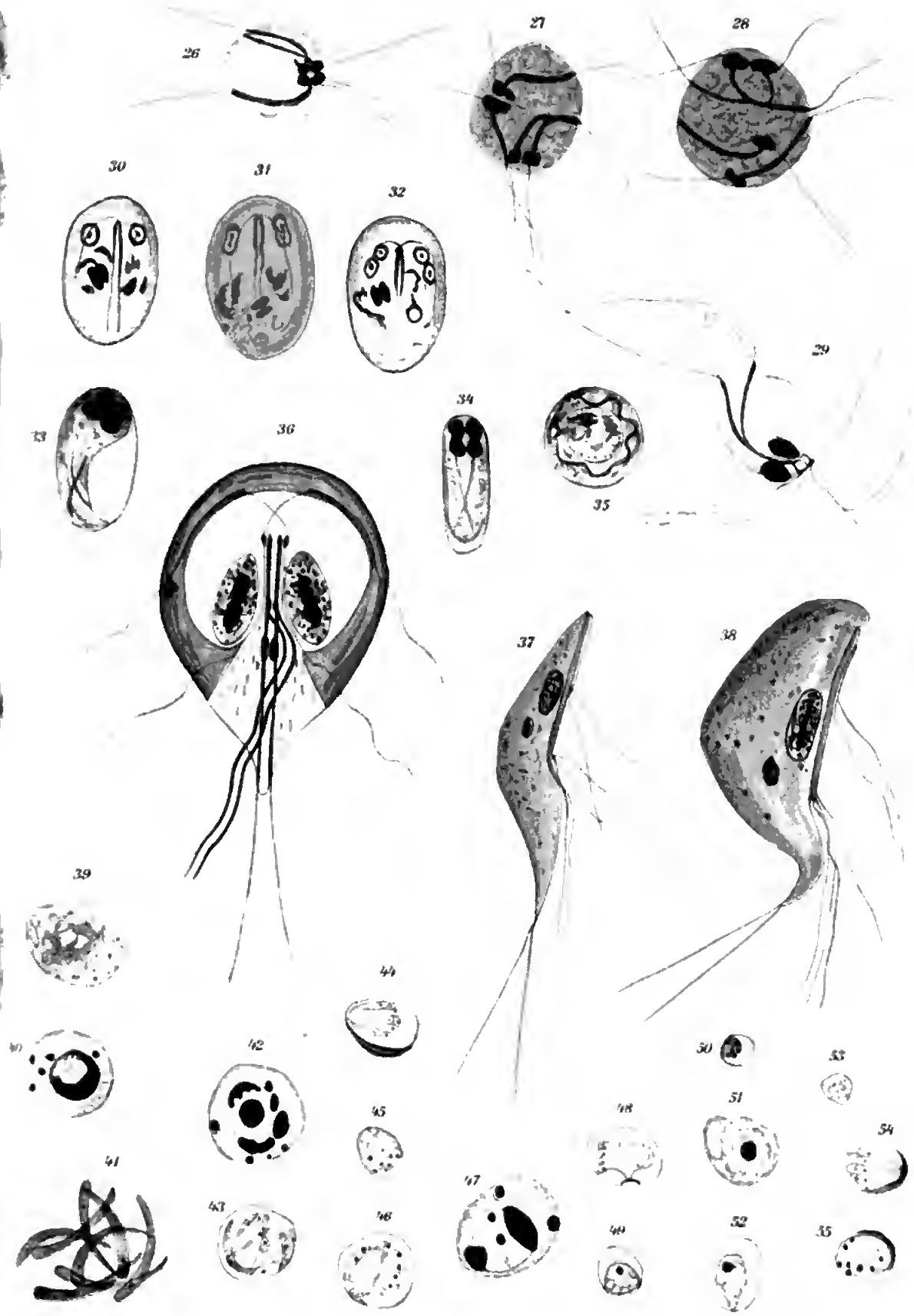

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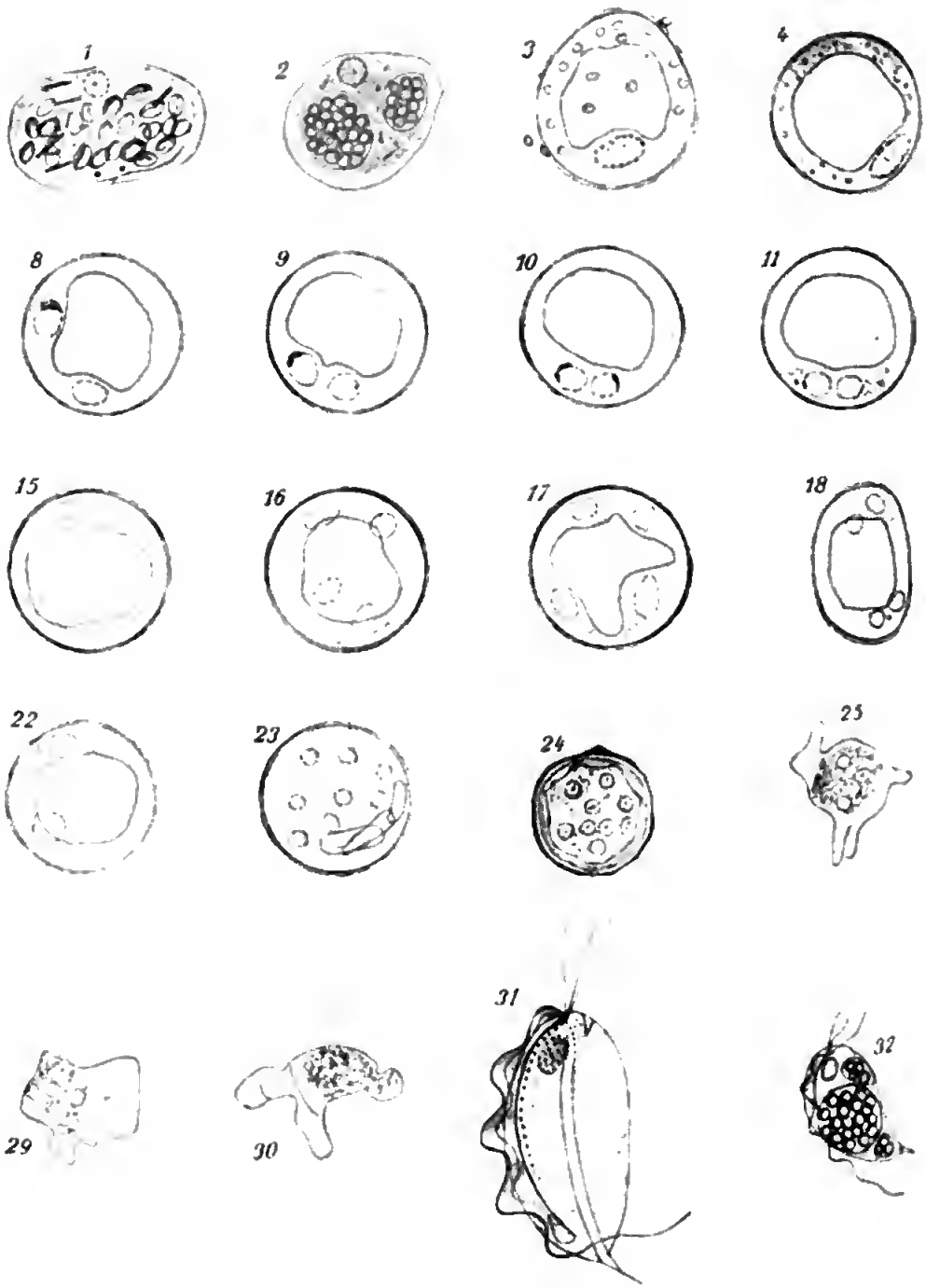

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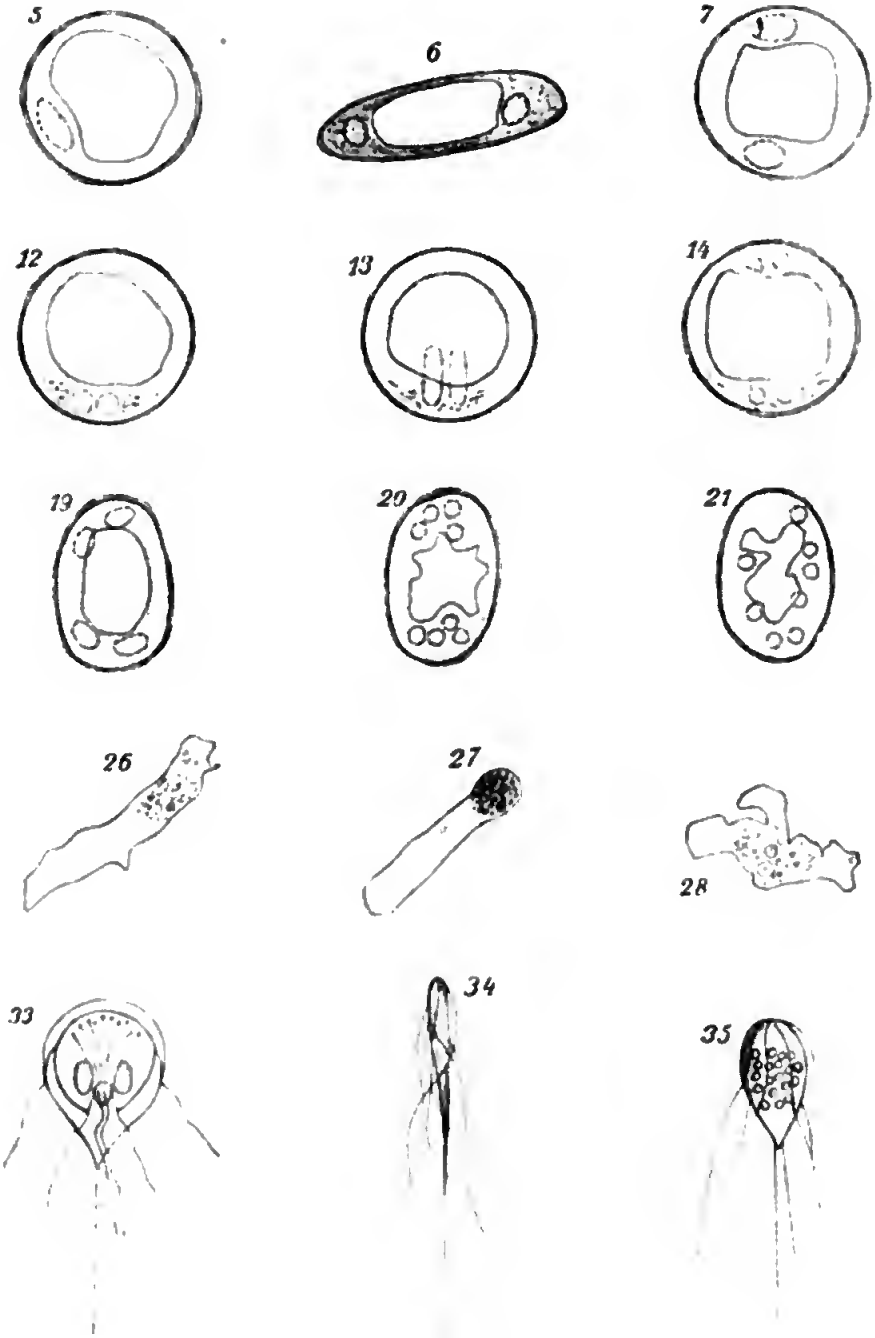

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