VIII. *Observations on Coprozoic Flagellates: Together with a Suggestion as to the Significance of the Kinetonucleus in the Binucleata.*

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[Plates 26–28.]

Contents.

Introductory.

Since publishing, with Mr. Lapage, the first account of the life-cycle of *Helkesimastix fæcicola*,* I have continued to work alone† on the biology and life-history of the flagellates occurring in simple dung-cultures. In the course of this investigation, I have made certain observations which I wish here to record, together with one or two suggestions which I have to offer. The work promises to occupy considerable time before it is completed, and in the case of some of the forms studied I am not yet able to describe the life-cycle in its entirety. Little or no attention has been paid hitherto to the protozoa active in dung, and the study of this fauna is probably not without interest and importance in connection with the subject of the soil-protozoa.

To distinguish those protozoa which are carried through the alimentary canal in a passive, encysted condition and become active and go through their life-history in the moist dung, Prof. Minchin has suggested, in the course of his lectures, the useful term *coprozoic*. The coprozoic fauna of goats and sheep is entirely different from their parasitic fauna, which has for its principal habitat the rumen. Neither the various specialised ciliates (of the fam. *Ophryoscolecidæ*) nor the flagellates (*Sphæromonas*, *Trichomastix* and *Callimastix*), some of which are invariably present

* 'Roy. Soc. Proc.,' B, vol. 88, p. 353.

† To my regret, Mr. Lapage has been obliged to relinquish his part in the research, owing to his having taken up medical studies.

in the rumen, ever occur in an active condition in dung-cultures; and, on the other hand, I have never found any of the coprozoic flagellates active in the rumen-contents, when freshly examined. These facts, readily determined because the sets of forms in the two cases are entirely different, afford important confirmation of the view, now generally accepted, that the *Entamœbæ*—the truly parasitic forms—are quite distinct from the *Amœbæ* which develop in fæcal cultures, *i.e.*, coprozoic species.

The Coprozoic Fauna.—The coprozoic protozoa occurring in goat- and sheep-dung are mainly flagellates. Ciliates are extremely rare: I have observed their presence (*Colpoda* sp., a holotrichous form) only on four or five occasions in all my numerous original cultures, over a period of more than two years, even when the cultures have been kept for several weeks. On the other hand, in simple infusions made of the hay which is the chief fodder of the goats and sheep under examination, ciliates (*Colpoda*, *Paramœcium* and another form, *Loxodes?*) soon begin to appear and become, one after another, abundant. Hence, either the cysts of these "ordinary" ciliates are not able, in general, to withstand the digestive juices of the alimentary tract, or else the dung-medium is not nearly so suitable an environment for their activities as the moistened hay alone. Conversely, I have never obtained certain very characteristic coprozoic flagellates (*Helkesimastix*, *Spiromonas*, *Phyllomitus* and *Copromonas*) in simple infusions of the fodder actually supplied to the animals in question. In addition, one of two varieties of *Amœba* (one being of the *limax*-type) often appears after a week or so in the dung-cultures, though not at all regularly. I have never seen *Mastigamœba*, though I have found this form in a fodder-infusion.

The flagellates which have been observed are the following:—*Monas* (*cf. vulgaris*), *Cercomonas* (*Cercobodo*) *longicauda* and perhaps another species, *Helkesimastix fæcicola* and *H. major*, *Bodo* (*Prowazekia*) *caudatus*, at least two "Bodos" without a kinetonucleus, one of which I refer to the genus *Heteromita*, Duj., the species being *globosa*, the other being regarded as a distinct type (*Heteromastix*, n.g.), *Spiromonas angusta*, *Phyllomitus undulans*, *Copromonas ruminantium* n.sp., and a most elusive uniflagellate form, which appears to be new, for which I propose the name *Proleptomonas fæcicola*. All these forms have developed from resting stages (cysts) present in the dung; there is no question of their being air- or water-contaminations. In nearly all cases, controls were made at the same time as the original culture, by boiling a portion of the fæces, in tap-water or normal saline, as the case might be, and then keeping under the same condition as the culture. These controls always remained absolutely sterile of protozoa. Moreover, one or two of these flagellates (*e.g.*, *Helkesimastix* and *Cercomonas*) will excyst and multiply in the rumen-contents, diluted with (boiled) tap-water and kept at the ordinary room-temperature, after four or six days (respectively).

Occurrence and Comparative Frequency.—Some of the flagellates occur, of course, more frequently and regularly than others, and there is also considerable variation in the time during which they remain present in the culture in an active phase. To

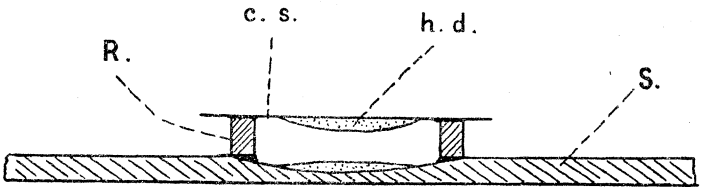
give a general indication, *Helkesimastix fæcicola* and *Copromonas ruminantium*, n.sp., occur most often (curiously, *H. major* is extremely rare, having been found only on one or two occasions, but I remain of the opinion that it is a distinct species, because of the uniformly larger size of its cysts). For some weeks I could not obtain *H. fæcicola* from the Lister Institute goats or sheep, although always from slaughter-house sheep; with that exception, however, it has cropped up in nearly all my cultures. I regard these two types as the most successful of the coprozoic flagellates, those best adapted, *i.e.*, to this particular environment. They both appear soon, always multiply rapidly, and soon conjugate and form cysts in large numbers. *Monas* is perhaps the next most important and regular member of this fauna. At first, it multiplies steadily, maintaining a fairly small form, but subsequently becomes larger and more sluggish. With this form, after a while, are associated large, highly refringent cysts, which are characteristic and unmistakable. Small individuals persist, however, in an active condition for a long time, until the culture is in the last stages of drying-up. *Spiromonas* has appeared more often of late than it did at first. Both of these forms, when present, begin to crop up after three days. *Spiromonas* also remains active, in a particular phase, so long as the culture is moist; a remarkable fact is that, in spite of all my efforts, I have not yet been able to find the cysts of this form (one reason for this will be given below). Perhaps the earliest form to appear is a small heteromastigine type, which I have found after two days; but it has occurred only seldom. *Bodo* (*Prowazekia*) *caudatus* has occurred more often, but neither of these types ever dominates the culture, so to put it, in the way that one or more of the other flagellates usually do. *Cercomonas* occurs irregularly and is generally the last to make its appearance, five or six days usually elapsing before I have noticed it. Neither does this form become very conspicuous by its numbers, though, as a rule, it persists for some time in an active condition. This form produces cysts much more readily in the nutrient medium than it does in simple dung-cultures.

The remaining types have been the rarest. *Phyllomitus* and the larger heteromastigine biflagellate (*Heteromita globosa*) have each occurred only in one original culture. The new uniflagellate (*Proleptomonas*) has been seen in three or four cultures, at long intervals. It occurs during the third to the fifth day, remaining scanty in numbers and then disappearing; and so far, I have been unable to obtain it again in sub-cultures.

Cultivation and Technique.

I have endeavoured to cultivate, separately so far as possible, all the above flagellates, with a view to obtaining their increased multiplication and more rapid development, and thus facilitating my efforts to ascertain their complete life-cycle. I have made use entirely of the observation-preparations referred to in our account of *Helkesimastix*, using dilute Lemco-medium as the "stimulating" agent. Without

exception, these coprozoic flagellates require an ample supply of air, if they are to thrive. Placing the cover-slip, on the under surface of which is the drop containing the flagellates, directly upon a hollowed-out glass slide does *not* provide sufficient air; the cover-slip must be supported on a ring, preferably about 1 mm. in thickness. It is hardly necessary to add that the ring must be well vaselined below, where it is in contact with the slide, and above, where the cover-slip rests upon it, or else the moisture will quickly evaporate. The thinner and more spread-out the drop of medium is—*i.e.*, the more it is a film and the less a hanging-drop—the better will the flagellates thrive as a rule. In order to allow for the evaporation of water-vapour



TEXT-FIG. A.—An observation preparation seen in vertical section. S, slide; R, ring; *c.s.*, cover-slip; *h.d.*, hanging drop. (×3.)

into the air-space and thus avoid concentration of the medium or even its drying-up, I put a small drop, well spread out, also in the hollow of the slide. I have successfully kept such an observation-preparation for more than a month, with a few "resistant" flagellates still active at the end of that time. The only drawback to the deep cell is that it rather interferes with illumination for high-power work. I find that a fairly low-power condenser, with a long working distance, is best; with a good beam of light on to the mirror, there is plenty of illumination for a 1/10th apochr. water-immersion, which is an excellent lens for the study of these forms in the living condition. (A sketch of an observation-preparation, seen in section, is given in text-fig. A.)

Most of the flagellates thrive well in the dilute nutrient medium. *Monas*, *Helkesimastix* and *Copromonas* succeeded from the first; *Bodo* (*Prowazekia*) had first to be accustomed to it, by adding a small quantity of the medium to the dung-liquid in which it was, that is to say, to three parts of sterile dung-infusion, one part of medium was added, and the drop for "cultivation" taken from this mixture. After a few sub-cultures, the strength of the medium was gradually increased, until this form also lived and multiplied rapidly in the dilute Lemco-medium alone. The cultivation of *Bodo* in this medium has led to an interesting result (see p. 399). On the other hand, I have not yet got *Phyllomitus* and *Spiromonas* to succeed in the nutrient medium alone, but have had to use equal parts of sterile infusion and medium. *Spiromonas* has proved very tantalising. A few months ago, I obtained a thriving culture, which I was able to sub-culture several times, and kept it going for more than a month. In recent attempts, however, for some reason or other it has always died off quickly in my observation-preparations, the forms becoming very

small and not going through the usual cycle, although I have tried many slight variants of the medium. Nevertheless, it persists for a long while in the original dung-cultures, although always too scantily to be of much use. I did not succeed in getting *Heteromita globosa* to thrive at all out of the dung-culture, from which, also, it soon disappeared.

I have also endeavoured to cultivate many of these flagellates on agar plates, using the same nutrient medium and having the plate of a thin consistency, containing not more than half per cent. agar-agar. Though I have not as yet persevered very much in these attempts, because of the success and much greater convenience of the observation-preparations, my experience has been that the thicker medium is not suitable for several of these forms. *Helkesimastix*, as already described, succeeds well. *Bodo caudatus*, after having become accustomed to the nutrient medium in observation-preparations, also thrives on plates; similarly *Cercomonas*. So far, I have had no success with *Heteromita*, *Spiromonas*, or *Phyllomitus* on plates.

The use of these observation-preparations also has the advantage that the cover-slip can be lifted off at any desired time and fixed. As a matter of fact, I first of all draw up part of the "hanging drop" into a fine pipette and then immediately drop the cover-slip on to the fixative. From the liquid in the pipette a smear is made and stained with Giemsa after fixation by osmic vapour. For the cover-slip film, I have found that an excellent fixative is a mixture of two parts of saturated aqueous sublimate and one part of absolute alcohol (or rectified spirit), to which is added 5 per cent. of acetic acid (S.A.A. mixture). This is better than either Schaudinn's fluid or aqueous sublimate-acetic mixture. I have also used Gilson's fluid. The best stain is iron-hæmatoxylin (using the long method); I often counterstain with alcoholic eosin (rarely with Lichtgrün picric), with a view to making the flagella more readily visible. The ring of vaseline remaining on the cover-slip presents no difficulty. Most of it is carefully scraped off some time after fixation and what is left is entirely dissolved in the xylol before mounting.

Movements.

An interesting point brought out by the study of these flagellates is the variety and distinctness of their movements. These furnish, in my opinion, a generic character of considerable importance. Any one of the above-mentioned forms can be distinguished at once by its movements alone, apart from any other character. In the case of certain forms (especially *Heteromita*, *Spiromonas* and *Phyllomitus*), the actual movements of the flagella in active individuals are very difficult to catch, excepting with the aid of dark ground illumination (using a so-called ultramicroscopic condenser and an oil-immersion objective), which is of very great assistance.

Monas.—In a certain phase or under certain conditions, small forms of *Monas* (*cf. vulgaris*) are elongated and oblong in shape (fig. 42). These have a steady forward movement, travelling fairly fast, by the lashing to and fro of the main

flagellum, as a whole. Larger, rounded forms, which constitute the more usual phase, have a more indefinite, irregular movement, performing usually a short glide or sweep, generally in an arc, then stopping and then going on again. In neither case is there any vibration or turning over of the body. When at rest, however, these forms frequently exercise little spasmodic jerks or vibrations, tending to partly rotate the body, but not causing displacement; these are produced by the small accessory flagellum, I think.

Cercomonas.—The movements of this form have been described by WENYON.* I need only draw attention to the fact that, when the flagellates are in the most active condition, being then pear-shaped or elongated, the undulatory vibration of the body is produced by the active movement of the attached tail-flagellum. On the other hand, the forward movement is chiefly caused by the somewhat irregular lashing of the long anterior flagellum, which often causes the body as a whole to swing sideways to some extent. This movement is very characteristic. In oval, more sluggish individuals, the only movement is produced by the anterior flagellum and I am inclined to think that, in these conditions, the posterior flagellum may become shortened or partly disappear.

Helkesimastix.—There is nothing to add to the account previously given. There is a great resemblance between the active undulatory movement (when the individual is not gliding on the surface) and the similar movement produced by the posterior flagellum in *Cercomonas*, and I am now inclined to regard *Helkesimastix* as derived from a *Cercomonas*-like form by the loss of the long anterior flagellum, rather than from a *Heteromita* or a *Bodo*.

Bodo (*Prowazekia*).—The movements of these forms are well known. In *B. caudatus*, I have never seen the constant, very energetic vibrations associated with the characteristic stoppages and sudden springing or dancing movement found in *B. saltans*. (I have observed this latter species once or twice in hay-infusions† and am able to confirm ALEXEIEFF'S discovery that it is binucleate.) Further, in *caudatus*, the posterior flagellum is scarcely ever trailed passively behind, for a considerable distance, as often occurs in *saltans*. The short, anterior flagellum is always directed forwards; it is usually in a state of vibration, but may be held for a short time stiffly, curved like a hook.

The Small Heteromastigine Form (Heteromastix, *n.g.*) *and* Spiromonas.—The type of movement in these forms is quite distinct from that of *Bodo*, lacking any vibratory character. The flagellar movements are similar in both, though the character of the progression differs somewhat in the two cases. In neither form is the shorter (anterior) flagellum kept mainly or entirely in front of the body, as in *Bodo*. In *Heteromastix*, both flagella act very much like oars, lashing or sweeping vigorously

* 'Quart. Journ. Microsc. Sci.,' vol. 55, p. 245 (1910).

† I think this species also occurred occasionally in the dung-cultures at the beginning of this research, when we were chiefly occupied with *Helkesimastix*, but I have never found it in them since.

to and fro, at the sides of the body. In *Spiromonas* the rather shorter flagellum (corresponding to the anterior one) behaves similarly, but the rather longer (posterior) one is frequently directed backwards and its movement reminds one more of a boatman propelling a boat by one oar, from the stern. The actual mode of progression of *Heteromastix* is easier to recognise than to describe. It progresses quickly, though not very steadily, rolling or wobbling slightly from side to side, though not turning over on its axis. Small forms can be distinguished from the small forms of *Spiromonas* (before the latter have become spiral) by the fact that their progression is not so extremely rapid and the individual never goes through more than one or two fields of a 1/6 lens before changing its direction considerably. *Spiromonas* displaces itself more rapidly than any other of these flagellates, with the possible exception of the darting uniflagellate. It is untiring; I have never seen it at rest except during division. Except possibly in the very youngest stages, the course of progression is a spiral, the body turning round on its long axis during the forward movement, now in one sense, now in the other. The rotating movement is produced by the action of the shorter flagellum, working laterally, helped by the spiral twist of the body in the spiral forms. The propulsion is caused chiefly by the more posteriorly directed flagellum.

Phyllomitus.—The interesting peculiarity of this form is that the long and short flagellum, doubtless representing an anterior and a posterior one, are partly joined together by a band, just as was shown by STEIN in his original figures, a fact which hitherto has never been confirmed. In consequence of this attachment, both flagella are always directed backwards in life, for the long one, which is much longer than the body, represents a trailing flagellum. The distal portion of the short flagellum is free from the band. It must be understood that neither the band nor the flagella are attached to the body; the former is not comparable to the undulating membrane, for instance, of a trypanosome. In the proximal region, where the band is, the joined flagella pass across a conspicuous depression or groove in the side of the body, towards the anterior end, which represents a peristome. The body shows a constant fine trembling or flickering, produced by the vibration of the short flagellum, modified by the band, which is very characteristic. This movement probably causes the band to function somewhat in the manner of the undulatory membrane of certain ciliates, aiding in wafting food-particles into the mouth-area, at the base of the depression. The long flagellum at times vibrates actively, but may be passively trailed behind. The body sometimes shows a tendency to rotate.

Copromonas ruminantium, n.sp.—The movements of this form are quite similar to those of *C. subtilis*, as first described by DOBELL,* and need not be again described.

The Acicular Uniflagellate.—The movement is unlike that of any other flagellate that I know. The creature darts very actively forwards, practically in a straight line, presumably by powerful lashing of the very long flagellum. Every now and

* 'Quart. Journ. Microsc. Sci.,' vol. 52, p. 75 (1908).

then it reverses the action in some manner and, as it were, backs along its own track abruptly for the length of its body or further; then it darts forwards again.

The Large Heteromastigine Biflagellate (Heteromita globosa).—This form has a very steady forward movement, progressing by the rapid, regular vibration of the short anterior flagellum. The long posterior flagellum trails passively behind, lying along the upper surface of the body for the first part of its length. This flagellum behaves, in short, very much like the single backwardly directed flagellum of *Helkesimastix*.

General Observations on the Flagellates.

I hope in time to obtain a complete knowledge of the life-cycle of most of these coprozoic forms. I propose here to give only a general outline of the observations already made, because of their bearing upon one or two suggestions which I wish to put forward. In the case of those forms in which I have now a good idea of the life-cycle, my attention has been directed particularly to ascertaining whether syngamy occurs, or is absent, and how to distinguish it from division.

Monas vulgaris.—Syngamy undoubtedly occurs in this form, and the process strongly recalls the syngamy of *Oicomonas termo*, as described by the late Lieut. C. H. Martin.* I have not seen the actual union of the two gametes, but the zygote, containing the two gametic nuclei (figs. 43, 44), is a large, rounded body, much larger than any of the single uninucleate individuals. There is no question of this phase representing a stage in division, because it proceeds to encyst, during which process its appearance very greatly resembles the corresponding stage in *Oicomonas* (*cf.* my figs. 45–47 with Martin's figs. 12–14, Plate 10). I have not yet come across cysts showing maturation or reduction changes in the gametic nuclei, nor the actual fusion into a synkaryon. The two nuclei seem to remain distinct for a long time. Very generally, there are numerous deeply staining granules in the cytoplasm—*not* representing bacteria—which form a zone or ring around a large vacuole. The cyst-membrane is at first very thin and only after an interval becomes thick-walled and refringent. Now and again, one of the gametic nuclei is slightly smaller than the other (fig. 46), perhaps indicating a slight difference in such cases in the size of the two gametes. Division is, of course, a quite different process, and takes place, as in all these flagellates, quickly; an early stage is seen in fig. 3 (Plate 26).

The Acicular, Monadine Flagellate.—As noted, this form is so elusive that I can only point out its morphological characters. It might seem almost impossible that a simple protomastigine uniflagellate—not a specialised parasitic form—of a new type yet remained to be described, but I believe this form is such a one. The body is very elongated and narrow, if anything more so in life than is seen from the stained preparations (figs. 4–6); it is not plastic or semi-amœboid at all. I do not think it ingests bacteria and regard it as a saprozoic form. The flagellum is very long and

* 'Roy. Soc. Proc.,' B, vol. 85, p. 393 (1912).

there are no accessory small flagella. An interesting point is that the flagellum is continued backwards for some distance from the anterior end (which is finely pointed), probably as a long rhizoplast, and terminates in a prominent basal granule. The nucleus is situated about the middle of the body. There is no kinetonucleus. Division has not yet been observed. It is certain that this form does not belong to either the genus *Monas* or *Oicomonas*.* It reminds me of nothing so much as a leptomonad, *without a kinetonucleus*. It may very well be a present-day representative of the ancestral type from which the genus *Leptomonas* and allied genera have been derived by the development of a kinetonucleus. I propose to call this form *Proleptomonas fæcicola*, n.g., n.sp. The dimensions of the body, I should add, are from 7 μ long by $1\frac{1}{4}$ μ broad to $8\frac{1}{2}$ μ long by $1\frac{3}{4}$ μ broad; the length of the flagellum, not including the rhizoplastic portion, is from 16 μ to 21 μ. These measurements are of individuals fixed with osmic acid vapour, so they are approximately correct.

Cercomonas (*Cercobodo*).—In the first place, with regard to the vexed question of the nomenclature of this type, there cannot be the slightest doubt, LEMMERMANN notwithstanding,† that the flagellates which have been described of recent years (*e.g.*, by WENYON‡ and ALEXEIEFF§) under the name *Cercomonas* are of the same generic type as those seen originally by DUJARDIN and STEIN, and thus named. The tail flagellum is so delicate and often so extremely difficult to see, that the earlier workers may well have been uncertain in regard to it, as distinct from a caudal prolongation of the body. But the long anterior flagellum, the form and very plastic or semi-amœboid character of the body in certain phases are exactly as depicted in the old figures; and, as I shall again show below, those old figures of the living creatures are wonderfully accurate. Further, from the recently published figures of *Cercobodo* (*e.g.*, as regards the nucleo-flagellar relations, the semi-amœboid body-form, etc.), it is clear that this form also belongs to the same genus. In choosing between *Cercobodo* and *Cercomonas*, there are points in favour of both names, and it is not easy to decide between them. On the one hand, this type is biflagellate and heteromastigine, whereas the family named after *Cercomonas* (*Cercomonadinæ*) comprises otherwise uniflagellate forms. But, on the other hand, this form is very unlike *Heteromita*, or *Bodo*, or *Spiromonas*, or other hetero-

* It is hardly necessary to add that there is no question of this form representing merely a young stage of *Copromonas*. Not only is the movement, both of the flagellum and of the body as a whole, entirely different in the two cases, but the new flagellate has no trace of a cytostome or oral groove characterising *Copromonas*. Further, although I have not actually seen the dissolution of the cyst-wall and the liberation of the active individual in *Copromonas*, in fresh observation-preparations of a "pure" (mixed) culture of *Copromonas*-cysts, after 24 hours there are numerous active individuals of the usual size and form of *Copromonas* and moving in the same steady manner, and no sign of anything like this new flagellate.

† 'Arch. Hydrobiol.,' vol. 8, p. 555 (1913).

‡ 'Quart. Journ. Microsc. Sci.,' vol. 55, p. 241 (1910).

§ 'Arch. Zool. Exp.,' Ser. 5, vol. 6, p. 491 (1911).

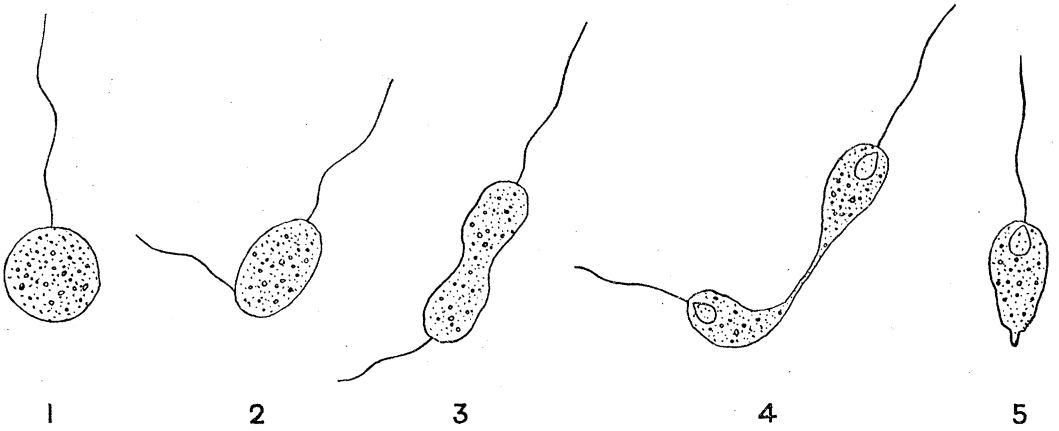
mastigine forms. In all the latter, the anterior flagellum is the shorter, the trailing flagellum being the longer and stronger; here it is the opposite. Again, in none of these others is the tail flagellum closely and permanently in contact with the body as in *Cercomonas*. Moreover, in the plastic and amœboid nature of the body, *Cercomonas* much more closely resembles *Monas* and similar forms. Further, the life-cycle, which, as I thought would be the case, very closely resembles that of *Helkesimastix*, differs considerably from that of the above-named types. Lastly, but not least, *Cercomonas* is the name that was given to the creature originally. Hence I am inclined to retain this name in preference to that of *Cercobodo*, as WENYON and ALEXEIEFF have done, and differing from LEMMERMANN and NÄGLER.* I may add that there is not the least necessity to create the name *Cercomastix* for *Cercomonas parva*, as LEMMERMANN has done.

With regard to the question of the different species of *Cercomonas*, in the case of some, at all events, I think the distinctions on which they are based are ill-founded. The selection from the original figures of *longicauda* and *crassicauda*, for instance, given by KENT in his 'Manual,' shows clearly that only one form is concerned. I have certainly worked with *longicauda* mostly. Now, not only does the body vary very greatly in size and form, especially when division is actively taking place, but the length of the tail flagellum is also extremely variable, much more so than that of the anterior one (*cf.* my figs. 48–53). It is useless to attempt to distinguish *crassicauda* from *longicauda* on those grounds, as ALEXEIEFF (*loc. cit.*) has done. His figures, purporting to be of *crassicauda*, are simply of young forms of *longicauda*. I think the species *crassicauda* must be merged in *longicauda*. (As regards the question of the size of the cysts, see below.) I have, on one occasion, obtained a species which I am inclined to consider as distinct from *longicauda*, though I am not yet certain. Large adults do not attain to the same size as those of *longicauda*, and, moreover, this form will not grow well on agar-plates, on which the latter thrives. It is, however, certainly not "*crassicauda*."

There is little to add to what is known about the morphology. The tail-flagellum, as is well seen in *lightly stained* Giemsa specimens, is distinctly thinner than the anterior one (*cf.* fig. 8). I am of the opinion this posterior flagellum may be at times temporarily absent, or at least partially absorbed. In life it is always in contact with the body, though not actually attached to the latter; on Giemsa-smears it is often seen separated from the body; rarely so in wet-fixed films. In nutrient media (especially on plates), but only very seldom in thin liquids (*e.g.*, the simple dung-cultures), definite, clear *purely ectoplasmic* pseudopodia are thrust out and withdrawn; this is a feature quite distinct from the general irregular and metabolic character of the body-protoplasm as a whole. Such forms are well figured by NÄGLER (*loc. cit.*, Plate 6). This feature never occurs in a true heteromastigine type, nor have I observed it in *Helkesimastix*.

* 'Arch. Protistenk.,' vol. 34, p. 138 (1914).

The process of syngamy is almost identical with that in *Helkesimastix*. Stages in it have been previously seen but regarded as representing division. Thus KENT'S fig. 20, Plate 14 (after STEIN), and WENYON'S text-figs. 1 and 4 (*loc. cit.*) showing forms with doubled flagella, really represent conjugation. A very great help in distinguishing between individuals undergoing fission and those undergoing syngamy (union), which from my long observations on these coprozoic flagellates will prove, I believe, of general application in such cases, is the fact that division is a very short process, whereas conjugation is a very long one. Moreover, in division, the body *practically* ceases to displace itself, only undergoing little jerky movements where, or so long as, the flagella persist. In all the forms which I have studied, division is usually a matter of only 15–20 minutes at the outside. In *Cercomonas*, the



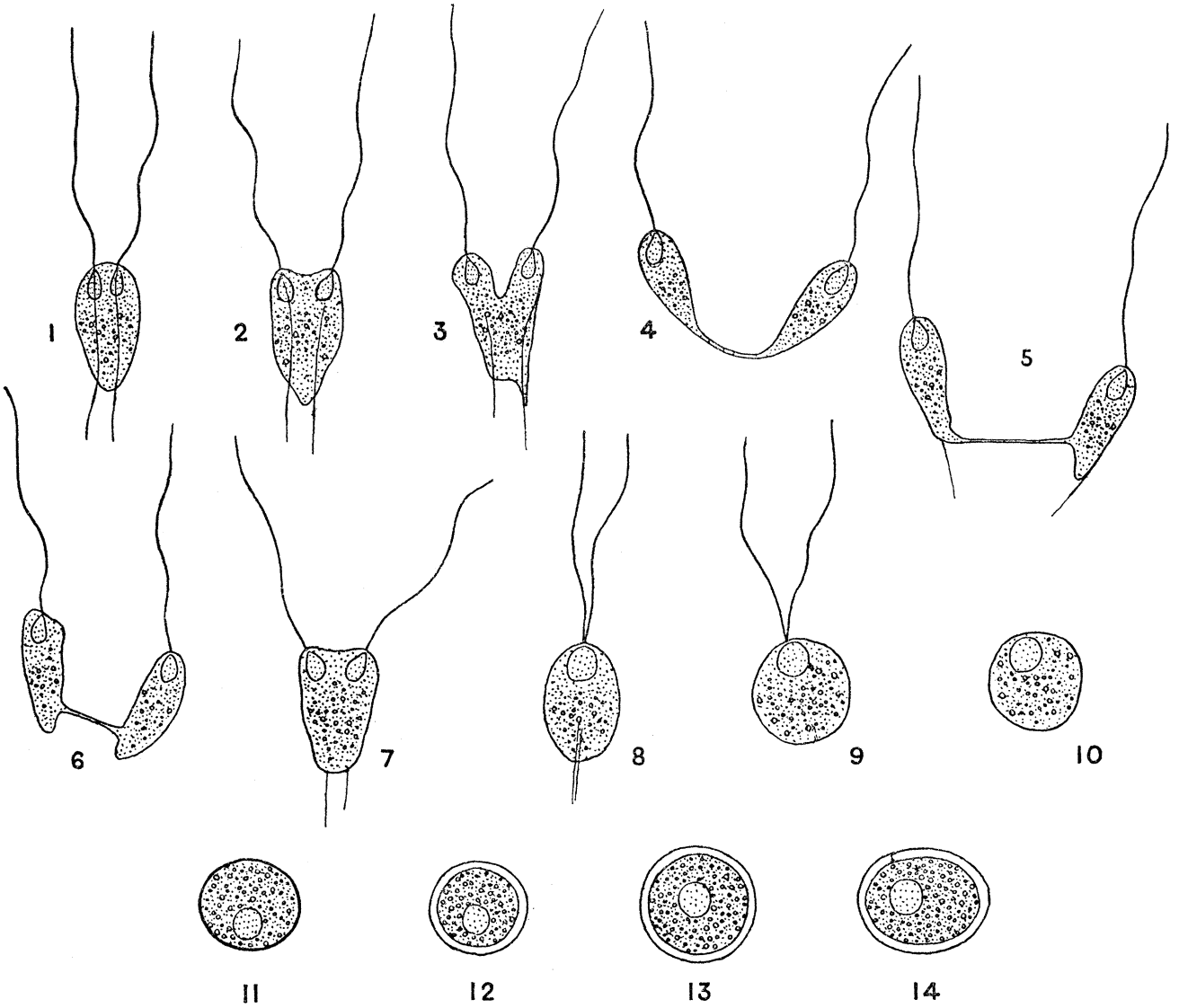
TEXT-FIG. B.—Division of *Cercomonas longicauda*. Tail flagellum not seen (either greatly shortened or disappeared). In 2 the daughter (anterior) flagellum has appeared. In 4 the daughter nuclei are reconstituted.

progression of an individual about to divide ceases, the body becomes rounded and the long anterior flagellum becomes shortened (sometimes considerably), and may, I believe, be occasionally quite absorbed (fig. 9, Plate 26). The tail flagellum is always much shortened and may also disappear (*cf.* below). After the body has been motionless for 5 minutes or so, the shortened anterior flagellum waving sluggishly, a new small (anterior) flagellum appears, at first near the old one, but then becoming displaced to one side (text-fig. B 2). The clear, pear-shaped (nuclear) area near the original anterior end of the body has disappeared and, owing to the granular contents of this form, I could not trace the origin of the two daughter-nuclei in life; but they become visible again about the time of the division of the body-protoplasm (fig. B 4). (I must repeat here, what was said in our account of *Helkesimastix*, that I hope, some day, to give more cytological details of the processes here described.) Next, the body from being rounded becomes ovoid, then elongated, and then dumb-bell shaped, a constriction appearing in the middle (figs. B 3 and 4). By this time, the new daughter-flagellum has passed to the opposite end of the body and, both flagella vibrating more actively, the two daughter-halves are drawn apart and at length

separate. I have been unable to see the tail-flagella at all during division (observing the process in life). They are often not present at first (*cf.* fig. 10), and, where present, are doubtless closely applied to the body and not distinguished in life. Both WENYON and NÄGLER indicate this variability (in different cases) in regard to the presence or absence of the flagella during the actual division. My figs. 9–11 of Giemsa-stained specimens show just the same nuclear bars as figured by WENYON from iron-hæmatoxylin preparations. I wish to emphasise the value of Giemsa-stained preparations for showing the flagella of these flagellates. In the case of an extremely delicate filament applied to the body, like the tail-flagellum of *Cercomonas*, I can always be sure whether it is present or not in individuals stained with Giemsa, but I cannot always feel quite certain in the case of iron-hæmatoxylin films. Properly used, the Giemsa stain is of very great value in the study of these flagellates.

After division has been going on rapidly throughout the culture for a couple of days or so, its place is taken by syngamy. Just as in *Helkesimastix*, numerous forms are seen, progressing more or less actively, with doubled flagella, two long ones, of approximately equal length, waving about in front, and two delicate ones trailing behind. I have not yet seen the actual union of two separate individuals, but I have no doubt that this happens just as in *Helkesimastix* (*cf.* our figs. 39 and 40, *loc. cit.*). These double forms vary somewhat in size, most being very large, but a small proportion are smaller. Equally here, these forms invariably go on to encystment, and do not divide. A series of stages in the sequence of events, from sketches made during life, is drawn in text-figs. C 1–10. At first the two sets of flagella remain well apart, *i.e.*, their points of origin are not approximated to one another, near the middle line of the body. The two somewhat pear-shaped areas, indicating the position of the two gametic nuclei, are also distinct. This condition may persist for three or four hours, perhaps longer in some cases. While this phase lasts there is just the same looseness of union and independence of the two gametic halves that characterises syngamy in *Helkesimastix*, at the corresponding phase. I have seen one conjugating pair break down twice into two halves, of approximately equal size, which remained connected only by the thinnest protoplasmic thread (fig. C 5); in one case I lost the thread for a second and was almost sure they *had* separated. But no! After 15 or 20 seconds the thread contracted, and the two halves ran together and "flowed" into one zygote again. It is remarkable that separation does not occur in such cases, when it takes place without the slightest hesitation in fission, often after a similar drawing-out of the cytoplasm. The next stage is that the two sets of flagella come to lie nearer together in the middle line, though there is no definite, prolonged "oval" stage, as in *Helkesimastix*. The two flagella soon appear to originate together, and the two posterior ones can scarcely be separated. Another point of difference is that the nuclear union takes place about this time, *i.e.*, earlier than in *Helkesimastix*. Instead of two nuclear areas, there is now only one large one (fig. C 8, also fig. 54).

In this stage, which may continue for quite a long period (two or three hours), the zygote can still be irregular and semi-amœboid. But at length it stops progressing, and gradually becomes rounded; the flagella then cease to move, and suddenly can be no longer seen; they have been absorbed. How long a period elapses before



TEXT-FIG. C.—Conjugation in *Cercomonas longicauda*, from sketches made at time of observation. The last three figures (12–14) show different sizes of fully developed cysts.

the definite cyst-wall is formed I am unable to say. In a culture which on one day contained numbers of these double forms, in various stages of syngamy, 18 hours later the great majority were encysted, the active forms remaining over being single individuals. As in *Monas*, there seems to be a single-contoured membrane developed first of all (fig. C 11), which is later transformed into the thick cyst-wall (figs. C 12–14). The fully developed cyst-wall is much more prominent than in

Helkesimastix. The cyst as a whole does not present, however, the characteristic refringent appearance of *Monas*-cysts; this is because, in *Cercomonas*, there is not a large space left between the body-protoplasm and the wall, as is the case in *Monas*. The size of the cysts varies appreciably; the diameter averages from 7 μ to 9 μ. Here and there a cyst of oval form is met with (fig. C 14). The cysts are not infrequently surrounded by a cluster of bacteria.

Bodo (*Prowazekia*) *and Heteromita*.—I leave a consideration of these types to the last.

Spiromonas angusta.—The flagellate which I am inclined to identify with DUJARDIN'S *Heteromita angustata*, and, following KENT, to place in the genus *Spiromonas*, on account of the very characteristic spiral twist of the body in one phase of the life-cycle, is a most interesting type, and shows very great polymorphism; indeed, I was at first uncertain whether the different phases all belonged to one form, notwithstanding the general similarity of their movements.

In describing the life-cycle in outline, I begin with the earliest stages, which make their appearance first in the fresh cultures. These are remarkably small forms, very active, and, until I had obtained stained specimens, I regarded them as short, thick bacilli. The body-form is elongate-oval, the size of the smallest individuals being not more than $2\frac{1}{2}\,\mu$ by $1\,\mu$ (figs. 20 and 21). The flagella of these minute forms are from two to more than three times the length of the body, the longer one representing the posterior flagellum. At first they arise close together at the anterior end, each originating from a distinct blepharoplast. Growth goes on rapidly during the two or three days after these forms appear, no division taking place at this time. Different stages in growth are seen in figs. 22–25. As the body elongates it gradually becomes spirally twisted, these individuals at last markedly resembling a very large spirillum, excepting for the fact that with good illumination the flagella can be seen. In this phase the creature is obviously the *Spiromonas* of the older writers. In this form the body averages about 12–13 μ in length by $1\frac{3}{4}$–2 μ in width. The two flagella do not now arise from the anterior end but from a slight distance behind, and their points of origin may be separate (though not always). The nucleus is usually situated about the middle of the body, though it may be nearer to the hinder end. There is certainly no mouth aperture, and I do not believe nutrition is ever other than saprozoic. I have never seen individuals containing ingested bacteria.

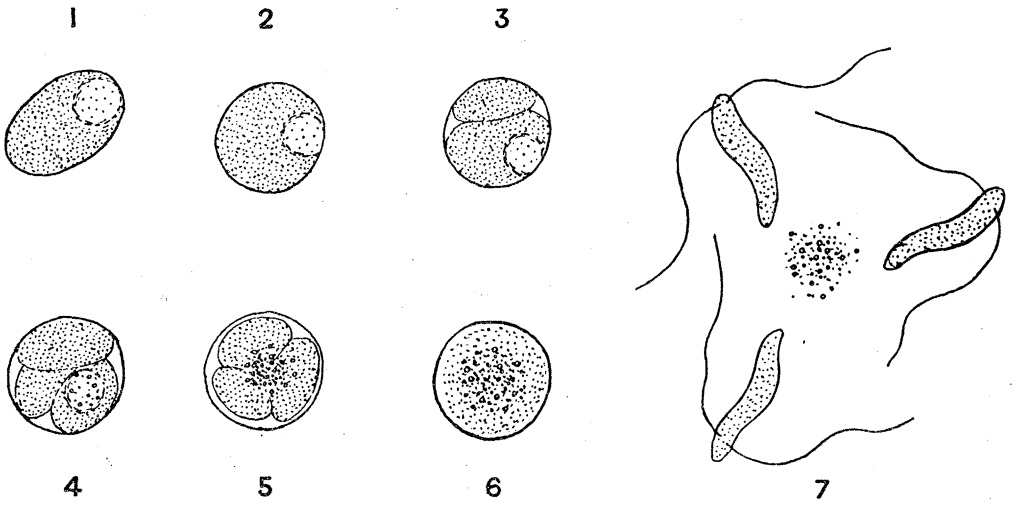
In the next stage, which I call the "bean" stage, the body is quite different in appearance. I am not sure yet whether the beans can be developed, in the first place, directly from the spiral individuals or only after conjugation, but I think it is likely that the former course can be followed (*cf.* fig. 26). The beans vary slightly in size, growth taking place to some extent after this form is attained. A large-sized individual (fig. 27) measures about 10 μ by $5\frac{1}{2}\,\mu$, the flagella being at the most not more than twice the length of the body. The anterior end is often very slightly pointed (fig. 59), the posterior end being broadly rounded; at times, especially in the

living condition, just a faint indication of the original spiral form can be detected. Neither in the spiral nor in the bean phase have I ever seen any change occurring in the body-form; *i.e.* it is never semi-amœboid. The nucleus is slightly in the anterior half of the body. A characteristic feature of the large beans is the presence of a clear area (in life) in the hinder region, which is usually also seen unstained in Giemsa preparations. Iron-hæmatoxylin preparations show, however, a number of closely packed granules or bodies in this region (figs. 59–63) which take up the stain intensely. I think these represent a secretion which aids, probably by swelling, in rupturing the delicate cyst-membrane formed during the process of division. This is an interesting point, because here there is no question of the cyst-membrane being dissolved by a fresh development of ferments or bacterial products in the environment (as occurs in the case of a fresh culture, when the resistant cysts of many of these flagellates are dissolved).

The large beans always proceed to divide. The body comes quite to rest, becomes rounded, and the flagella entirely disappear (figs. 61 and 62); then a very delicate membrane is formed, *i.e.* division occurs inside a cyst. This is invariably the case; I have never seen fission, whether of spirals or of the beans, taking place in an active condition, and do not believe it ever occurs. Division is most usually tripartite, three daughter individuals being formed; exceptionally, rather smaller beans may divide into two and rather larger ones into four. From the time a bean becomes rounded and motionless the process of division takes as a rule from 15 to 20 minutes; in rather old observation-preparations, where the environment is becoming unfavourable, it may take longer.

Successive stages in tripartite division are seen in text-figs. D 1–7, from sketches made during the actual observations. First, about one-third of the body begins to be segmented off; after an interval another segment makes its appearance, and the body protoplasm is now divided up into three approximately equal portions (text-figs. D 4 and 5). The clear area has undergone alteration. It is not so compact and has apparently become resolved into a lot of more or less separate granules (*cf.* also fig. 65). A moment before the actual liberation of the daughter-individuals the whole mass swells, and the outlines of the segmented individuals are no longer distinct (fig. D 6). Suddenly, instantaneously, the three daughter-individuals rush away in three different directions, leaving nothing behind but a small cluster of unused granules. The actual separation may be so rapid that it cannot be seen; one simply observes the three individuals swimming away out of the field. The liberation is not, however, always so rapid. Sometimes the cyst-wall is only dissolved at one point, and the daughter-individuals squirm out, one after another, from this one point; after the first has come out, the other two (or, rarely, three) can be seen rolling over one another inside the cyst-membrane for an instant or two before they are liberated. Lastly, in old observation-preparations, the division may take a comparatively long time (three-quarters of an hour or more) and the daughter-individuals become active

and squirm about inside the cyst for a couple of minutes or so before coming out. The daughter-individuals are always spirals (fig. D 7), not quite so slender in form as those first developed in a culture.



TEXT-FIG. D.—Division in *Spiromonas angusta*. (From sketches made at time of observation.)

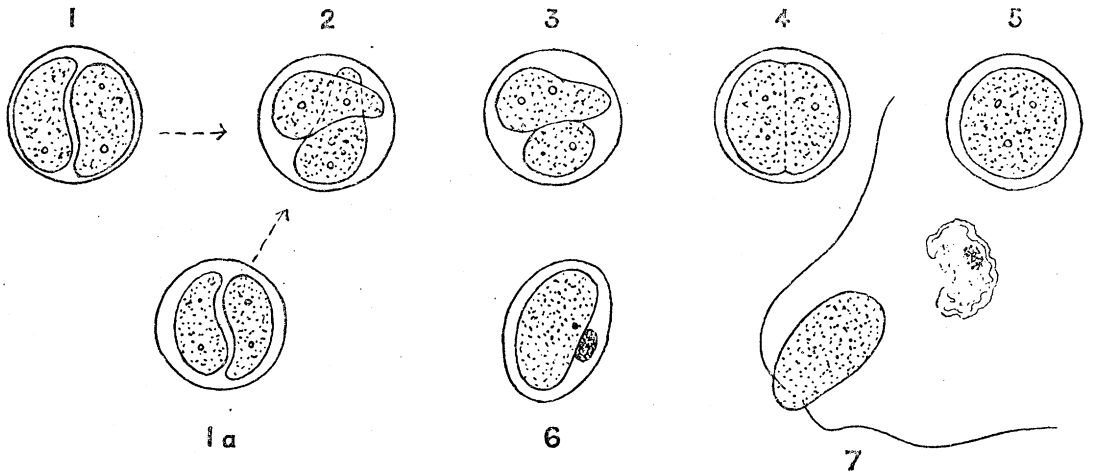
As regards the division of the nucleus, nothing could be made out during life. From stained preparations (figs. 63, 64), it is seen that the first nuclear division is unequal, a smaller portion (the first daughter-nucleus) being cut off from a larger part. I have not yet found a stage in the act of nuclear division, but this undoubtedly takes place very quickly, probably by a simple form of promitosis. I should say the larger nucleus then divides again, to produce the two remaining daughter-nuclei, for the three, when formed, are about equal in size (fig. 65).

Many of the spirals thus produced certainly grow into beans, which again undergo division; fig. 58 shows an individual in a condition intermediate between a spiral and a typical bean. On the other hand, the spirals may conjugate, but I am not sure whether syngamy always occurs between individuals of this form. Probably young bean-like forms may also unite (*cf.* below). Unlike the other flagellates in which I have found syngamy, however, the process in *Spiromonas* appears to occur spasmodically; in the case of this form, the great majority of the individuals in a culture do *not* at length cease multiplying and undergo syngamy, prior to encystment. When conjugation does occur, it is not followed by the formation of a resistant cyst. For these reasons, the process is much more difficult to study in this flagellate; nevertheless, I have obtained a good idea of it, though unfortunately I have as yet no permanent preparations showing stages in it.

The process varies to some extent, as a result, I believe, of different conditions in the environment. The whole process is so long (it may take up to 12 or 14 hours, if not longer) that I have not been able to follow it all through in an individual case, though I have stayed up till 4 o'clock in the morning over it, when my eyes ached so

much that I was obliged to relinquish the observation. I desire to express my indebtedness and thanks to Miss RHODES for relieving me on many occasions in this tiring and tedious watching, not only in the case of *Spiromonas* but also in the observations on other forms. I also thank my laboratory assistant, Mr. GEORGE KAUFFMAN, for similar help on several occasions.

Describing first what is, I think, the more usual and perhaps more normal method of syngamy (text-fig. E), the earliest stage I have observed is of two individuals

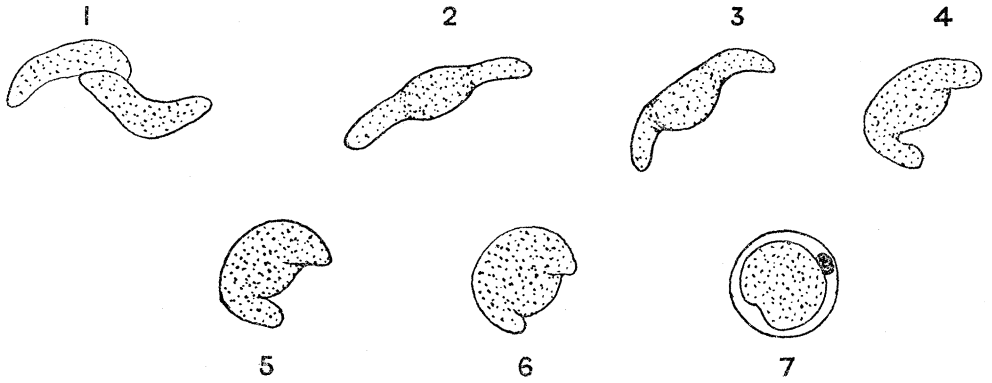


TEXT-FIG. E.—Conjugation in *Spiromonas*. First method—probably the more typical or normal. (From sketches at time of observation.)

circling round and rolling over one another inside a delicate cyst-membrane; the cyst itself undergoes no displacement, remaining in the same position the whole time. The flagella must be present, perhaps shortened, but I have never been able to see them. These two gametic individuals may resemble the "beans" (fig. E 1), or may be like rather short, stout spirals (fig. E 1*a*). The two individuals are quite separate, as yet, inside the cyst. They usually show one or two highly refringent granules. The nuclei could not be made out. Figs. E 2 and 3 show different positions assumed by the gametes inside the cyst. This period of rotation round and round may go on for at least six or seven hours, though I cannot say whether it always lasts so long. At length, probably when the nuclear preparation for syngamy is completed, the two gametes cease to move and become closely applied along their length to each other (fig. E 4). After a few minutes, the line of separation between them disappears (fig. E 5), and the zygote is constituted. The body of the zygote next becomes elongated and bean-shaped, and the cyst-membrane also often becomes slightly oval. After a few minutes more, the body begins to partially rotate in different ways inside the membrane, and as it circles round there can be distinctly seen a small body, consisting of unused (residual) protoplasmic material, including the refringent granules, lying at one side (fig. E 6). Sometimes the liberation of the zygote from the cyst may occur almost immediately after it begins to move, but, on the other

hand, it may remain thus for several hours. A minute or two before liberation is about to occur, all the active beans in the vicinity are violently attracted to the zygote-cyst, and there may be six or seven butting up against it. The cyst-membrane is not dissolved but ruptured, and the zygote swims away as a typical bean, leaving the remains of the cyst and the residual mass behind (fig. E 7). Very soon afterwards the other beans still on the spot are no longer attracted and also swim away: they do not, of course, ingest any of the residual mass.

In an observation-preparation which is three or four days old, or in a sub-cultured one, conjugation of typical spirals takes place in a quite different manner (text-fig. F). Two spirals become attached by the sides of one extremity of the body, I think the anterior ends, as in fig. F 1, and there is a gradual fusion of the two bodies. The conjugants continue to move actively the whole time, circling round and round in a very characteristic manner, in addition to progressing. For the first couple of hours the movement is extremely rapid, but afterwards slows down somewhat. To follow such a pair for some hours is the most difficult experience I have ever had with a mechanical stage. As the fusion progresses, the united portion of the zygote becomes larger and larger (figs. F 2 and 3), the free ends of the original spirals becoming smaller and smaller, and resembling two short, curved tails. (The flagella are not indicated, because I could never see them in active *Spiromonas*-individuals, except with the dark-ground illumination, and that is not feasible in the case of observation-preparations.) At length, after 6 or 8 hours, the zygote appears as in fig. F 6, only two small protuberances remaining to show the ends of the spirals. It is now very sluggish, no longer displacing itself, but only rotating now and then. I have not actually followed it to the next stage, because, as mentioned, I was obliged to break off the observation at this point. But when I left off, there were



TEXT-FIG. F.—Conjugation in *Spiromonas* (second method). Flagella are undoubtedly present up to about Stage 6, but as they could not be made out clearly they are not represented.

several zygotes in the condition of fig. F 6, and the next morning their place was taken by forms such as that shown in fig. F 7. The same condition has now been attained as described above (*cf.* fig. E 6). (I may mention that in preparations in which syngamy according to this method is taking place, there are no cases of two

separate gametes inside a cyst, *i.e.*, a stage like that of fig. F 7 has not been reached by the first method.) The rest of the process is just as above; here, however, the zygote apparently always goes on spasmodically rotating inside the cyst for several hours before being liberated. Syngamy, according to this second method, takes several hours longer than it does by the first method. I never saw this method occurring in observation-preparations freshly made from a dung-culture.

Syngamy is not always brought to a successful conclusion. Once, such a form as that of fig. F 7, after being watched for several hours, at last burst inside the cyst; and, on another occasion, in a case of syngamy by the first method, where the two gametes differed appreciably in size, first one and then the other eventually died. The conjugating elements must be, apparently, of approximately equal size; but the conjugating pairs (and the zygote-cyst) may vary appreciably in size, in different cases.

The result of syngamy is a typical, active bean-shaped form, which, I have no reason to doubt, may undergo division, though I have not yet been able to follow a zygote-bean until it divided. The remarkable feature is that I have not yet observed the encystment of this flagellate. Probably, under certain conditions, the beans at length encyst, for they represent the adult phase of *Spiromonas*; but I have not yet been able to induce them to do so.

In an old dung-culture, there are only beans still present, and few in number. Assuming they encyst eventually, the cyst must be of fairly large size. Now the first forms of *Spiromonas* to appear in a fresh culture are extremely minute forms, as described. Hence it looks very much as though the cyst in this case produces a swarm of very minute individuals at the commencement of the cycle, and will prove to be a multiplicative cyst. More than this I cannot say at present.

Phyllomitus undulans.—The body is usually somewhat pear-shaped, the anterior end being the more pointed one (figs. 13–16). The two flagella arise close to the anterior end, their origin being just on the side where the oral depression lies. The relations of the two flagella have been dealt with under "Movements." The size of the body varies from 6–8½ μ by 3½–4½ μ (in ordinary small forms) up to as much as 13 μ by 8 μ (in a few very large forms). The length of the short (anterior) flagellum is 6–9 μ; that of the long, posterior one, which is always at least twice as long as the body, is 17–30 μ. In most of the individuals on Giemsa-stained smears, the band joining the two flagella is quite distinct and definite; but in some cases it has apparently broken down into two or three fine, flagella-like threads (fig. 14). I regard this as an artificial condition, produced in the making of the smear, because I have never seen separated flagellar threads in life. Curiously, in wet-fixed films, stained by iron-hæmatoxylin, the connecting band is never visible at all; sometimes the two flagella are very close together (figs. 67, 68), when it is probably really present, but in other cases the flagella appear quite separate (fig. 69), and there is no sign of any connection. This is another instance where the Giemsa

method gives a truer indication of the actual state of affairs. I do not think, however, that this peculiarity in wet-fixed films explains the erroneous view of KLEBS and ALEXEIEFF, based upon the form named *P. amylophagus*, that the genus *Phyllomitus* had no connecting band between the two flagella, because KLEBS' figures are from life, when the connection (if present) is very obvious; moreover, the disparity in length of the two flagella is much greater in the true *Phyllomitus* than in the case of the other form, as figured by either KLEBS or ALEXEIEFF. Hence I do not think that the species *amylophagus* belongs to the genus *Phyllomitus* at all.

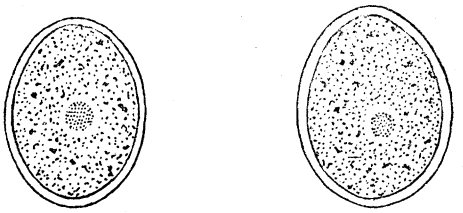
The nucleus is situated near the anterior end of the body, but it is always spherical, never drawn out in the direction of the basal granules of the flagella (as in *Cercomonas*), and appears to be unconnected directly with them. There is no kinetonucleus. The cytoplasm often contains several ingested masses of food-material or Bacteria (figs. 13, 14, 16), or else food-vacuoles (fig. 15). When one of these coprozoic flagellates feeds usually in a holozoic manner, there is not the slightest doubt about it; the cytoplasmic contents, in stained preparations, reveal the fact.

I have seen the later stages of binary fission occurring in the living individual and have also obtained indications of the process in stained preparations. I have not yet observed the earliest stages in the process and so cannot say whether the dividing individual becomes first of all quite motionless for a short period. From the time when the body begins to be elongated and constricted about the middle in a dumb-bell-shaped manner to the ultimate separation of the two daughter-individuals, a period of only about 9 minutes elapses. Hence division in *Phyllomitus* undoubtedly takes place in the same rapid manner that it does in the other coprozoic flagellates; probably the entire process does not occupy more than 15–20 minutes. From the time when the dumb-bell form is attained, the dividing body undergoes active, spasmodic vibrations, and the flagella of both daughter-individuals are now present; this is clearly seen from figs. 18 and 19 from a stained preparation. In the individuals found at about this stage (or perhaps a rather earlier stage) of fission on iron-hæmotoxylin films, I have been unable, in spite of all efforts, to make out any flagella (figs. 70 and 71). Not having seen the earliest stages in life, however, I cannot be certain whether the flagella may be at first absent, though I think this is quite possible; or, for some reason, they may not have taken up the stain, though the same preparation shows the flagella of single individuals.

An interesting question is whether the band uniting the two flagella represents really several (three or more) short flagella fused together, or whether it is a purely periplastic development, *sui generis*. If the former is the case, the flagella-like streaks or threads, often seen instead of the band in Giemsa-stained individuals (*cf.* fig. 14 and the dividing form of fig. 18) doubtless represent the constituent, short flagella; and *Phyllomitus* is then a polymastigine form (perhaps related to forms like *Chilomastix* and *Tetramitus*). But I hardly think this is the case, because on wet-fixed

films, which do not for some reason or other show the connecting band, never more than two flagella are seen, and there is no doubt that this is the normal condition in life, only the long and short flagellum are always joined together by a band. I incline, therefore, to regard this as a biflagellate, heteromastigine form, specialised in this particular manner. The remainder of the life-cycle, including cyst-formation, has still to be worked out.

Copromonas ruminantium, n.sp.—To this form I have paid attention only incidentally, because from all that I have observed the general features of the life-cycle agree closely with those of *C. subtilis*, as first described by DOBELL (*loc. cit.*); I have on several occasions noticed stages both in fission and conjugation, the latter process leading directly, at any rate, usually, to cyst-formation. There is, therefore, no necessity to describe here the life-history. The chief point of importance is whether the form which I have found in the fæces of goats and sheep is a new species. To this question I am inclined to answer in the affirmative. In the first place, the habitat and environment is, of course, different in the two cases, *C. subtilis* occurring in the fæces of frogs and toads. DOBELL himself considers that a form which he found in a newt is probably distinct from *C. subtilis*, on account, also, of its rather smaller size. With regard to the comparative size of *C. ruminantium* and *C. subtilis*, so far as the cysts are concerned, there does not appear to be much difference. The cysts of *C. ruminantium* are nearly always ovoid, and usually 8–8½ μ in length by 6½–7 μ broad (text-fig. G); in *C. subtilis*, the cysts may be either spherical or ovoid, and when of the former shape are 7–8 μ in diameter. The average size of a large "adult" individual of *C. ruminantium* is 12–13 μ by 7–8 μ (fig. 7); I have rarely seen forms much larger, but, of course, often smaller individuals. The average length of *C. subtilis* is given as about 16 μ, distinctly longer than my form.



TEXT-FIG. G.—Cysts of *Copromonas ruminantium*, n.sp. (Drawn from life.)

"*Bodos*" *with a kinetonucleus* (Bodo, syn. Prowazekia) *and without one* (Heteromita *and* Heteromastix, n.g.).—As is well known, the genus *Prowazekia* was founded for certain heteromastigine flagellates because of their possession of the binucleate condition; apart from this character they greatly resembled *Bodo*. ALEXEIEFF has since shown, however, that many species of *Bodo*, including *saltans* and *caudatus*, in reality also possess two nuclear organellæ, corresponding in appearance to a trophonucleus and a kinetonucleus respectively. Now it is most probable, according to STILES,* that the type-species of *Bodo* is *saltans*. Hence the generic name *Bodo* must be restricted to those forms with a kinetonucleus, and the name *Prowazekia* vanishes as a synonym. ALEXEIEFF maintained, however, that all "Bodos" would be found to be binucleate. In this view he was certainly mistaken, as I became aware

* 'Zool. Anzr.,' vol. 25, p. 689 (1902).

early in the course of this work. In my dung-cultures I have come across at least two simple, heteromastigine, "Bodo"-like flagellates, which never show the least sign of a kinetonucleus, and are, moreover, in other respects very different from true Bodos, such as *caudatus*. They are much more like certain of the species described by SAVILLE KENT under the generic name *Heteromita*. Incidentally, I may point out how much better KENT appreciated the important distinctions, even as observed in life, between such forms as *Bodo* ("*Diplomastix*"), *Heteromita*, *Spiromonas*, etc., than did German workers like BÜTSCHLI, KLEBS, SENN, and others, who placed them all indiscriminately in the same genus *Bodo*.

Considering first the *Heteromita*-like forms, beyond ascertaining definitely that they are quite distinct from *Bodo*, I have not yet been able to pay much attention to them, and have still to work out the life-cycle. The larger form, which has only occurred in one recent culture, is very probably *Heteromita globosa* (Stein). The smaller form may represent the species *lens* (Müller), placed by KENT in the same genus *Heteromita*, as a biflagellate; but, in any case, I do not think it belongs to the same genus as the larger form. The behaviour of the flagella and the movements are quite distinct in the two cases. In the larger form the longer flagellum is passively trailed behind, along the surface of the body, which is never the case in the smaller form (*cf.* under Movements). The cytoplasm of the larger form, also, shows numerous characteristic refringent granules (not bacteria), often occurring in the hinder part of the body (figs. 29–31, and *cf.* figs 61, 62, Plate 15, of KENT); these are never present in the small form. Moreover, while the smaller form is saprozoic and does not ingest bacteria, etc., the larger form is holozoic, at any rate partially, for cocci, etc., are often present in the cytoplasm. These points of difference were clearly indicated by the older writers among the forms placed in *Heteromita*. The whole appearance of these two forms which I have observed is different, and does not suggest that they belong to the same type. Now *globosa* is manifestly very similar in character to the type-species of *Heteromita*, Duj., namely *ovata* ("*Bodo ovatus*"); I retain the name *Heteromita*, therefore, for the large form (*H. globosa*). For the smaller one, since it is certainly not a *Bodo*, I propose the name *Heteromastix*, n.g.; the species with which I have worked may be *lens*, but I am not certain. The size of the body, in the small form (figs. 32–34), is typically about 6–7 μ by $2\frac{1}{2}$–3 μ; in the larger form it is about 9 μ by $6\frac{1}{2}$–7 μ in average-sized individuals. In both cases the body is typically elongate-ovoid and is not amœboid. In none of these forms, I wish to point out, is the nucleus directly connected with the flagella, as is usually the case in *Cercomonas*.

Unfortunately, I have never noticed the mode of division. As pointed out early in this paper, I have not yet obtained good cultures of these forms, and I am afraid on one or two occasions the small form has occurred at a time when I have been too much occupied with some other flagellate to observe it carefully.

As regards the question of syngamy, I have certainly evidence that it occurs in

the large form (*Heteromita globosa*). In one observation-preparation, which I made before this form vanished from the dung-culture, there were several typical "double" forms, *i.e.*, large ovoid individuals with duplicated flagella.* These individuals reminded me of the oval conjugating stage of *Helkesimastix*, the chief difference being, of course, that in addition to the pair of parallel, closely approximated trailing flagella, they had a pair of short, actively vibrating anterior flagella (*cf.* fig. 31). After about 3 hours' steady progression, one of these which had been followed was seen to stop moving, become rounded, and then lose its flagella; in other words, it was proceeding to encyst. The next morning I found three or four cysts in the preparation, and several were seen in the dung-culture from which the double forms were taken. The cyst-membrane appeared very thin and delicate (fig. 74), but would probably thicken in time. Here again, therefore, just as in *Helkesimastix* and *Cercomonas* (*cf.* fig. 54), I think there is no doubt that the large, active forms, with duplicated flagella, of usual length, are conjugating, and not dividing forms.

The only possible indications of syngamy which I have so far noticed in the small form (*Heteromastix*) are the stages, from permanent preparations, figured in figs. 37 and 38. Of course I cannot feel certain in regard to these, and at first sight it may seem hazardous to regard them as representing conjugation rather than division. Nevertheless, in the light of all the observations above recorded, I think they may be stages in conjugation. Here, also, the two pairs of flagella are present, fully developed, not in process of arising. The two nuclei are either close together, but not yet united (fig. 37), or the nuclear union has taken place and the synkaryon is formed (fig. 38). In all the cases of division which I have observed in these coprozoic flagellates, at a corresponding stage, the daughter-flagella (or some of them) would be short or rudimentary, they would be well separated, so would the daughter-nuclei, and the body would be either rounded or dumb-bell shaped, beginning to undergo constriction. I draw attention particularly to the close approximation of the two nuclei in fig. 37; this is quite unlike the appearance and relation of the two daughter-nuclei immediately after dividing. Further, fig. 38 also recalls strongly the late stage in conjugation of *Cercomonas* (*cf.* fig. 54). However, I have no wish to press this instance unduly until I have observed syngamy and its sequel in the living creatures, the surest test in the first place.

Bodo (*Prowazekia*) *caudatus*.—Different species of the true Bodos, under the name of *Prowazekia*, have been so much studied of late years (*e.g.*, by ALEXEIEFF,† KÜHN and SCHUCKMANN,‡ MARTIN,§ and SINTON,‖ to name only some workers),

* There were no indications of division occurring in the observation-preparation; it was probably over.

† 'C. R. Soc. Biol.,' vol. 70, p. 130 (1911); 'Arch. Zool. Exp.,' Ser. 5, vol. 6, p. 491 (1911).

‡ 'Ber. Natf. Ges. Freiburg i/B,' vol. 20, p. 35 (1913).

§ 'Zool. Anzr.,' vol. 41, p. 452 (1913).

‖ 'Ann. Trop. Med. Parasitol.,' vol. 6, p. 245 (1912).

that it is unnecessary to say much here with regard to the morphology and division. I include a few drawings of different individuals of *caudatus* (figs. 39–41, and 75), for comparison with those of *Heteromita* and *Heteromastix*. The body varies very much in size and form under different conditions; sometimes it is elongated and typically *caudatus*-like; at other times it is ovoid or nearly round. This variation was also found in *B. urinaria*, Hassall, by SINTON, which is doubtless also a coprozoic, and not really a parasitic form. Many of the more recently described species are most probably only older known ones, found in a strange habitat, as SINTON himself suggests; and, to begin with, I have little doubt that *urinaria* is a synonym of *caudatus*, the two forms appearing very similar.

Division in *B. caudatus* is just the same rapid process that it is in all the other flagellates I have observed, occupying only 15–20 minutes altogether. A dividing form ceases to displace itself but remains actively vibrating all the time until the two daughter-individuals are at length fully developed and separate. Division has been described and figured by most of the above authors (*cf.* especially SINTON, text-figs. 4–9, for observations in life, and ALEXEIEFF*). It is important to note that in all the forms binary fission, and this only, is described; and from my own observations I entirely agree that only binary fission, occurring in the active condition, takes place. MARTIN and LEWIN† are undoubtedly mistaken in referring their cases of multiple division inside a cyst to *B. caudatus*. Their fig. 37, showing four daughter-individuals just being liberated from a cyst, strongly recalls the multiple division in *Spiromonas*, in which, as I mentioned above, four daughter-individuals may occasionally be formed. From their figure it is clear that this case of division has nothing to do with *B. caudatus*, because each of the daughter-individuals is uninucleate and shows no sign of the binucleate condition, and if one thing is certain, it is that in all binucleates the division of the kinetonucleus and of the trophonucleus is quite independent, and each of these organellæ is always present, as such, from the start, in any newly-formed individual. I do not consider that multiple division, inside a cyst, occurs in the true binucleate Bodos.

The most important fact about *B. caudatus*, from the point of view of this paper, is that, so far as can be ascertained, *no* process of syngamy occurs in its life-cycle. I have carefully studied thriving cultures of this form in observation-preparations at different periods, both towards the end of the cycle, when cyst-formation is beginning to occur and numerous individuals are seen which have ceased to displace themselves and have become sluggish and are about to commence encystment, and also at the beginning of the cycle, when numbers of individuals have been recently liberated from their cysts. In no case have I ever been able to find any double individuals, *i.e.*, with duplicated flagella or any other indication whatever of syngamy. This is entirely in accordance with the observations of the above-mentioned workers, many of

* 'C. R. Soc. Biol.,' vol. 70, p. 130 (1911).

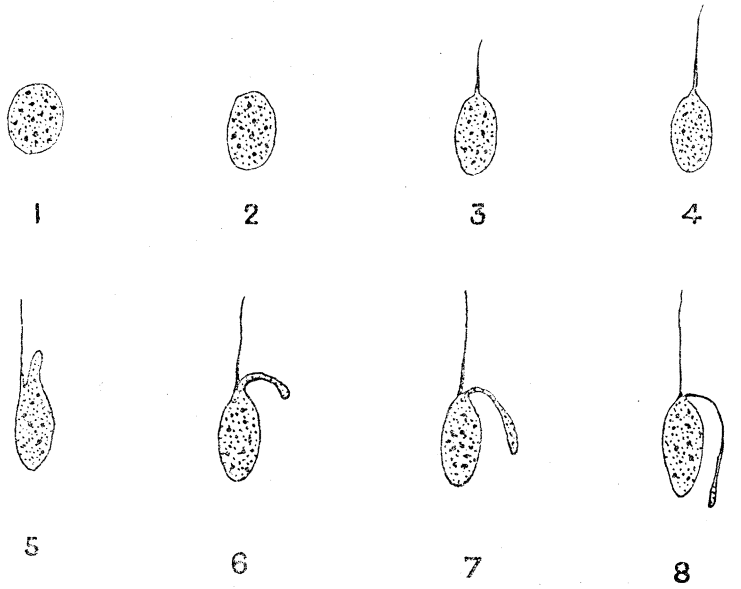
† 'Phil. Trans.,' B, vol. 205, p. 77 (1914).

whom expressly mention that, in the case of the particular form which they studied, solitary individuals became encysted and no signs of conjugation could be found. For my part, I certainly believe I should have found syngamy in my cultures, if such a process of union occurs in the life-cycle of *B. caudatus*. I conclude, therefore, that syngamy is lacking in the binucleate genus *Bodo*.

The process of excystation differs from that occurring in, at any rate, *Helkesimastix* and *Copromonas*, in that the cyst-wall is not dissolved as a whole, but is ruptured at one point and, when the newly active individual has emerged, the remains of the cyst are left behind. This fact was mentioned in our account of *Helkesimastix* and we can entirely corroborate SINTON's description and text-figs. 12–17 (*loc. cit.*).

But in the course of the "intensive" culture of this flagellate on agar-plates, I have found that an interesting modification has taken place in the character of the "encysted" individuals and the mode in which they become active again. In an old culture, the appearance of the resting quiescent forms is quite different from that of the normal refringent cysts. The body is rounded or ovoid, and of course the flagella are completely lost, but there is no sign of any conspicuous refringent cyst-wall. Indeed, I cannot assure myself that any definite membrane is present, even of the delicate character found, for instance, in *Helkesimastix*. On putting these forms into fresh medium, after about an hour or so, they again develop into active individuals. In the first place, as there is no rupture of a resistant cyst-wall, there is, of course, no wriggling through an aperture and no escape from the empty cyst to be observed. I have watched very carefully and I cannot be sure that any membrane is dissolved. There is no alteration in the appearance of the edge of the body to be noticed, such as occurs in the excystation of *Helkesimastix*.

The mode of origin of the new flagella is very peculiar; text-figs. H 1–8 show



TEXT-FIG. H.—Showing development of active form of *Bodo caudatus* and mode of formation of flagella, from quiescent resting stages.

different stages in the process. The body becomes rather more elongated. Then slight "working" movements of the protoplasm near one end (which will be the anterior end) occur; the body, as a whole, may undergo very slight jerky movements from side to side. Suddenly a short flagellum is shot out, which is distinctly thicker in its proximal portion and tapers gradually (figs. H 3 and 4). This vibrates to and fro, slowly and spasmodically at first. The body appears to be increasing somewhat in size, and all at once a short, thick, finger-like process is thrust out at the anterior end, close to the origin of the first flagellum (fig. H 5). So far as can be seen, this process is similar in nature to the general protoplasm. It gradually elongates, thinning out in so doing and becoming curved (figs. H 6 and 7). This peculiar process is nothing else than the second flagellum in course of development. As it lengthens it begins to wave about sluggishly. The proximal part becomes more and more flagellar-like as the whole lengthens and thins out. But for quite a quarter of an hour or more the distal part remains thickened. The flagellate is now actively vibrating by the movements of the anterior flagellum, but the movements of the posterior one remain for a while stiff and jerky. Ultimately the only sign of the thickening still left is a little bead at the tip of the flagellum, which doubtless also disappears in time.

This manner of development of the flagella, particularly of the posterior one, recalls in some measure the development of the flagellum as it has been described in the origin of the *Leptomonas*-form of *Leishmania*, from the "resting," aflagellate stage, though I have seen nothing comparable to the "flagellar vacuole" which occurs there.

The difference in the behaviour, as regards the formation of "resistant" phases, of *Helkesimastix* and *Bodo* after intensive cultivation is very noteworthy. The former has lost all power of developing cysts or resistant stages of any kind, equally with the loss of syngamy. The latter, in which there does not appear to be any process of conjugation in the first place, no longer forms definite cysts with a well-marked membrane, but is able to form resting, "resistant" phases—resistant to some extent (*i.e.*, to the toxic products in the medium), but whether resistant to drying-up I am not yet able to say. From these quiescent forms active individuals can be again developed in a manner markedly different from that in which they arise normally from the cysts.

Biological Note: The Relations of the Coprozoic Flagellates to the Bacteria Present in the Environment.

The cultivation of the different flagellates above enumerated has afforded me the opportunity of making certain general observations bearing upon the question of their effects, if any, upon the bacterial population in the surrounding medium. I think it is worth while to consider these observations briefly, since they may, perhaps, be of assistance to those engaged in the study of the correlated subject of the effects of the soil-Protozoa upon the soil-Bacteria. For it is not at all

unlikely, especially in the case of soils well manured with dung, that the Protozoan fauna in these is of a similar character to that with which I have been working.

I should say, in the first place, that it has not come within the scope of my present work (on the life-histories of the flagellates) to enter into this question from a definitely quantitative standpoint; nevertheless, from a long and close acquaintance with these "mixed" cultures, certain general features can be pointed out without hesitation. To begin with, however successful the Protozoan (*i.e.*, Flagellate, or Flagellate+Amœbæ) development is, and however numerous these forms become, the bacterial development is always enormously greater; and this is the case not only in the special nutrient media, but also in the simple dung-cultures, those, *i.e.*, which are very dilute, to which nothing but water (or saline) has been added, and which correspond, therefore, more closely to natural conditions. Again, as regards the flagellates, many are certainly *not* holozoic, but saprozoic. For instance, *Helkesimastix*, *Heteromastix*, *Spiromonas* and *Proleptomonas* obtain their nutriment very largely or entirely by osmosis. This group, therefore, can be at once eliminated, so far as regards any active diminution of the bacterial flora by ingestion. On the other hand, forms like *Monas*, *Cercomonas* (at any rate, partially), *Bodo*, and *Copromonas* feed by the ingestion of Bacteria and organic particles. Nevertheless, I consider that far too much stress has been laid upon this behaviour as a possible factor in the appreciable diminution of the bacterial population. Certainly in my experience, even in the case of markedly bacterial-eating Protozoa, like *Bodo*, *Monas*, or the Amœbæ, however great their multiplication is, it has little or no *practical* influence upon the numbers of the Bacteria present. Conditions that favour the Protozoan development invariably favour the bacterial development; but the converse does not by any means always hold. For instance, especially in old cultures, I have not infrequently failed to get any Protozoan activity worth mentioning on again sub-culturing, although the bacterial development has been as usual.

One very important biological fact which does not appear to me to have been taken sufficiently into account in the discussion of this subject is that the Protozoa (at all events, the Flagellates and Amœbæ) are themselves markedly affected by the Bacteria. (The soil-Bacteria being the special object of solicitude, the question has always been as to what action the Protozoa have upon them.) Now, as CROPPER and DREW showed in the case of Amœbæ, and as LAPAGE and I have indicated in the case of *Helkesimastix*, the Bacteria on their side exert, either directly or indirectly, an important, indeed a vital, influence upon the life-history of these and, doubtless, other coprozoic Protozoa.

Starting with the commencement of the cycle of activity, what is the first thing that happens in a freshly made culture (or sub-culture), or, to put the matter in other words, when the environment in which are the encysted Protozoa and the spores of the Bacteria (or their quiescent stages) is altered—diluted and purified—by the

addition of fresh nutrient medium (or, ultimately, fresh water)? The first thing to occur is that the Bacteria rapidly become active and multiply again. In the case of many, at all events, of the coprozoic Protozoa, *e.g.*, some of the flagellates and most of the Amœbæ (including the *limax*-forms), it is not until the Bacteria have produced to a sufficient extent some ferment or substance capable of dissolving the cyst-wall* that these components of the protozoan population can again become active. That is to say, the very activity and multiplication, after a period of encystment, of these forms (including the *limax*-Amœbæ, which must be, in any case, a most important protozoan-factor of the soil) is directly dependent upon an ample bacterial development.

Again, after a period of activity, shorter or longer, depending, I think, mainly upon the degree of the bacterial development rather than upon the extent of multiplication of the Protozoa themselves (though this is doubtless a factor), the great majority of the individuals present in any culture encyst; when this has occurred, the further influence, direct or indirect, of the Protozoa upon the Bacteria is almost *nil*, since, although a few active individuals often (*e.g.*, especially in *Monas*) persist sporadically, these may be regarded as negligible compared with the vast majority which have encysted. But the Bacteria always go on thriving for some time after the Protozoa have become encysted. Ultimately they too, for the most part, cease to be active, and either enter upon a resting stage or persist only as spores. (The distinction between quiescence and activity is not quite so pronounced in the case of the Bacteria as in that of the Protozoa; in an old culture, where there is no sign of Protozoa otherwise than encysted, there are always a certain number of active bacteria to be found, but this is a very different thing from the marked bacterial activity in a young culture.)

As Lapage and I pointed out (*loc. cit.*), there is no doubt that the principal cause of the cessation of protozoan activity is the development to an excessive degree of toxic products of metabolism in the environment. Now, what is the cause of the cessation of the bacterial activity as a whole? As shown above, the Protozoa cannot be held responsible, for the bacterial activity is at its greatest when the Protozoa become encysted. Moreover, it may be repeated, I have only too often found the protozoan development unsuccessful and quickly overcome by the bacterial development, but I have never seen the least indication of any failure of the bacterial development or of this being overcome by the protozoan activity. The cause responsible for the quiescence of the Bacteria must be either hunger, *i.e.* the lack of some essential food-constituent, or else (and more probably) here also the too great

* In the case of those forms in which the cyst-wall is definitely ruptured, *e.g.*, *Bodo*, the principal factor is probably the lowering of the tonicity of the environment, leading to the absorption of water by the encysted protoplasm and its consequent swelling. I hope very much to ascertain exactly how excystation takes place, for example, in *Monas* and *Cercomonas*.

concentration in the medium of some harmful substance, produced as a result of the bacterial metabolism, or of the Bacteria themselves.

From the evidence afforded me, therefore, by the above general biological observations on a large number of cultures, which have included at different times many different protozoa and manifestly different kinds of bacteria, I have no hesitation in saying that the coprozoic Protozoa are very much more dependent on and influenced by the bacterial development, than are the Bacteria by the protozoan development. Indeed, I cannot find that the Bacteria are in any practical degree affected at all by the Protozoa. If one can deduce anything from these conclusions, in regard to the relations of the micro-organic population of, at any rate, well-manured soils, it is that the Protozoa (at all events, the Flagellates and the Amœbæ) are most probably *not* the main factor limiting or inhibiting the bacterial activity in the soil. I am more inclined to consider that this limiting factor is one which operates, equally or unequally, upon both the Bacteria and the Protozoa, and may perhaps be in some way connected with an excessive development of certain toxic products or of certain abnormal conditions in the immediate environment.

The Significance of the Kinetonucleus in the Binucleate Flagellates.

Of the infusion-flagellates which I have observed in dung-cultures, sexual union (syngamy) certainly takes place in the following:—*Monas*, *Cercomonas*, *Helkesimastix*, *Spiromonas* and *Copromonas*; further, I have evidence that it takes place in *Heteromita globosa*. Very probably, also, syngamy occurs in the other forms, *e.g.*, the acicular monadine (*Proleptomonas*) and *Phyllomitus*; as stated, I have not yet observed these forms often enough to ascertain their complete life-cycle. On the other hand, in the case of the one binucleate generic type represented in this coprozoic fauna, namely *Bodo* (*Prowazekia*), I have carefully followed the course of the simple life-cycle ending in cyst-formation and have seen no indication whatever of syngamy; the only individuals ever seen with duplicated flagella are in the act of division. Moreover other workers who have studied other species of this type latterly, *e.g.*, Sinton, Martin, Kühn, and Schuckmann, have also seen no signs of conjugation. Further, in all the parasitic Binucleata leading up to the trypanosomes, which have been more studied than any other flagellates of recent years, and in which a special look out has been kept for the occurrence of syngamy, no one has ever found any process which can for a moment be regarded as indicating conjugation. As Mr. Lapage and myself have pointed out, we consider that conjugation is now entirely lacking in all these forms. This view receives additional support from the fact that I have not found it (nor has anyone else) in *Bodo*.

If we can feel assured that the binucleate flagellates do not undergo syngamy—a negative is, strictly speaking, impossible of proof—and when we see that other (quite probably all) more or less closely related forms among these lowly proto-mastigine flagellates, which possess no kinetonucleus, *do* undergo syngamy, we have

an important pair of facts, which may well be correlated. I suggest, therefore, that there is a close connection between these two developments, namely, the presence of the binucleate condition and the absence of syngamy.

In any endeavour to explain this connection, however, we are at once presented with the difficult problem whether the binucleate flagellates lost the process of syngamy first and developed the binucleate condition subsequently, or whether the latter arose first, and, as a result, all necessity for syngamy became lost. Further, since in all probability the forms from which the Binucleata are descended underwent a process of syngamy and possessed only a single nucleus, I am obliged to refer in the first place to the much-debated question of what is the significance of conjugation itself, notwithstanding the opinion of a well-known protistologist that one might as well inquire the meaning of the moon.

One view or theory, certain aspects of which have attracted considerable support, has been put forward and subsequently elaborated by such workers as Bütschli, Geddes and Thomson, Schaudinn, Hartmann and Doflein, to explain the necessity for the union of two independent cells which takes place in syngamy. (For a fuller discussion of this question, see Minchin's 'Protozoa,' Chapter VIII, "Syngamy and Sex.") Stated briefly, this theory is that, by the continued division of the cell-individual (in the Protozoa), the relative proportions of certain substances or constituents, exerting different physiological activities, are altered, owing to a certain inequality resulting from the more primitive methods of nuclear division. Two of the most important physiological activities concerned are the kinetic (relating to motility) and the trophic (relating to nutrition). To quote from Minchin (*loc. cit.*):—"If we suppose that these two manifestations of physiological activity have each a distinct material basis in the cell, then it can be easily imagined that the imperfections of cell-division may lead to the production of cells in which one or other substance predominates. As a result . . . some cells acquire more 'male' properties, others more 'female'; the cells preponderatingly male show greater kinetic and motile energy, those that have more female qualities show greater trophic activity. With continued division these opposite tendencies tend to accumulate in certain cells. . . . Thus a want of balance in the vital functions is brought about, which may reach such a pitch that the organism is unable to assimilate and reproduce, and must die unless the equilibrium is restored by syngamy with an individual that has been specialised in the opposite direction."

Now nuclear division in these protomastigine flagellates is very generally of a quite primitive type, a simple form of promitosis amounting to little more than "étranglement." The separation of the trophic and kinetic chromatinic elements and their localisation into two distinct centres, undergoing independent division, would doubtless facilitate the approximately equal partition of these two material constituents during cell-division. On the above theory, therefore, if certain forms first of all developed a kinetonucleus, by this means a balance between the trophic

and kinetic elements (and the related activities) might be sufficiently maintained throughout successive generations to enable the necessity for equalisation by an act of syngamy to be dispensed with.

There is, however, another and, it seems to me, more fundamental standpoint from which the question has to be considered. A large amount of experimental work has been carried out by various workers (*e.g.*, CALKINS, ENRIQUES, and especially WOODRUFF) upon *Paramœcium* and other ciliates, which has shown that the necessity for syngamy may be apparently indefinitely postponed, provided the culture is kept in a specially favourable environment. Nevertheless, conjugation can still take place if descendants from such a culture are kept under "proper" conditions for such a consummation. Hence there is no evidence yet that a non-conjugating race of *Paramœcium* (*i.e.* one which can no longer conjugate) exists. WOODRUFF comes to the conclusion that, under favourable environmental conditions, syngamy is not necessary for the continued life of the race; in other words, that the protoplasm of a single cell may be self-sufficient to reproduce itself indefinitely, under favourable conditions, without recourse to conjugation. This result indicates, he considers, that "senescence" and the need for fertilisation are not primary attributes of living matter. With this last statement I am inclined to agree, but chiefly because we have no evidence whatever of syngamy in bacteria, *i.e.*, in organisms in which the cell-unit is of simple constitution, the protoplasm not being differentiated into a definite localised nucleus and cytoplasm.

With regard to the conclusion that syngamy is not a necessity for continued existence (in *Paramœcium*, or other Protozoa, for that matter), and that Ciliates are able to live and multiply asexually for an indefinite number of generations, I think the force and importance of this conclusion are very greatly minimised by the qualification which invariably has to be attached, namely, *in favourable conditions.* How often in nature can a *Paramœcium*, or other protozoan, expect to find itself every day in a fresh "constant" environment, with the toxic products, present in ordinary environmental conditions, removed? Excepting in the case of certain parasitic forms, rarely or never. Even if we agree that syngamy is not a fundamental attribute of living matter, we know now that, under natural conditions, this process, in one form or another, is of widespread occurrence among the Protozoa, and appears to be, in fact, practically speaking, a necessity. This is, indeed, at the present day, almost a truism, but it is one which is badly in need of re-statement. In my opinion, the most important deduction to be drawn from these Ciliate experiments is not that under certain special circumstances they can exist without syngamy, but that it is the ordinary conditions of the natural environment which are primarily responsible for and necessitate the occurrence of syngamy in the Protozoa.

In this connection, the case of the non-conjugating strain of *Helkesimastix* is of very immediate interest. I have now cultivated this form for nearly 18 months, or for between 2500 and 3000 generations, without any conjugation or cyst-formation

taking place. Moreover, in this non-conjugating strain, successive generations of individuals have never been kept isolated, so that there has always been ample opportunity for syngamy to occur, if any of the individuals could or would conjugate.* This, however, they are never able to do; as the age of any culture increases, the flagellates gradually all die off. Certainly in this case, the loss of syngamy must be considered due to the ample supply of "constant" nutrition and to the removal of the excess of toxic products from the environment (by continually sub-culturing).

So far as these facts lead, therefore, they appear to me to indicate that syngamy is not due to some inherent factor in living protoplasm (*i.e.*, for the purpose of inducing variations),† but is *primarily* a reaction of the differentiated cell to its environment.

In a recent paper‡ WOODRUFF and ERDMANN have described a process of nuclear reorganisation, without cell-fusion, occurring in one of their races of "non-conjugating" *Paramœcium*, which they term "endomixis." From their observations they conclude that the cell has an internal regulatory phenomenon, endomixis, which is self-sufficient for the life of the race. From other observations made on mass-cultures, they consider that endomixis and conjugation may occur simultaneously among different individuals of the same culture, thus strongly suggesting that the same general conditions lead to both phenomena and that both fill essentially the same place in the economy of the creature. It is evident, they continue, that in the life-history of *Paramœcium*, periodically, a dynamic reorganisation occurs, either by endomixis or by syngamy. Lastly, while endomixis is not regarded as exactly comparable with parthenogenesis, for instance, both are nevertheless apokaryomictic phenomena, which must be contrasted with karyomictic ones such as conjugation.

If, therefore, endomixis is still necessary for the continued existence of a race of *Paramœcium*, which is (artificially) prevented from conjugating, does not this fact still further diminish the value of any generalisation that syngamy is not an essential process? For, according to the opinion of the authors themselves, endomixis is only a variant of conjugation, both processes having a similar function; and emphasis is laid upon molecular rearrangement as the result common to both.

I am strongly of opinion that it is in this direction that we must look to find the primary cause of, or reason for, syngamy. In the protozoan cell-individual, the cell possesses that characteristic complexity of structure, namely, differentiation into

* With regard to the "double" forms referred to in our account of this non-conjugating strain, I have now come to the conclusion that they are instances of very long delayed division, and *not* of union at all. About six months ago, moreover, I started another, entirely fresh non-conjugating strain, which I have kept throughout in observation-preparations (*i.e.* in dilute broth-medium), and this has never shown these peculiar forms. This strain has now for more than five months equally lost the power of conjugation and cyst-formation and is equally dependent for its survival upon sub-cultivation.

† On the contrary, I am more inclined to agree with ENRIQUES, and also MINCHIN, that syngamy tends (secondarily) to maintain the fixity of species.

‡ 'Journ. Exp. Zool.,' vol. 17, p. 425 (1914).

definite nucleus and cytoplasm, which distinguishes all organisms above the grade of bacteria. After a certain time, and after a varying number of generations, the cell-protoplasm has in all probability undergone important changes in chemical constitution and molecular disposition and balance, as the effect of changes in the environmental conditions ordinarily prevailing in nature. These changes necessitate drastic molecular re-arrangement and re-organisation, especially, perhaps, of the nuclear material, if life with all its activities is to be maintained. To give a concrete instance, the balance of the material elements associated respectively with the trophic and kinetic functions may be thus upset. To effect the requisite re-adjustment, the cell undergoes syngamy, as a result of attraction between individuals in a different chemical or molecular condition. Phenomena like endomixis are probably secondary or specialised modifications, since, so far as we are aware, union of two separate cell-individuals is the rule.

It will be seen, therefore, that the disturbance in the balance of the cell-constituents is to be ascribed to a more fundamental cause than the act of cell (or nuclear) division, though this latter factor may well accentuate the disparity, especially where nuclear division is of a primitive character. For, as MINCHIN says, "it can hardly be supposed that intensive culture can diminish consequences arising from defective cell-division"; that is to say, if defective cell (or nuclear) division were really the primary cause of syngamy, it would not be possible to cultivate different protozoa, especially simple flagellates, for long periods without their conjugating. Notwithstanding various authors, one must, it seems to me, regard "rejuvenescence" or, as I prefer to term it, *recuperation* of the cell, as the essential primary result of syngamy.

Returning now to the binucleate flagellates, so far as the parasitic forms are concerned, they are living under conditions closely comparable to those of an "intensive" or specially favoured culture, as was explained in our first account of *Helkesimastix*. Hence, in their case at all events, I am certainly inclined to think that syngamy was first of all lost, the organisms being able to continue existing in those favourable circumstances without the necessity for this process. Then, probably, as a useful adaptation, the binucleate condition arose. As indicated above, the double nuclear division must undoubtedly effect a more equal partition of the trophic and kinetic nuclear elements. (The peculiar delay in division occurring at certain times in my non-conjugating strain of *Helkesimastix* may stand in relation with the fact that here syngamy is absent, but no kinetonucleus is present.) On the other hand, in the case of the non-parasitic Bodos (Prowazekias), *e.g.*, *caudatus*, there is not the same *a priori* reason for supposing that syngamy was first lost; because these forms may occur in the same environmental conditions as the other coprozoic flagellates, which conjugate. There is at present no evidence to indicate which development occurred first. Forms like *Bodo* may have developed the binucleate condition independently, in the first place; and this development may have been

of sufficient advantage to the cell to enable it to dispense with syngamy. Here, it seems to me, the principal factor in the maintenance of the proper cell-balance may be the actual separation of certain nuclear elements, and their localisation into two distinct nuclei. We have no idea how closely associated and intermingled these two constituents are in ordinary (single) nuclei, or what the effect of their interaction may be.

If the sequence of the loss of syngamy and the development of a binucleate condition has been different in the two cases of the uniflagellate binucleates (*Leptomonas—Trypanosoma*) and the biflagellate (heteromastigine) forms (*Bodo—Trypanoplasma*), there is one interesting point which may be thereby accounted for. This is the fact that the organella termed kinetonucleus does not appear to be of quite the same type in these two different lines of forms. As has been clearly shown by WENYON for *Herpetomonas muscæ-domesticæ* and *Leishmania*, the kinetonucleus in the uniflagellate forms is of definite nuclear structure and in its division the basal granule of the flagellum acts unmistakably as a centrosome. On the other hand, the kinetonucleus of a trypanoplasm is a large, unwieldy and apparently more inert organella; indeed in some intestinal forms it is usually found in two or three pieces. During cell-division, it appears to divide by simple constriction, without any directing mechanism (MARTIN). And apparently the kinetonucleus in *Bodo caudatus* divides in a similar manner (ALEXEIEFF).

To summarise the matter, it may be suggested provisionally that in *Bodo* (and the other biflagellate binucleates), a development took place by which a certain nuclear (chromatinic) constituent was removed from the immediate sphere or zone of the nucleus and came to occupy an independent position in the cell; this permanent change in the molecular arrangement of the nuclear material facilitated the maintenance of the nuclear or nucleocytoplasmic stability, so that the cell became able to dispense with the necessity for periodic re-organisation or re-arrangement of its nuclear material by syngamy, with the result that this process was lost. In the uniflagellate binucleates, all of which so far known are parasitic forms, the special environmental conditions reacted so favourably on the general metabolism that here, too, the cell-balance could be maintained without recourse to conjugation, which at last no longer took place. But, as a sequel, a double nuclear condition was also developed, by which the more equal division of the trophic and kinetic nuclear constituents was facilitated. The kinetonucleus in these uniflagellate forms may be more closely and primarily associated with the kinetic activities than the organella in the heteromastigine forms to which the same name is usually applied. MARTIN, indeed, has expressed the opinion that in these latter there is no definite evidence to show that the kinetonucleus is associated with kinetic activities, and if this second nucleus has originated under the rather different conditions I have suggested, in the two sets of forms, its constituent elements and functions may be somewhat different in the two cases.

I am fully aware that the explanation I have endeavoured to outline is very hypothetical. Nevertheless, I certainly consider that the evidence available points strongly to a close connection between the development of a binucleate condition and the loss of syngamy, or the loss of syngamy and the development of a kinetonucleus, as the case may be.

Summary.

The paper deals with the first results of a comparative study of the coprozoic flagellates of certain ruminants (goats and sheep). The coprozoic fauna comprises those forms which pass through the alimentary canal in a resting, encysted condition, and undergo all the active phases of their life-cycle in the (moist) dung. These forms are entirely distinct from the parasitic forms, which are never found active in the dung.

The following flagellates have been observed:—*Monas* (probably *vulgaris*), *Cercomonas* (syn. *Cercobodo*) *longicauda* and perhaps another species, *Helkesimastix fæcicola* and *major*, *Bodo* (syn. *Prowazekia*) *caudatus*, at least two heteromastigine, Bodo-like forms without a kinetonucleus, one of which is probably *Heteromita globosa*, the other being regarded as a new type (*Heteromastix*, n.g.), *Spiromonas angusta*, *Phyllomitus undulans*, *Copromonas ruminantium*, n.sp., and a uniflagellate form which appears to be quite new, for which I propose the name *Proleptomonas fæcicola*. Many of these forms have been separated and cultivated on special media and in observation-preparations.

The movements of all these generic types are characteristic and can be readily distinguished one from another.

Many of the heteromastigine forms are the same as those described long ago by early British workers (*e.g.* Saville Kent) and rightly regarded as independent genera. There has been far too much tendency, especially amongst German workers, to place these forms indiscriminately in one comprehensive genus "*Bodo*." I revive, therefore, certain of the names. The genus *Bodo* itself is binucleate, and *Prowazekia* is a synonym. *Spiromonas* is an extremely polymorphic flagellate, which undergoes division, usually tripartite, inside a delicate cyst. *Phyllomitus* has the two flagella, short and long, partially united by a peculiar band; I can thus corroborate Stein's original description. *Proleptomonas* is very like a *Leptomonas*, but differs in having no kinetonucleus and in not being parasitic. It may represent the ancestral form of the Leptomonads and the related series of uniflagellate Binucleata.

I am able to confirm the statements of early British workers (Dallinger and Drysdale, Kent) that syngamy (conjugation) does occur in many of these lowly "infusion" flagellates. Besides *Helkesimastix*,* I have found it in *Monas*, in the well-known genus *Cercomonas*, where it is very similar to the corresponding process

* *Vide* Woodcock and Lapage, 'Roy. Soc. Proc.,' B, vol. 88, p. 353 (1915).

in *Helkesimastix*, and in *Spiromonas*; and I have strong evidence that it occurs in *Heteromita globosa*. I have no reason to doubt that I shall find conjugation takes place also in the other forms, *e.g.*, *Phyllomitus*, *Proleptomonas*, when I have been able to study them sufficiently. Distinction can readily be made between individuals undergoing fission and individuals in the act of conjugating.

On the other hand, the only type in which it can be said with certainty that syngamy does not occur is the binucleate genus *Bodo*; in this important respect, this form agrees with all the other known Binucleates. I consider that there is a close relation between these two features, namely, the binucleate condition and the absence of syngamy, and discuss this suggestion together with the question of the significance of the conjugation.

From the general observations afforded by my cultures of coprozoic protozoa, I consider that the Flagellates (and the Amœbæ) are probably not the principal factor inhibiting bacterial activity in well-manured soils.

EXPLANATION OF PLATES 26–28.

(The magnification of all the figures is 3000 times linear. All have been drawn and coloured by Miss RHODES, to whom I am greatly obliged for the care and time taken over them. The figures on Plates 26 and 27 are from preparations stained with Giemsa, also figs. 48–53 on Plate 28. All the other figs. on Plate 28 are from iron-hæmatoxylin preparations.)

PLATE 26.

Figs. 1–3, *Monas vulgaris*.—Fig. 1, small form. Fig. 2, large form. Fig. 3, large individual undergoing division.

Figs. 4–6.—Different individuals of *Proleptomonas fœcicola*, n.g., n.sp.

Fig. 7.—*Copromonas ruminantium*, n.sp.

Figs. 8–12, *Cercomonas longicauda*.—Fig. 8, lightly stained individual, to show the difference in thickness of the two flagella; the tail-flagellum cannot be followed where it runs along the body (but *cf.* figs. 48–53). Figs. 9–11, dividing forms; fig. 9 has only a single nucleus, the other deeply staining body being an ingested food-mass. Fig. 12, late stage in conjugation (*cf.* text-fig. C 9).

Figs. 13–19, *Phyllomitus undulans*.—Figs. 13–16, individuals of different sizes. Figs. 17–19, dividing forms; in fig. 17 the nucleus is still single but about to divide; the other deeply staining bodies are ingested food-masses (*cf.* figs. 13 and 16). Figs. 14 and 16 show the oral groove.

Plate 27.

Figs. 20–28, *Spiromonas angusta.*—Figs. 20 and 21, minute forms. Fig. 22, young spiral. Figs. 23–25, spirals. Fig. 26, stout spiral transitional to bean-form. Figs. 27 and 28, large beans. Fig. 27 shows well the apparent clear area in the hinder part of the body, filled with secretion or secreted granules (*cf.* figs. 59–63). Fig. 28 is probably a bean becoming rounded prior to division; it may be slightly flattened.

Figs. 29–31, *Heteromita globosa.*—Figs. 29 and 30, single forms; that of fig. 30 is very large. Fig. 31, late stage in conjugation, very similar to the corresponding stage in *Cercomonas* (*cf.* figs. 12 and 54), except that there are apparently two karyosomes, not yet united, in the synkaryon (*cf.* also fig. 74). The paler bodies in the cytoplasm represent ingested cocci, etc., which are quite distinct from the characteristic numerous small granules.

Figs. 32–38, *Heteromastix*, n.g. (cf. *lens ?*).—Figs. 32–34 are typical forms. Figs. 35 and 36 are of individuals belonging to a slightly different type, met with in a later culture and may possibly represent a distinct species. Figs. 37 and 38: These are in all probability stages in conjugation (of the typical form); in fig. 37, the two nuclei are still separate, but in fig. 38 there is a single nucleus (synkaryon). (*Cf.* the figs. of *Cercomonas* and *Heteromita globosa.*) Note throughout the absence of a kinetonucleus.

Figs. 39–41, *Bodo caudatus.*—Note the kinetonucleus. The little beak, behind which lies the (functional) cytostome, is well seen.

Plate 28.

(The individuals on wet-fixed films are usually more or less shrunken and afford no reliable indication of the size: this is the great disadvantage of such preparations.)

Figs. 42–47, *Monas vulgaris.*—Fig. 42, medium-sized, slender, active form. Figs. 43 and 44, zygotes preparatory to encystment. Figs. 45–47, encysted forms, the last figure showing the typical appearance of the ripe cyst. In fig. 46 the two gamete-nuclei are slightly unequal in size.

Figs. 48–56, *Cercomonas longicauda.*—Figs. 48–53, different stages transitional between small forms and large ones, showing that they all belong to one species. Fig. 54, late stage in conjugation (*cf.* fig. 12). Fig. 55, rounded zygote, which has lost the flagella, preparatory to encystment. Fig. 56, cyst (the cyst-membrane has become contorted in the fixing).

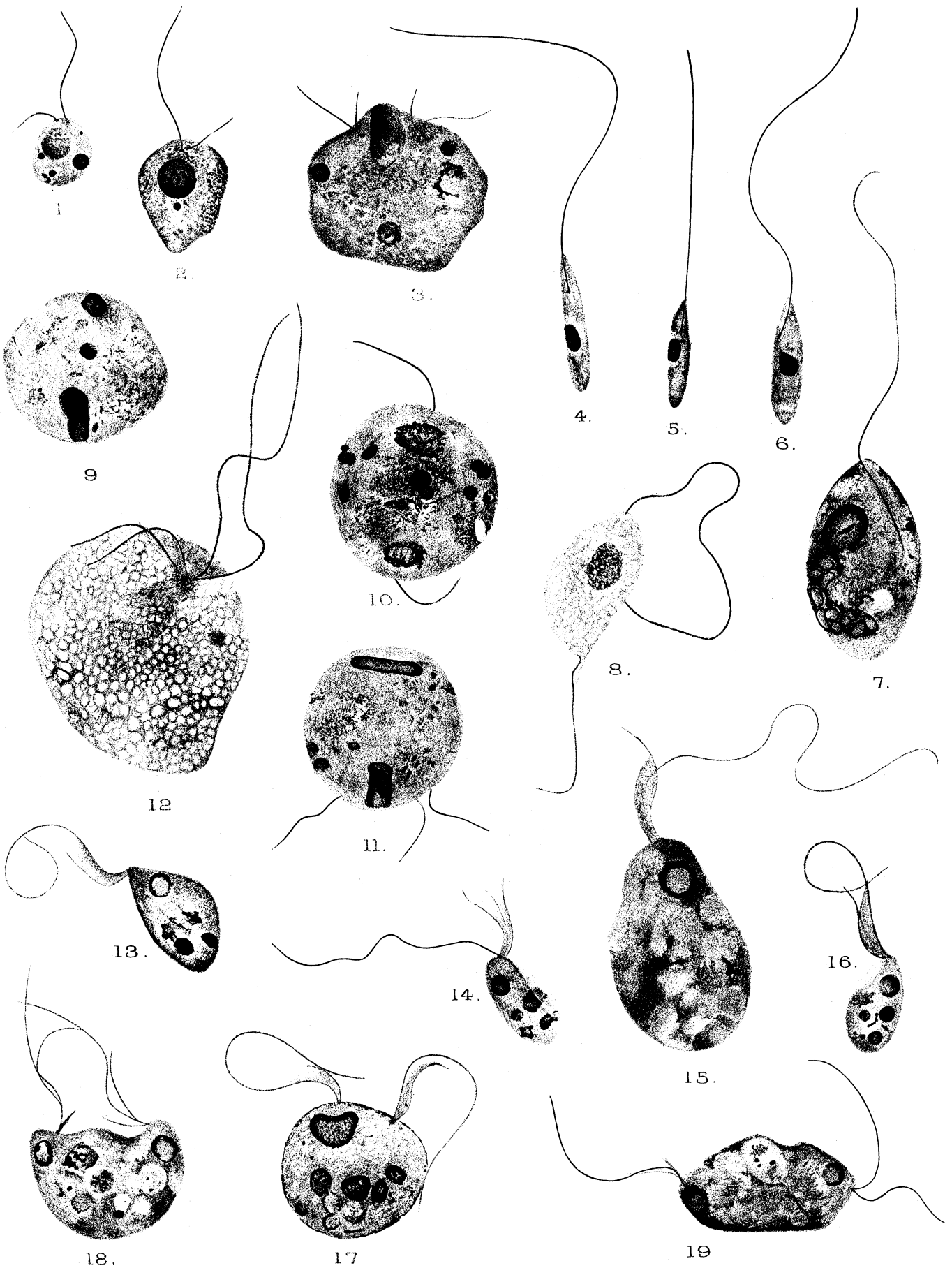
Figs. 57–66, *Spiromonas angusta.*—Fig. 57, spiral. Fig. 58, young bean. Figs. 59 and 60, large beans. Figs. 61 and 62, beans rounded off prior to division; the latter shows the delicate cyst-membrane formed. Figs. 63–66, different stages in tripartite division; in the last, the flagella are developed and the daughter-individuals are about to separate.

Figs. 67–71, *Phyllomitus undulans.*—Figs. 67–69, single individuals. Figs. 70 and 71, dividing forms. The stage of fig. 71 is about that of fig. 19 (with regard to the flagella, see text). (*Note.*—For some reason or other, individuals of this form, showing the flagella in the natural position, as in life, are very rare, both on Giemsa smears as well as in wet-fixed films.)

Fig. 72, *Heteromastix* (cf. *lens?*).—Many individuals on wet-fixed films show, as here, finely granular, deeply staining substance, around the nuclear membrane; individuals on Giemsa smears do not show this.

Figs. 73 and 74, *Heteromita globosa.*—Fig. 73, medium-sized, single individual. Note the absence of a kinetonucleus. The posterior flagellum is considerably shorter than usual in this individual (*cf.* figs. of Giemsa-stained forms), or else we have not been able to see its entire length. Fig. 74, zygote-cyst, early stage. There are apparently two karyosomes in the synkaryon, about to unite (*cf.* fig. 31).

Fig. 75.—*Bodo caudatus.*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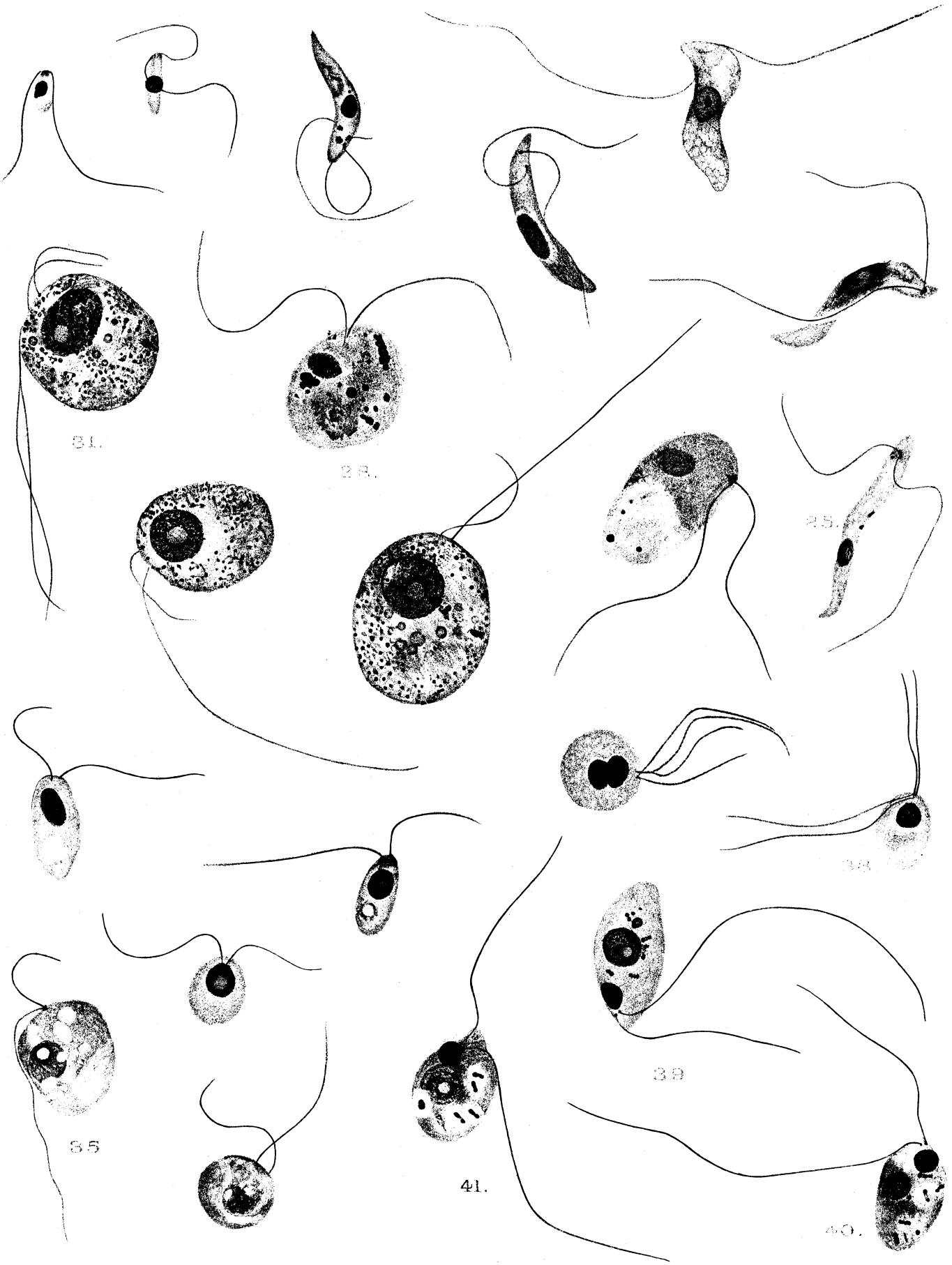
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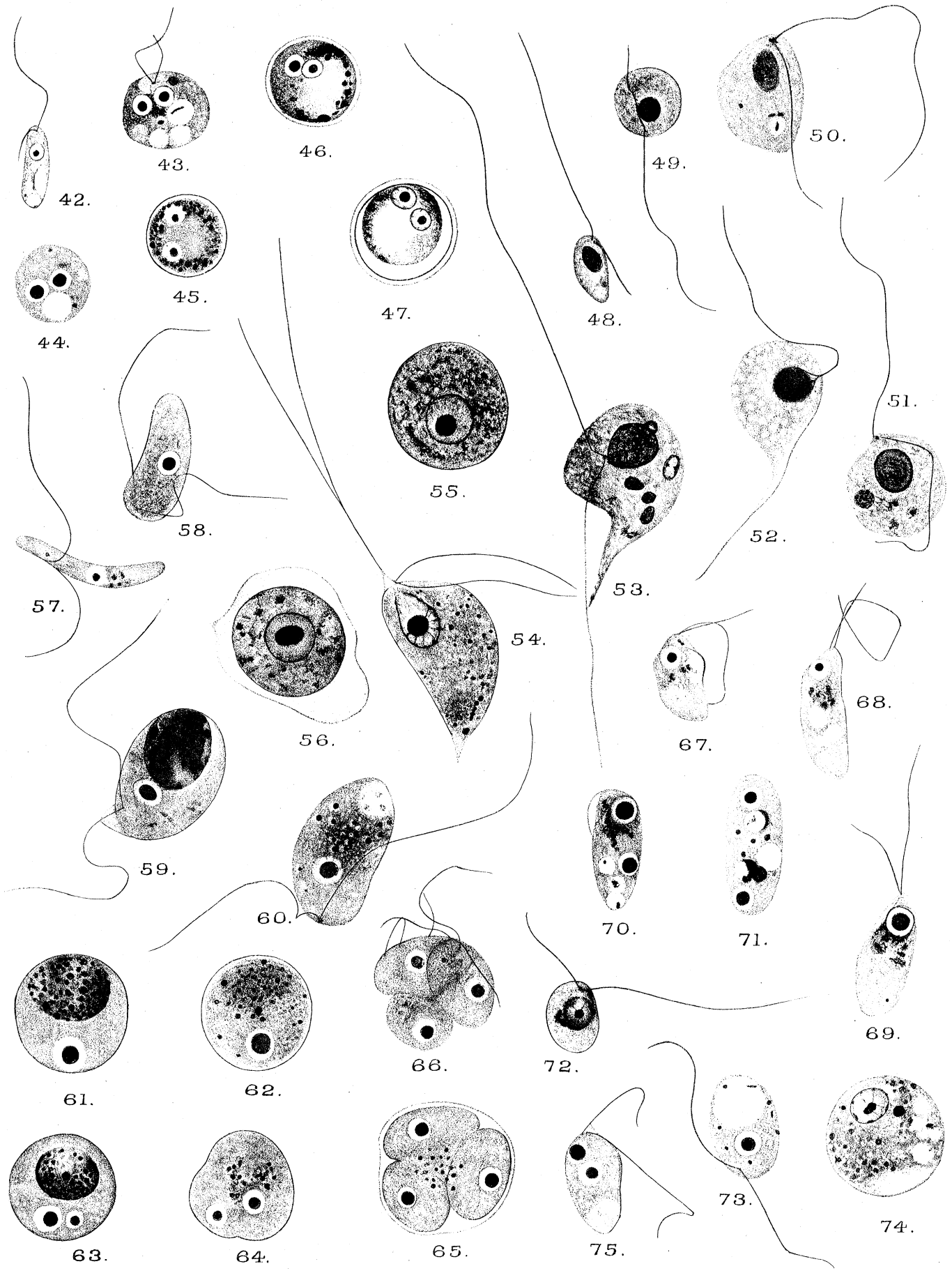

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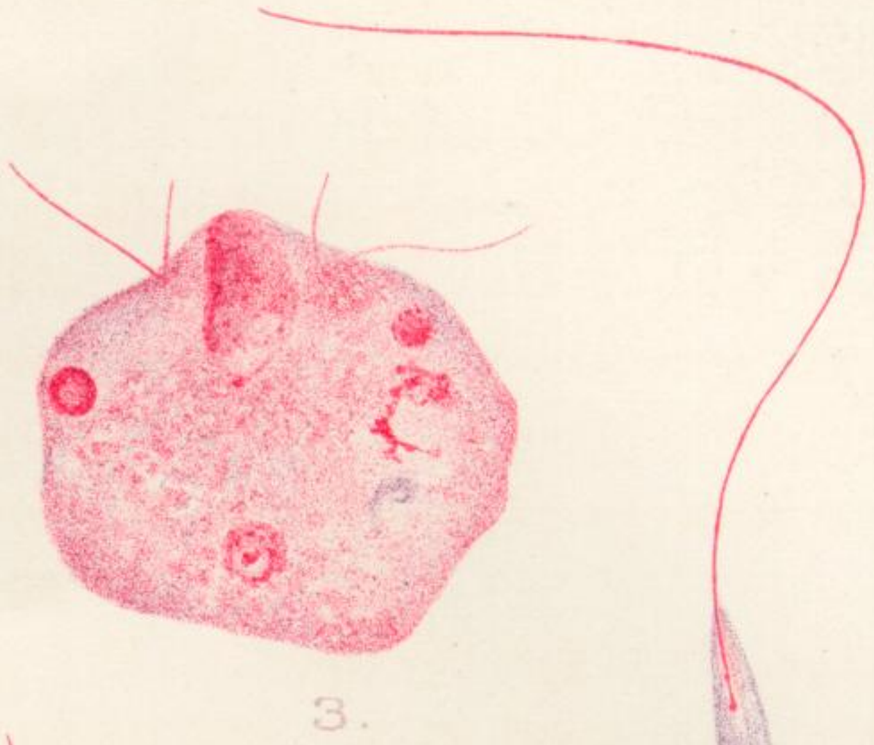

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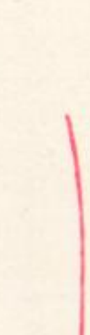

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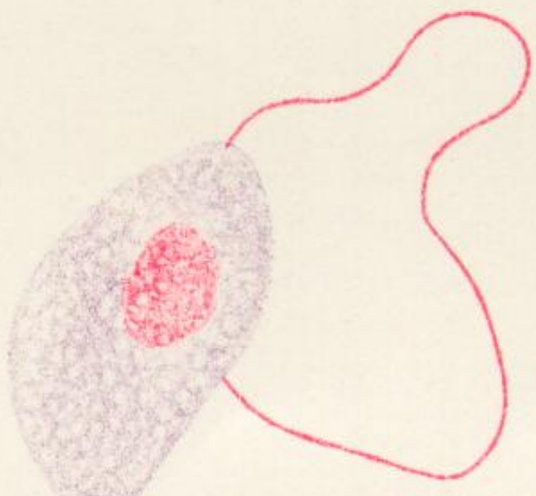

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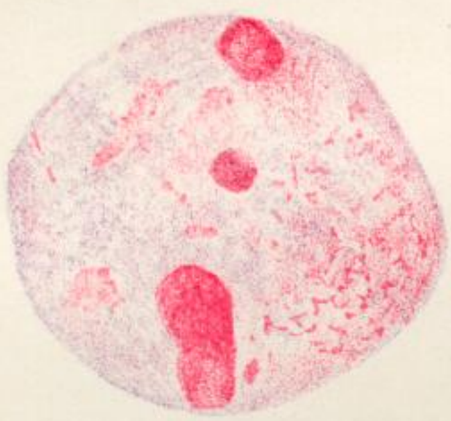

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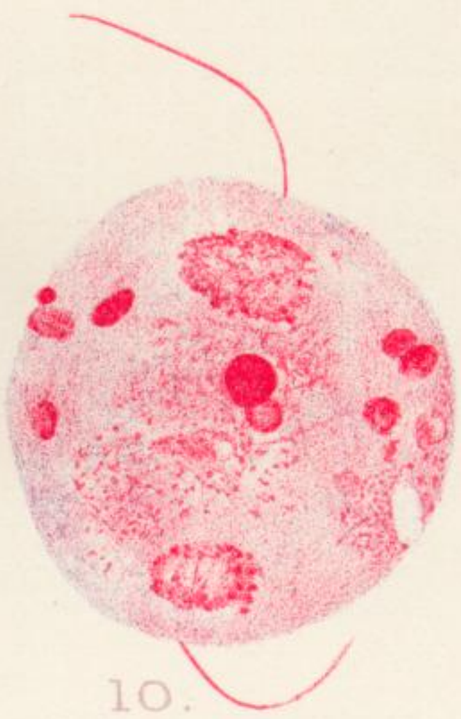

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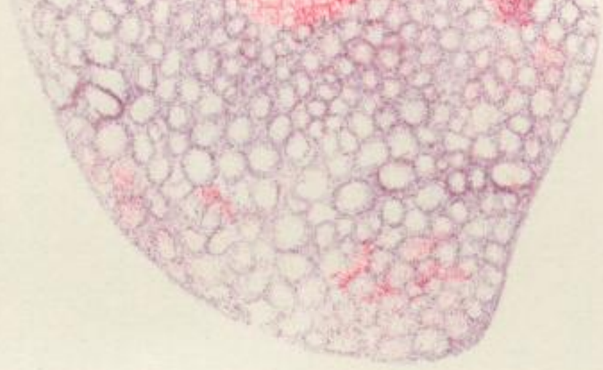

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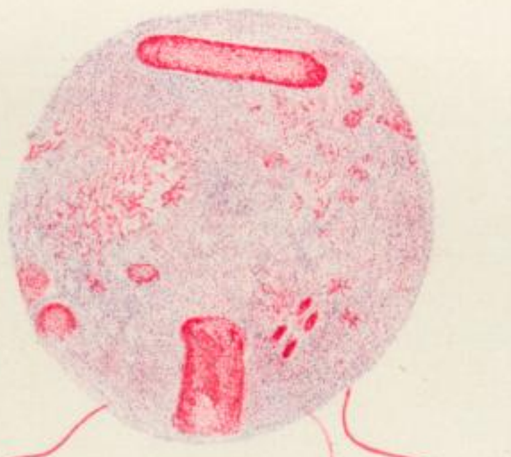

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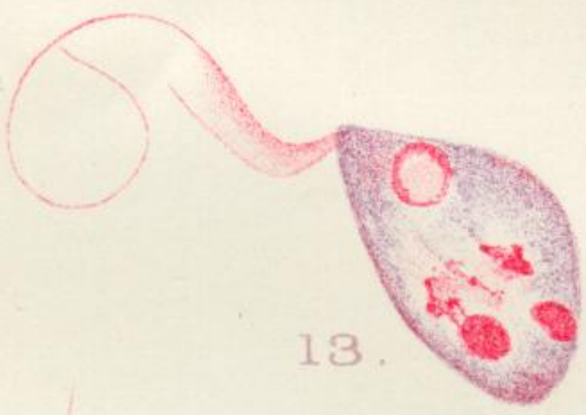

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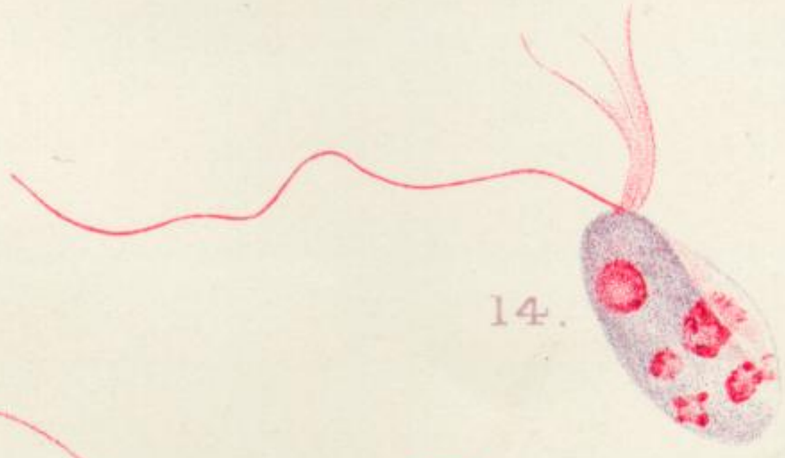

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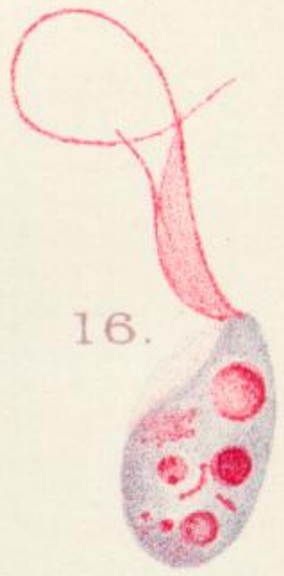

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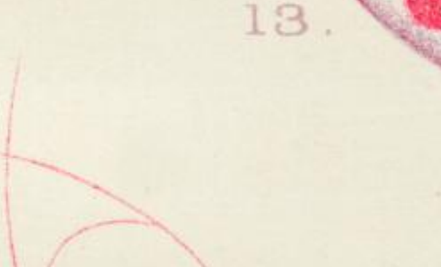

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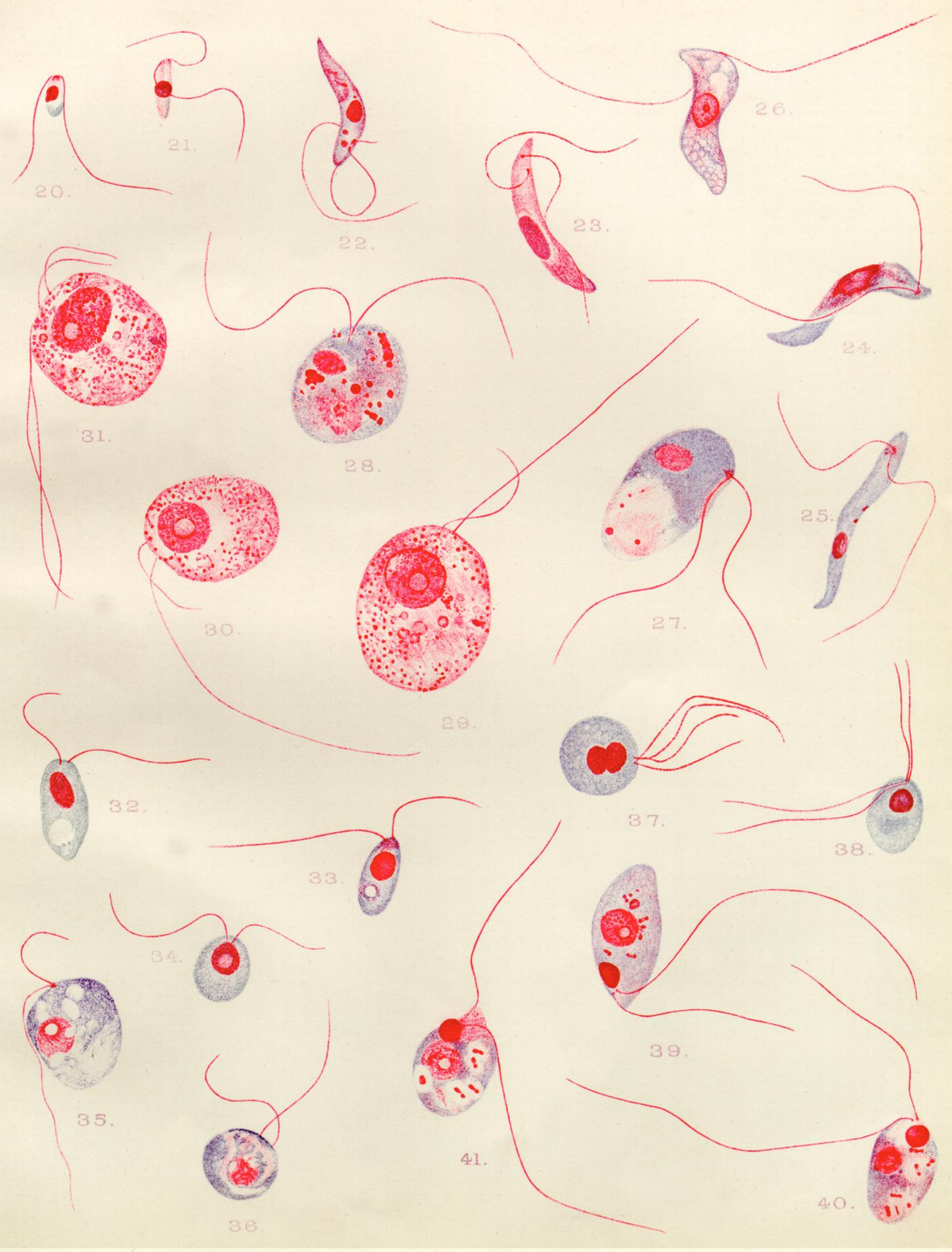

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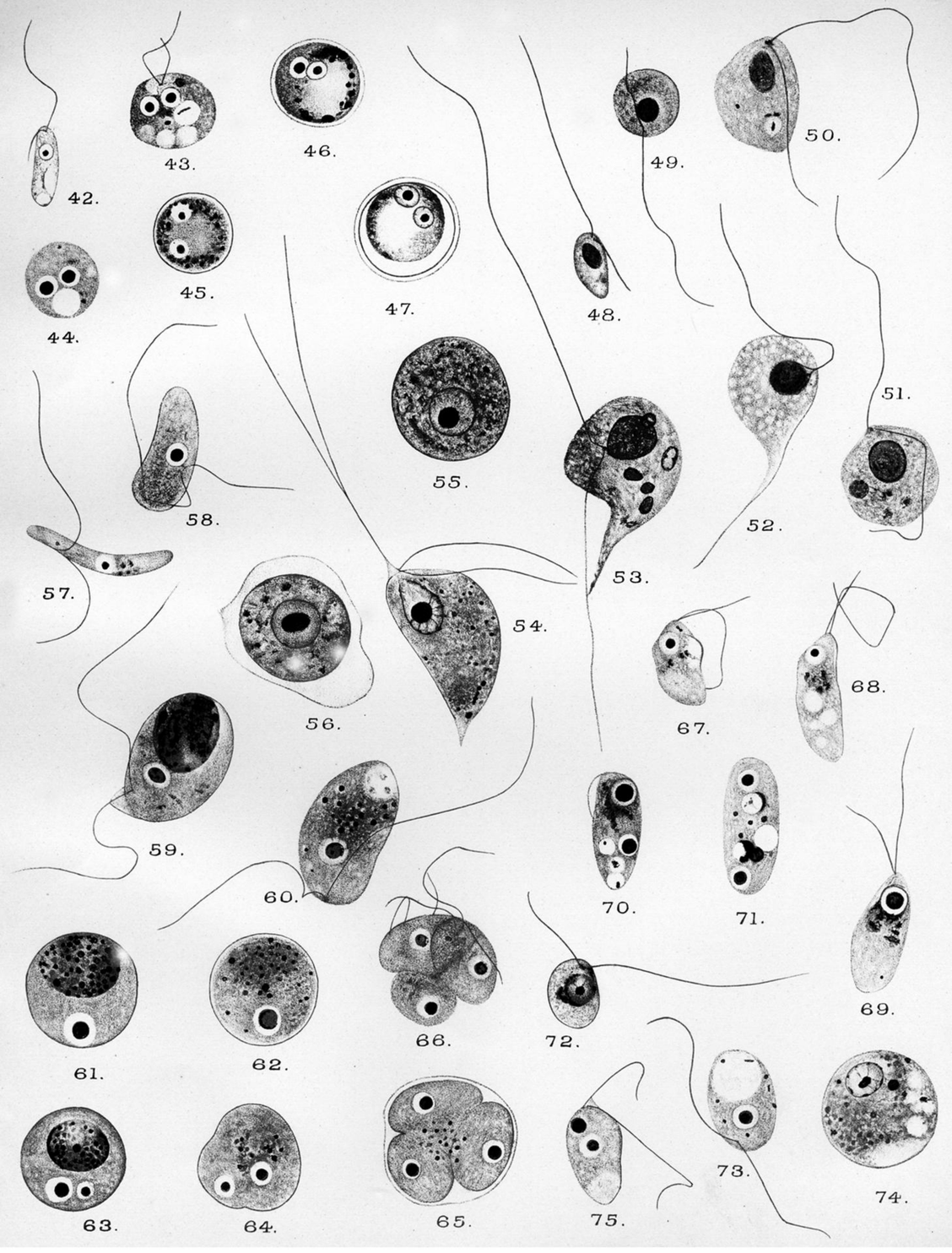

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