Observations on the Life-Cycle of a New Flagellate, Helkesimastix* fæcicola, *n.g., n.sp.: Together with Remarks on the Question of Syngamy in the Trypanosomes.*

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[Plates 13 and 14.]

This new flagellate occurs frequently in goat-dung; we have found it also in sheep-dung. It is a "passenger," being carried passively through the alimentary tract, in an encysted condition. When the dung is moistened with water—probably, in nature, when it is deposited on damp grass or earth—the flagellate emerges from its cyst and goes through its life-cycle, ultimately encysting again. The cysts are doubtless swallowed by the goat with its fodder. We have cultivated *Helkesimastix* under various conditions, which will be described in our full account later. In order to obtain this form in large numbers and study its life-history without any fear of being misled by stages in the life-cycle of other flagellates, we succeeded in isolating it from the other forms occurring in simple dung-cultures and cultivating it on agar-media, on which it multiplies rapidly. The medium which we have used principally is weak Lemco-agar, *i.e.* the same medium as used for blood-agar, but considerably diluted. For our continuous observations we have used hanging-drop preparations in sealed cells; in these cases we used very dilute Lemco-broth, without any agar, as the medium for the development of the flagellates, because in the denser agar-medium it is very difficult to see the flagellum. In all these media the flagellate forms the protozoan component of a "mixed culture," since there is, of course, an even greater and more rapid bacterial development.

Commencing the account of the life-cycle with the permanent cyst, this is a small spherical or slightly ovoid body about 3–3½ μ in diameter (figs. 1–3). The cyst-wall is well defined, but not very thick; it appears to consist of a single membrane, there being no differentiation into inner and outer envelope such as is found, for example, in many *Amœba* cysts. Sometimes bacteria are adherent to the cyst-wall (fig. 3). The protoplasm is

* The generic name is formed on the analogy of the Homeric epithet, ἑλκεσιπεπλος, trailing mantle or cloak. We are indebted to Prof. Minchin for suggesting this appropriate name.

fairly homogeneous, or contains fine granules. There is frequently, however, a conspicuous, somewhat refringent grain, situated near the periphery (Plate 13, figs. 1 and 2), but no vacuole is present. The nucleus can usually be seen as a clearer area, and at times the contained karyosome can be distinctly made out as a dull body in the centre. No division takes place inside the cyst, which is therefore a resting or "Dauer" cyst and not a multiplicative one.

Excystation.—We have followed the process of excystation in cysts which had been for about 22 hours in fresh medium (Liebig broth). This is not quite the minimum period required, before the flagellate will emerge from its cyst, for in this particular preparation some half-dozen individuals were already active by this time, though the great majority were still encysted. The period which must elapse depends, we consider, upon how soon the multiplication of the active bacilli in the new environment has taken place to a sufficient extent to produce, in sufficient quantity, the ferment or chemical substance in solution which is either directly or indirectly the cause of the dissolution of the cyst-wall. For we are strongly of opinion that the explanation of both the encystment and the excystation of *Helkesimastix* (and probably equally of other dung- and infusion-flagellates) is to be found in the view put forward by Cropper and Drew* in their recent important and suggestive work on the causative factors of the corresponding phenomena in *Amœbæ*. We hope to study our new flagellate from this biological standpoint subsequently, and will here merely indicate in passing certain facts we have observed which bear upon the question. We have found excystation occurring only in those cultures, plates, or observation-preparations in which there was a plentiful development of an (or of more than one) active, markedly aërobic bacillus, which we have not yet more closely determined. We have kept cysts in ordinary cover-slip preparations in different media (the preparations being sealed, of course, to prevent evaporation), for several days, without the emergence of any active individuals taking place; and in such preparations, the medium not being in contact with free air, no noticeable development of active bacilli took place.

In a cyst from which the flagellate is about to be liberated the wall is noticed to become gradually less and less obvious, until at length one can no longer say that there is any envelope present, apart from the delicate membrane or pellicle limiting the body-protoplasm (fig. 4). There is no definite rupture or bursting of the wall at any point, through which the flagellate could pass out to the exterior; in other words, when the creature has again become active and moved off, there is no cyst-wall left behind. We were the

* 'Researches on Induced Cell-reproduction in Amœbæ,' London, John Murray, 1914.

more convinced of this because we had previously seen the excystation of a species of *Bodo* from other dung-cultures, and in that case the rupture of the cyst-wall was very evident, and when the *Bodo* had moved away the empty cyst-wall was left behind. To return to *Helkesimastix*, when a sphere from which the cyst-wall had disappeared was watched it was seen gradually to change its shape, and in about seven minutes had elongated somewhat and become ovoid (fig. 5). Near one extremity a small vacuole had made its appearance, which represented the contractile vacuole; active metabolism had again begun. Very slight, spasmodic movements were noticed at intervals, and then suddenly it was seen that a very short, delicate flagellum was present, apparently emerging from about the middle of the body (fig. 6). This waved slowly to and fro, though we do not feel at all certain that it was causing the jerky movements. The flagellum could at first be made out only when it was projecting from the side of the creature, and not when it lay over the body-protoplasm. Meanwhile the flagellate was growing in size rapidly, and five or six minutes later it appeared as in fig. 7: the flagellum had lengthened considerably and was more prominent and stouter, and its point of origin was now near to the anterior end. The nucleus had doubtless also passed towards the anterior end, but unfortunately it could not be clearly made out; this was owing partly to the jerky movements and partly to the fact that the protoplasm was becoming very granular. The jerky, to and fro movements of the body, producing no displacement of the creature, are very characteristic of certain phases; we describe them as "knicking" movements (*cf.* below, p. 358). Ten minutes later the flagellate was beginning to make short, gliding movements of progression, and these alternated for some time with the knicking movements until, after about an hour, it moved steadily for the first time out of the field of vision.

During this period the same process had been going on throughout the preparation, and many actively gliding individuals were now present. These were all growing rapidly before beginning to multiply, and attained a size considerably larger than the average individual size found later on; the protoplasm was usually full of refringent granules.

Form and Structure.—The body of the ordinary active individual of *Helkesimastix fæcicola* is typically elongated and fairly cylindrical, with one end (the anterior one) bluntly rounded, the other (posterior) one tapering away more and being at times somewhat pointed (figs. 8, 10, 13). The broadest part of the body is generally nearer to the anterior end. We can most aptly compare the form with that of the fleshy part of a small carrot. Sometimes the hinder extremity is drawn out into a narrow prolongation (fig. 11). This hinder part of the body is often very plastic and irregular, and reminds us somewhat of the posterior extremity of *Cercobodo* (*Cercomonas*), with its long cytoplasmic "tail," though in *Helkesimastix* it is never, in normal conditions, drawn out to anything like the same extent. The body is usually about 6μ to 7μ long by $2\frac{1}{2}\mu$ or 2μ broad. Under other conditions the shape of the body may be oval or slightly pyriform (figs. 9, 18); this is frequently the case in smaller individuals, and also when the flagellates are sluggish, in a rather denser medium than usual.

There is a single flagellum, generally about two and a half to three times as long as the body, or even longer, inserted at, or very near to, the anterior end. This new flagellate is remarkable in having its flagellum *always* directed backwards, *i.e.* it possesses only a trailing flagellum, whence the generic name. In life, the flagellum is usually contiguous to the body for practically the entire length of the latter, and the proximal portion is always in very close contact with about the anterior third or so of the body, from which it never becomes free. This is seen very clearly when a steadily progressing individual makes a sharp turn and goes off in another direction. The proximal portion of the flagellum also turns immediately, along with the body, but the remaining part is for the moment free from the latter and turns more gradually into the new direction (*cf.* fig. 14). The flagellum lies along the middle of the upper (dorsal) side of the creature; in ordinary conditions it is never on the under side. Sometimes the flagellate turns on its side, when the close adherence of the flagellum is well shown (fig. 10). Yet, in spite of this close contact, there is certainly no attaching membrane developed, for, in individuals killed by osmic acid, the flagellum is sometimes seen to stand off completely from the body (*cf.* fig. 12). Frequently there is a definite row of three or four conspicuous granules along the line of contact of the body with the flagellum (figs. 10, 11). The nucleus can generally be seen as a clear, spherical area near the anterior end; in individuals in motion it is sometimes difficult to see the karysome, but in those quiescent, or in individuals killed by osmic acid vapour, this body can also be made out (*cf.* fig. 12). *Helkesimastix* is not a binucleate; it has no kinetonucleus.*

There is certainly no cytostome or definite mouth-aperture present. We are not quite certain, however, whether the creature does or does not ingest solid particles, such as small cocci, etc.; we are strongly inclined to think it does not. In this connection, a peculiar mode of food-ingestion which we have observed in *Cercomonas* (or *Cercobodo*) *longicauda* is of interest. While

* We may add that, as seen in stained preparations, the nucleus is of the usual flagellate character, consisting of a nuclear membrane, a clear zone (the nuclear sap), and a large central karyosome, connected with the membrane by delicate radiating fibrils.

an individual was moving sluggishly along, the posterior, plastic part of the body would come into contact at some point with a small extraneous particle. A small portion of the protoplasm of the flagellate in the immediate neighbourhood of the point of contact would remain adherent to the still stationary particle for a few seconds, gradually engulfing it, while the *Cercomonas* moved calmly on. Thus most of the body was quickly separated from the small portion of cytoplasm remaining behind, until often only an extremely thin thread or line of protoplasmic substance still joined the two parts; this thread might be as long as the whole body of the flagellate. Suddenly the tension of the connecting thread overcame the resistance of the stationary particle, and the latter, together with the small portion of protoplasm surrounding it, was safely hauled up again into the main body. We often thought the thread must break, but it never did!

As our new flagellate shows a resemblance in some respects to *Cercomonas*, we have watched particularly for the occurrence of anything corresponding, which, however, we have never seen, although analogous appearances are seen during conjugation (*cf.* below). We have never been able to satisfy ourselves that *Helkesimastix* does engulf solid particles at the hinder, plastic end, but do not deny the possibility of it doing so. We certainly consider, however, that its principal mode of nutrition is by osmosis. The natural habitat of the creature, namely, moist dung, is, of course, rich in organic matter in solution, when the bacteria have been active for a little time. In *Helkesimastix*, therefore, we have an instance of a form which is, at all events, mainly saprozoic.

The contractile vacuole is usually small, that is to say, it contracts before it attains a large size. It is generally situated in the hinder part of the body, to one side (figs. 8, 10). Now and again, however, owing doubtless to some condition of the medium, the contractile vacuole becomes very large (fig. 15). Immediately after it has ruptured the protoplasmic wall and its contents have passed out to the exterior, the body of the flagellate presents for a short time the curious appearance of fig. 16; the hinder end is forked, like the two arms of a V. After a little while it becomes triangular (fig. 17), and ultimately assumes again its normal shape.

Another point illustrating the looseness or plasticity of the body is a method of turning round often shown by a sluggishly moving individual. It will come to rest, and the body becomes more ovoid (fig. 18). Then the side with which the flagellum is in close contact begins to show what we term "working" movements; at first the peripheral protoplasm moves in slow, short, irregular waves to and fro, the line both of the contiguous part of the flagellum and of the row of granules (if these are present) becoming meanwhile indented and uneven (fig. 19). These peripheral movements of the protoplasm can perhaps be compared, on a very small scale, with the wave-like movements of the ectoplasm of *Amœba verrucosa*. Next, the anterior portion of the body-protoplasm on this side is moved as a whole backwards, around the central part, as it were, carrying with it the anterior part of the flagellum and the granules, and also, doubtless, the nucleus (*cf.* fig. 20). Finally, the creature elongates again, having the anterior part of its body now where the posterior part formerly was (fig. 21), and is ready to swim away in just the opposite direction.

Movements.—*Helkesimastix* possesses two distinct and characteristic methods of locomotion. One, the more usual mode, is very interesting, because it is very difficult to explain; in fact, we do not know quite how to explain it. The creature glides forwards with a steady, almost unwavering movement, the flagellum trailing behind in a straight line. While the rate of progression is not as rapid as that of a *Monas* or a *Bodo*, for example, the movement cannot by any means be called slow; indeed, it is often surprisingly fast, considering how little there seems to be to account for it. There is certainly no vibration of the free, distal part of the flagellum at all; in this method of movement, the flagellum does not act as a pulsellum. The creature is always at the surface of the medium when moving in this way (or it is gliding along the under surface of the cover-slip), the flagellum in both cases being uppermost. This fact leads us to think that surface-tension plays some part in this type of movement. The body is, as it were, suspended along its length to the flagellar thread, as a gymnast may be suspended along a tight rope. The body is often seen to swing sideways partially around its flagellum, appearing then as in fig. 10; but it never swings right above the flagellum. The only movement of the body which can be noted is a very slight "knicking" movement of the anterior end, *i.e.* the anterior end may make little tentative jerks from side to side, perhaps caused by slight contractions of the anterior end of the flagellum. But the anterior end of the body is not displaced laterally by more than half its width, if as much, and the strength of these slight movements appears wholly insufficient to produce the steady forward progression; moreover, the creature may glide for quite a considerable distance without even these.

The other characteristic mode of progression occurs when an individual is in the middle of the fluid, *i.e.* completely surrounded by it. Then it performs vigorous, more or less undulatory movements of its whole body and flagellum, the latter, as regards its applied portion, never becoming separated from the body, and with its free, distal part lashing actively behind. The movement of the body is very like that of a fish's tail, and not at all unlike the movements of certain trypanosomes. Yet, in spite of all this activity, the rate of progression is no greater than is obtained by the quiet surface gliding.

One other variety of movement—not of progression—remains to be noted. An individual which has been gliding about will become anchored by the end of its flagellum to some particle of débris or small clump of bacteria. The body will then execute sharp bending or knicking movements about its narrow, posterior end, where the flagellum becomes free, often turning through nearly 180°, and then turning back again. This anchoring process recalls the anchoring of some Bodos by the trailing flagellum, but the body movement in *Helkesimastix* is not at all of the vibrating or dancing character seen in the *Bodo,* because it lacks the anterior, vibratile flagellum.

Multiplication.—After the flagellates have been active for some hours multiplication begins. We have not observed it occurring up to six hours after excystation has taken place, but by the end of 20 hours it was proceeding actively and had evidently been going on for some time. An individual about to undergo division always comes to rest in the first place. The body becomes ovoid and then practically spherical (figs. 22, 24, 27). The subsequent course of events is not always quite uniform, though the variations are only slight. In the great majority of cases, by the time the body-form has become rounded, or even after it has been ovoid for a short while, the flagellum is no longer visible. The free part has entirely disappeared, and we are strongly inclined to think that the attached part also goes, though we cannot write with absolute certainty because it is extremely difficult to detect a motionless flagellum lying over, and closely applied to, the body. However, we think that, in many cases, it is probably entirely withdrawn and absorbed, especially as, just prior to this stage, small "working" movements of the peripheral protoplasm occur at the side where the flagellum lay. At this stage the clear nuclear area can frequently be seen to be elongated and to possess now two karyosomatic bodies (fig. 22), the parent-karyosome having already divided. Next, the body begins to elongate again (figs. 23, 29), and in a minute or two more becomes slightly dumb-bell shaped (fig. 25). The two daughter-nuclei are practically reconstituted and have begun to separate by this time (figs. 23, 29); they can usually be seen because the body remains motionless during this period. (We hope to give information with regard to the cytological details both of multiplication and conjugation in a subsequent memoir, when we have studied fixed and stained preparations). The time of appearance of the first new (daughter) flagellum varies somewhat. As a rule, it does not appear until the dumb-bell stage is reached, when it can suddenly be seen projecting out from the side of the body, a short distance from one end (fig. 23), and waving slightly to and fro. Occasionally, however, it can be seen while the creature is still rounded (fig. 24), and in such a case it may represent the old flagellum, which has been only partially withdrawn. The constriction at the middle of the elongated body now rapidly increases (figs. 25, 30), and about this time the second flagellum appears, always some distance away from the first and in the other half of the body, not far from the second nucleus. Very generally, the second new flagellum projects out from the side of the body opposite that where the first one is. The flagella increase in length and the body undergoes little irregular, jerky movements. Its middle part becomes narrower and more drawn out, the whole body having now the appearance of a double pear. Usually the two flagella have their free ends directed towards the middle, the bluntly rounded extremities becoming the anterior ends of the two daughter-individuals; though sometimes the second daughter-flagellum (the later developed one) starts from near the middle of the body, *i.e.* near to the constriction connecting the two halves, and points outwards. Ultimately, helped by the movements of the flagella, the two halves of the body are drawn still farther apart and the small daughter-individuals at length separate, gliding away in opposite directions. The cytoplasmic "tail," which each at first possesses, rapidly contracts and the typical body-form is attained (fig. 31).

In the above type of division, which is the most usual one, we regard the cytoplasmic fission as being approximately transverse to the original long axis, so that in this case we have the flagellate undergoing transverse division of the body. The whole process is fairly rapid. From the time when an individual has become ovoid and practically motionless to the time of separation of the two daughter-individuals only 10 to 15 minutes usually elapse. Now and again, however, the process is slower, taking upwards of 25 minutes; but this is of rare occurrence. In such a case, moreover, we have noticed that the body becomes divided before the second flagellum is formed, so that one daughter-individual swims away, leaving the other motionless for a few minutes longer, until it has developed its flagellum.

On one or two occasions we have observed a modification of the above method of division, a second flagellum being formed while the old one is still present, having only been withdrawn (shortened) a little (fig. 32). The shortening proceeds further (fig. 33), but in this case the old flagellum is not entirely absorbed but forms the basis of one of the daughter-flagella. In this variety of division we regard the fission of the body as being more in the long axis, rather than transverse. This may represent a more primitive mode, which has been largely relinquished in favour of the other.

Syngamy.—In a fresh culture (either agar-plate or observation-preparation), after the flagellates have once emerged from their cysts, multiplication goes on, often at first with amazing rapidity, for two days or so, until by about the third day—or sometimes even earlier—an epidemic of conjugation sets in. The only important point in the whole life-cycle in regard to which we are not yet certain is whether definite conjugating individuals, gametes, morphologically distinctive from the usual forms, are developed, and, if they are, whether these are anisogamous or not. Our difficulty arises from the fact that in a culture in which conjugation is beginning, the flagellates present show more or less the customary variation in size and form ; and, further, we have not yet succeeded in seeing two individuals actually come together and unite. In most of the cultures in which we have observed conjugation, the majority of the individuals belong to one of two slightly different types. One of these is rather characteristic and distinctive, we think, of this period. It has the posterior end of the body gradually tapering and always turned definitely to one side (figs. 34, 35), usually, though not invariably, the right side, when the flagellum is dorsal or uppermost. The curved tail-portion differs from the irregular extension sometimes seen at the hinder end in ordinary individuals (*cf.* fig. 11), in being fairly constant and not so changeable or metabolic, now retracted and now drawn out, as in that case. The other type is distinctly smaller, but is not so readily distinguishable from an ordinary individual (fig. 36) ; it is oval in shape, and the hinder end is usually more bluntly rounded.

In an observation-preparation in which syngamy has begun, two individuals are often noticed to come into contact and glide along together for a short while (fig. 37). The members of such a pair are very frequently—though, again, not invariably—of the two distinct forms just noted. The larger one of the two often appears to stick, or become attached to the other by its curved, hinder end (fig. 38) ; when this happens, both individuals get very excited and actively jerk themselves about for a moment or two ; then they will either separate abruptly or glide along together for a short distance again, and then move apart. Unfortunately, we have never seen this process followed by actual union, and therefore cannot say whether it represents a tentative seeking out of each other by definite gametes. Only in one case, up to the present, have we caught the two gametes in close contact, with the body-protoplasm of each still separate just for an instant before joining up (fig. 39) ; and, in this case, so far as could be gathered from our momentary impression before the two protoplasmic masses ran together, as it were, into one, the two conjugating individuals were not very dissimilar. We leave the matter there for the present, but hope to settle it before publishing our detailed account.*

Of course, the actual coming together and uniting is a matter of a few seconds, and therefore difficult to catch; but the gradual fusion of the two gametes and the subsequent development of the zygote is a long process, and we have observed every stage in it, on many occasions. The actual cytoplasmic union is lateral (fig. 40), and immediately after it has occurred one would think that a single protoplasmic entity was now constituted. But the subsequent behaviour is amazing and unique, so far as we are aware, in the history of conjugating elements, and well illustrates the looseness of the first union and the fluidity of the protoplasm in *Helkesimastix*—and, we doubt not, in other of these dung- and infusion-flagellates. As soon as the actual cytoplasmic union has occurred, the definite form of the two gametes is practically lost, and there is for some time a remarkable lack of attraction, or reluctance to unite, between the two essential parts, if we may thus regard the nuclei and associated elements together with the portion of cytoplasm immediately surrounding them. (This is probably because the nuclei have not yet undergone a process of maturation.) We cannot do better than describe the sequence of form-changes undergone by the zygote immediately following the union, in the instance referred to.

The conjugants of fig. 40 had not been joined for more than a minute or so before they separated again in the anterior region, one individual being desirous of steering off to the right, while the other preferred to keep straight on (fig. 42). This little difference being composed and the two individuals joined up again, one began to lag behind the other, the combined body appearing now like an irregular rhomboid (fig. 43). The conjugants were progressing forwards, more or less steadily, all the time. In the next instant the smaller half had slipped still further back, and the body had now the appearance of fig. 44. A few seconds later it was quite behind the larger half (or individual), the two being connected together only by a narrow cytoplasmic thread (fig. 45). After progressing thus for a short distance the smaller half rapidly overtook the one in front by a kind of "slithering" movement along it, and the protoplasm of both again joined up along the side (fig. 46). A few seconds later a very characteristic stage was reached, in which the combined body of the two conjugants

* We have since reconsidered the above point and now think that the tendency to adhere in couples in this way is probably purely a matter of surface tension or attraction. We have observed the same phenomenon in observation-preparations of a non-conjugating strain of a closely allied species (*vide* p. 366, *et seq.*).

was almost square, the whole zygote, with its two flagella trailing along near the outer sides, having almost the appearance of a procession banner (fig. 47). (At a later stage the body becomes more definitely rectangular and banner-like.) In this particular instance the zygote remained thus for a couple of minutes or so, moving along steadily, the two "halves" with their flagella at times approximating slightly, causing the common cytoplasm to sag, as it were, just as a banner does when its pole bearers do not keep their line. Now and again the zygote turned on its side, when it appeared as in fig. 41. At length it altered again completely, a portion of the body, apparently about a third of the bulk, advancing quickly in front of the remainder (fig. 48), until the two portions were only connected by a very narrow thread of cytoplasm, which was at first extended along and in contact with the greater part of the flagellum (fig. 49). We have no doubt that in this remarkable dissociation of the zygote into two portions, one nucleus goes with each half, just as the origin and proximal part of the flagellum can clearly be seen to do. Unfortunately, we could not make out the nuclei at all during these living observations of the conjugation-processes; this was due partly to the fact that in the earlier stages the conjugating pairs are very active and constantly undergoing form-changes, and partly because, in the later stages, the nuclei are most probably undergoing maturation prior to nuclear fusion. This separation, which may amount almost to disruption, of the common cytoplasm into two portions reminds us of the behaviour of the protoplasm of *Cercomonas* (*Cercobodo*) above alluded to, but in *Helkesimastix* it is not a passive leaving behind of a certain amount of protoplasm, but a separation actively brought about by a difference in behaviour of the two gametic energids. Here, again, in more than one instance (*cf.* fig. 54), we felt sure that actual rupture was going to occur, but we have observed this remarkable process on several occasions and the two halves never broke loose. To return to our particular zygote, the small leading portion suddenly turned right round, its flagellum becoming at the same time mostly free from the cytoplasmic thread (fig. 50), and then joined up again to the main portion (figs. 51 and 52). The zygote next assumed the form of a pear (fig. 53), and after another minute or less the stage of fig. 45 was repeated, with the difference that the smaller half was this time in front. The hinder part then "slithered" up along the other and the banner form was again arrived at.

All the above changes took place in a period of about 14 minutes from the instant of first union. This time the banner was fairly permanent, and after following it for a little while longer we left it, as we knew exactly what the subsequent behaviour would be from numerous earlier observations. The above described remarkable behaviour of the two conjugants—or, at least, of the two essential portions, since the cytoplasm is apparently indiscriminately divided at times — is not at all an exceptional occurrence; indeed, we think something similar usually happens during the earlier stages of syngamy. On other occasions we have seen both the "slithering" of two slightly unequal portions and the almost complete separation of the two halves which remained connected only by a delicate thread, just as in the above case. The joining up again in such a case occurs, we consider, as a result of the rapid contraction of the connecting thread, in just the same manner as the lagging portion of protoplasm was suddenly hauled up into the main body of the *Cercobodo* (*cf.* above). Further examples of these early conjugation stages are given in figs. 54–57, from different zygotes. Even after the banner has been definitely formed for as long as an hour, we have seen it suddenly break down into two portions, one behind the other.

Different forms assumed by the banner, when at length permanent, are seen in figs. 58–62. As time goes on the banner gradually loses its square shape and becomes oblong (fig. 63), the two parallel flagella also gradually coming to lie nearer to the mid-dorsal line, and so to each other. Most probably, by this time, the maturation processes of the gamete-nuclei are completed. Further, the two flagella are slowly shortening in length. In its earlier period, however, the banner is still capable of performing the active, undulating (free) movement, as well as the more characteristic gliding movement. From an oblong the banner now turns to an oval—really, of course, to an ovoid—which is ultimately almost as deep as it is broad. We have followed a banner from the time when it had about just become permanent (*e.g.* as in figs. 61 and 62), up to the definite oval (fig. 65), the time occupied being about three hours. In an observation-preparation in which syngamy has been going on for some time, banners and ovals are quite numerous; this will be apparent when it is remembered that every cyst formed is a zygote-cyst. At a later stage the two flagella have become much shorter (figs. 66 and 67), and the flagellate moves very sluggishly; it soon ceases to displace itself and only performs little turning and "knicking" movements. The protoplasm becomes contracted and somewhat denser, and the oval nearly always leaves the under surface of the cover-slip by this time and sinks to the lower level of the medium. The flagella are at length quite absorbed and the body becomes spherical. We have followed an individual oval with two flagella close together, as in fig. 65, up to the time when it became a motionless, rounded body at the lower level, and this change took about six hours. The whole process up to this stage takes from 9 to 10 hours, according to our actual observations; of course, this may not be the minimum period required, though we should say it is not far from it.

We have not actually watched a motionless, rounded zygote through its encystment period, but we noted the position of the above individual and of others at the same time late in the evening, and on looking at them next morning we found they had become the very characteristic "shrinkage" cysts (figs. 68 and 69). The cyst-wall has been formed and the protoplasm has continued to contract, so that a space is left at one side between the body and the wall. We are inclined to think it is a space containing no liquid, because it is always very clear. The space appears of different size, according to the age of the cyst (figs. 68 and 69). When small the space is spherical, but when it attains its maximum size it is lens-shaped. A curious fact is that after the cyst has shown this condition of shrinkage for some time—the period may vary from two days or less up to longer—the space disappears entirely and the cyst becomes a permanent cyst, as described at the commencement of this account. This finishes the life-cycle.

Biological Notes.—As, in a normal culture, the great majority of the flagellates form cysts, one could not have a more convincing and readily obtained demonstration of the regular occurrence of syngamy in the life-cycle of these simple dung-flagellates. Although all the above observations on *Helkesimastix* have been described either from plate-cultures or from observation-preparations, this is only because, on account of the more rapid multiplication and development under those conditions, the individuals are very much more abundant than in a simple, moistened dung-culture, and therefore the different stages are more readily found. We have not the slightest doubt that the life-cycle is exactly similar under the more natural conditions, the only difference being that it is probably slower, *i.e.* a longer period may elapse before the whole life-cycle is completed. For instance, it is usually three or four days before this flagellate is observed in the active condition in a dilute dung-culture, and it may persist in the active condition for a week or more before forming cysts. The reason for this is, we consider, because the watery medium is not nearly so rich in nourishment as the beef-extract medium. The bacterial development is not nearly so great as in the latter case, nor is the multiplication of the flagellates so abundant. Therefore, on the one hand, a somewhat longer time most probably elapses before the cyst-wall is dissolved; and, on the other hand, the medium does not so soon become excessively full of the "toxic" products, or whatever chemical substances induce the cessation of multiplication and the tendency to conjugation, whether formed from the flagellates, or the bacteria, or from both. As, however, we are at present engaged in making a full study of this interesting flagellate from a biological standpoint we will not further discuss these questions at present.

Loss of Syngamy and the Power to form Cysts.—We have discovered one very interesting and important fact, however, which deserves to be mentioned. By sub-culturing the flagellates* while they are still all in the active condition, *i.e.* before cysts have been developed, on to a fresh plate of the same medium, we have found that multiplication will continue for a further period (of two days or so), before conjugation sets in. The important fact, however, is that, after a certain number of sub-cultures have been thus made, the flagellates, although they are still able to thrive and multiply actively on each successive transference, no longer undergo syngamy, followed by cyst-formation, and have, so far as can be seen, entirely lost the power to do so, at all events under the existing conditions.† Up to the present time (October), we have thus kept a "strain" for more than 20 weeks, through 35 sub-cultures on to fresh "constant" medium, and in each sub-culture the flagellates have multiplied enormously. In the sub-cultures up to the fourth a very few isolated cysts were still found. *But in none of the subsequent ones has a single cyst ever been seen.* The flagellates, instead of conjugating and forming great numbers of cysts, as usual, degenerate and die off, only a small proportion remaining still alive. Yet, at the end of 10 or 12 days, sometimes more, a few individuals still persist alive, and the transference to a fresh sub-culture can be successfully made. The flagellates which remain alive are rounded, granular, very sluggish forms, with the flagellum sticking straight out and almost motionless; but, in the fresh medium, they are capable of quick recuperation and renewed multiplication.

During the earlier weeks, after this new strain was fairly started, we never observed any biflagellate forms, comparable to the conjugating pairs above described, although thousands of individuals must have passed under our eyes. Then, during the 8th and 9th weeks, respectively, we observed on two occasions, in different observation preparations, a single biflagellate individual. After this, we looked carefully for others, at intervals, but no more were seen until the 14th week (in the 23rd sub-culture), when another was observed. Since that time, in most of the sub-cultures, a few biflagellate individuals (banner-like forms) have been found and the number of these has gradually tended to become less infrequent, although they still constitute a very small

* This experimental work, it may be mentioned, has been carried out upon another species of *Helkesimastix*, with which we have worked latterly. This form (*H. major*, n. sp.) also occurs both in sheep and goats, and differs only from *H. fæcicola* in the larger size of the adult individuals and also of the cysts, rendering it more convenient for study. The life-cycle is similar in both.

† We tried the experiment of re-introducing the non-conjugating "strain" into the original dung-culture (sterilised) with the object of seeing whether the ability to undergo syngamy would be restored. This also gave negative results.

proportion only of the total number of flagellates present. We have been able, on several occasions recently, to follow the further behaviour of these forms, and have ascertained the important fact that they always divide, and never become rounded-off and form cysts. Moreover, we have observed a certain number of these forms which were very large and possessed not two, but three, or even four or five flagella (and nuclei, as ascertained from permanent preparations). We have seen an individual with three flagella, and also one with five, actually divide into two rather unequal portions; in the former case, one half had two flagella and the other one, and in the latter case, one had three and the other two flagella (and nuclei).

We will assume, for the present, that these forms represent a union of two or more individuals, rather than a long-delayed division.* When we first observed the isolated instances of biflagellate forms in this strain, we considered that they represented true syngamy, which, although occurring very rarely was apparently still "latent." We now know (1) that these "unions" may be either binary or multiple, apparently more or less indifferently; (2) that these forms always divide, the nuclei and flagella being apportioned out, often unequally, between the two halves, and that they never proceed to form cysts—the invariable sequel, normally, to conjugation in *Helkesimastix*; (3) and, lastly, that these forms, or the products of their division, are equally incapable of persisting ultimately (unless, of course, they are transferred to fresh medium). Hence, we feel certain that none of these unions represent true syngamy. Bearing in mind the extremely plastic character of the cytoplasm of this creature, we consider these unions are due to physical, rather than "vital" factors, and result from continued cultivation and the condition of the culture at the time.

In our opinion, these peculiar cases do not invalidate the following general conclusion which we wish to draw from our observations on this non-conjugating strain. The "intensive" culture of this flagellate has resulted in the loss of the power to undergo true syngamy and to form cysts. The further existence of this "strain" is now dependent on continued transference to fresh "constant" medium. Minchin, in his 'Introduction to the Protozoa' (London, Arnold, 1912), in the chapter on "Syngamy and Sex," has pointed out (p. 161) that "intensive culture, whether artificial or

* We cannot yet write with certainty upon this point, because we think, from the evidence obtained so far, it is possible that these forms represent a long-drawn-out mode of division, due to the effect of the cultural conditions, in which the nucleus and flagellum divide, it may be more than once, before the cytoplasm does. There are other cases known of a corresponding behaviour under conditions which are probably not quite normal; an example which is particularly interesting in relation to the present discussion is that of multiple longitudinal fission in certain lethal trypanosomes.

natural, as in parasitism, seems to diminish the necessity for syngamy"; from this stage it is only a step further to find the capacity for syngamy lost.

Bearing on the Case of the Trypanosomes.—It appears to us that this remarkable experimental fact has an important bearing on the question of the trypanosomes, and may afford an explanation of why conjugation (or syngamy) in these parasites, though assiduously sought, has not been observed in any authenticated instance. Probably it never does occur, because these forms have lost the power to conjugate. Just as, in the case of the above-discussed strain of *Helkesimastix*, the rapid, successive transferences to fresh, non-toxic medium at first removed the necessity for conjugation (and encystment), and then have led to the loss of the ability to undergo syngamy (and, in this case, to form cysts), so a similar development has very likely occurred in the trypanosomes and related forms. As is obvious, the conditions of life in these parasites are readily comparable to those above described. The trypanosomes live in a rich nutritive medium, namely blood, in the vertebrate, or blood in various stages of digestion, in the invertebrate host; and, as is well known, rapid multiplication in both hosts is usually found. In the invertebrate we get, more or less frequently, replenishment, *i.e.* a fresh supply of the "constant" medium (namely blood), together with removal of toxic products. In the blood of the vertebrate, where there is at first abundance of medium, but not any "fresh" supply, it is very interesting to note that, in the case at all events of many lethal trypanosomes, which, as Minchin has pointed out, are not yet completely adapted to their hosts, we get the production eventually—after active multiplication has gone on for some time—of the well known "involution" forms. These, at any rate in our opinion, as in that of many French workers, simply die off after a time, if left to themselves; but they are capable of being "rejuvenated" and of again multiplying actively if passed into a fresh host. And a similar state of affairs has been met with by one of us in cultures of avian trypanosomes. Now it seems to us that these two cases present a very close parallel to what we have observed in *Helkesimastix*. It may be pointed out, also, that the non-conjugating strain of *Helkesimastix* is the result of an abruptly originating and more or less artificial intensive culture, whereas in the case of trypanosomes and allied parasitic flagellates the corresponding conditions have operated naturally and over a long duration of time.

On the supposition that syngamy has now been entirely lost in the hæmoflagellates, the explanation which we here put forward is much more probable, we think, than the idea, which has also been suggested, that the stimulus afforded by the change of hosts is accountable for the loss of this process in the trypanosomes. We have always considered that there are two or three serious objections to this latter view. Briefly stated, these are as follows:—(1) Trypanosomes can be successfully inoculated, without limit, into fresh vertebrate hosts, *i.e.* the same "constant" physiological medium, without showing (so far as is known) conjugation. (2) Binucleate flagellates of insects alone (*e.g.* certain leptomonads) have no alternation of hosts, and syngamy is apparently just as little likely to be found in these parasites as in the trypanosomes themselves. (We are inclined to think, indeed, that syngamy may have been lost in the ancestral trypanosome form, before ever it acquired an alternation of hosts.) Lastly, we have the important case of the Hæmosporidia, intracellular parasites which all have the change of hosts, and in all of which conjugation regularly occurs. Why should not the physiological stimulus afforded by the change of environment have influenced them also? And, on the other hand, there is no reason to doubt that the primitive ancestors of the trypanosomes underwent a process of syngamy, since it appears likely that many of these lowly protomonadine forms, among which the origin of the trypanosomes is to be sought, possess this feature.

We consider, therefore, developing the ideas expressed by Cropper and Drew (*loc. cit.*) along the line indicated by the experimental facts adduced above, that the loss of syngamy is due to the surfeit of nutrition, together with the non-toxicity of the medium, that is to say, the absence (in excess) of the chemical substance or substances to which the flagellates react normally by the cessation of multiplication and the onset of conjugation. It will be readily apparent, from what has been just pointed out, how these factors have prevailed in the case of the trypanosomes. And we think that a similar explanation can be applied, not only to the case of insectan Binucleata, but probably to that of many other parasitic flagellates as well.

EXPLANATION OF FIGURES.

All the drawings, with the exception of fig. 12, are from sketches made directly at the time of the living observation. Fig. 12 is a camera lucida drawing of an individual fixed with osmic acid vapour. The magnification throughout is approximately 2250 times linear, arrived at after a comparison of other individuals fixed with osmic. We are indebted to Miss Rhodes for kindly tinting and sharpening up the drawings.

In our figures, we have shown the nucleus or omitted it, according as to whether we were able to observe its position in the particular individual represented, or not. As mentioned in the text, we were unable to make out satisfactorily the nuclei in life, in the conjugating pairs.

Note with regard to the flagellum :—As mentioned in the text, the length of the flagellum varies in individuals otherwise similar. In order to save space, we have drawn the flagellum usually short, except in fig. 10; the flagellum of the individual of fig. 12 is, of course, natural length.

PLATE 13.

Figs. 1–3.—Cysts.

Figs. 4–7.—Excystation and the development of the flagellum.

Figs. 8–11.—Different forms of individuals.

Fig. 12.—Individual fixed with osmic acid vapour, to show that the flagellum is not actually attached by any membrane to the body.

Figs. 13 and 14.—To illustrate the behaviour of the flagellum in turning of the body.

Figs. 15–17.—Rupture of a large contractile vacuole and form-changes of the body.

Figs. 18–21.—To illustrate a mode of turning of a stationary individual (see text).

Figs. 22–26, 27–31, and 32 and 33.—Different instances of division (binary fission).

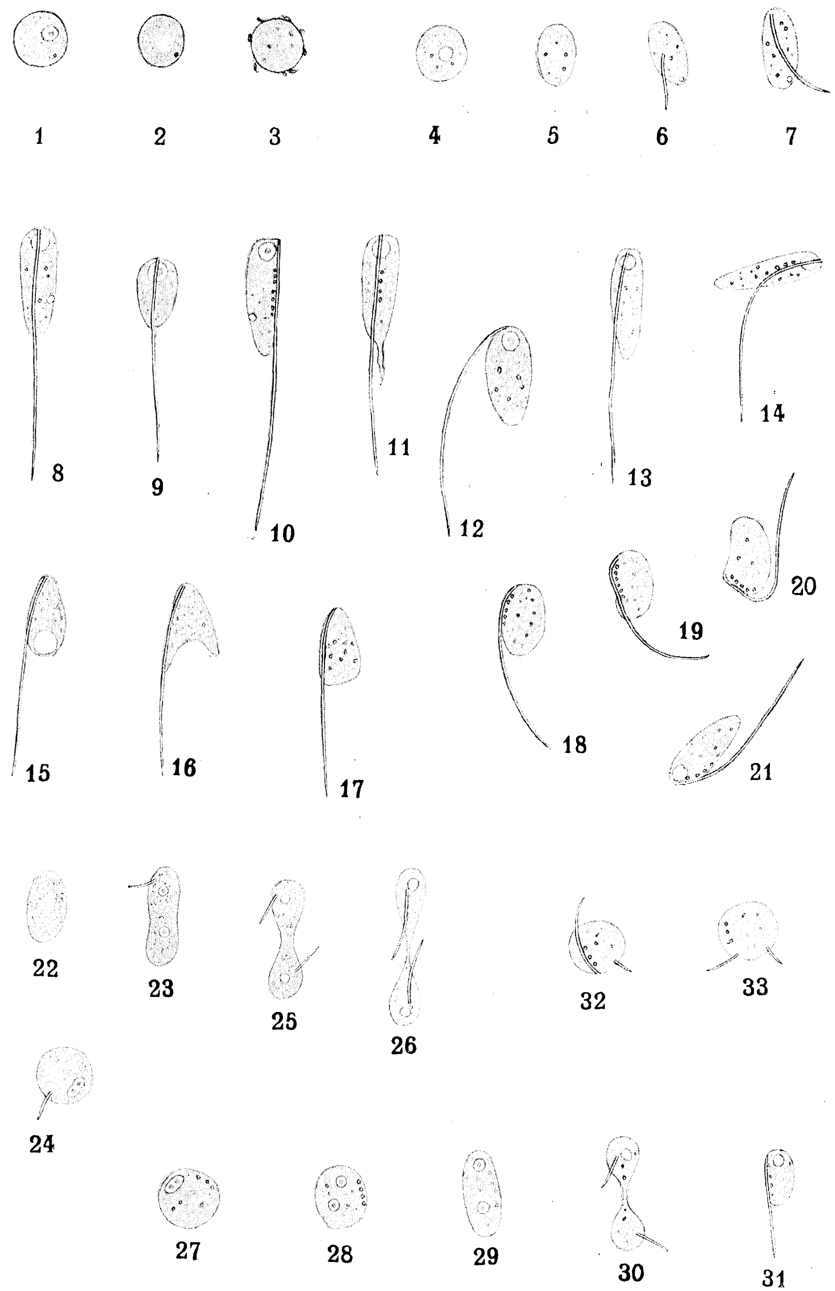
PLATE 14.

Figs. 34–36.—Different forms predominating in a culture about to start conjugation.

Figs. 37–62.—The whole process of conjugation (syngamy), showing the different form-changes during the progress of the fusion.

Figs. 63–67.—The gradual contraction of the body and absorption of the two flagella of the zygote, prior to encystment.

Figs. 68 and 69.—The cysts as first formed, so-called "shrinkage" cysts, with a vacuole or space of varying size.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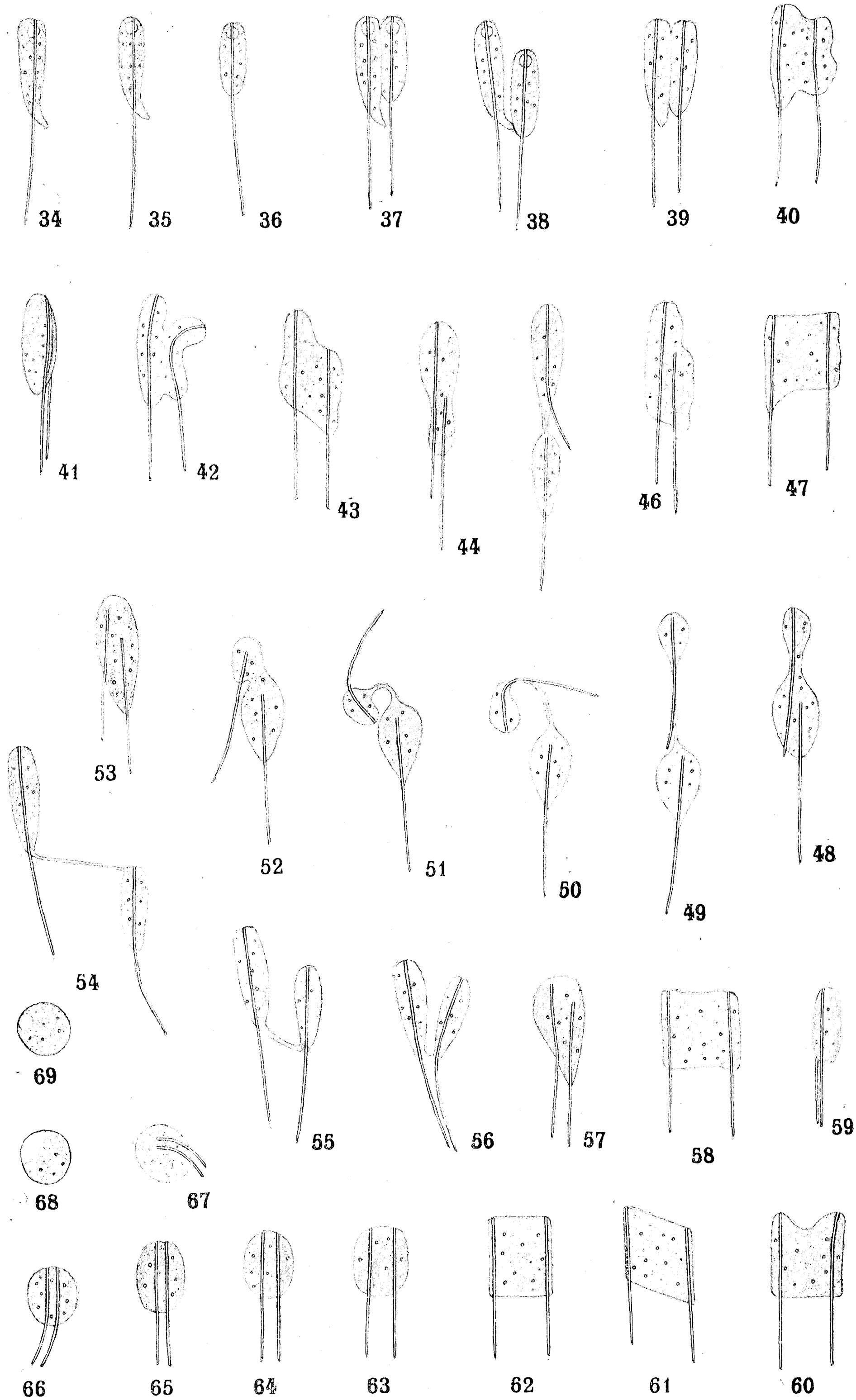
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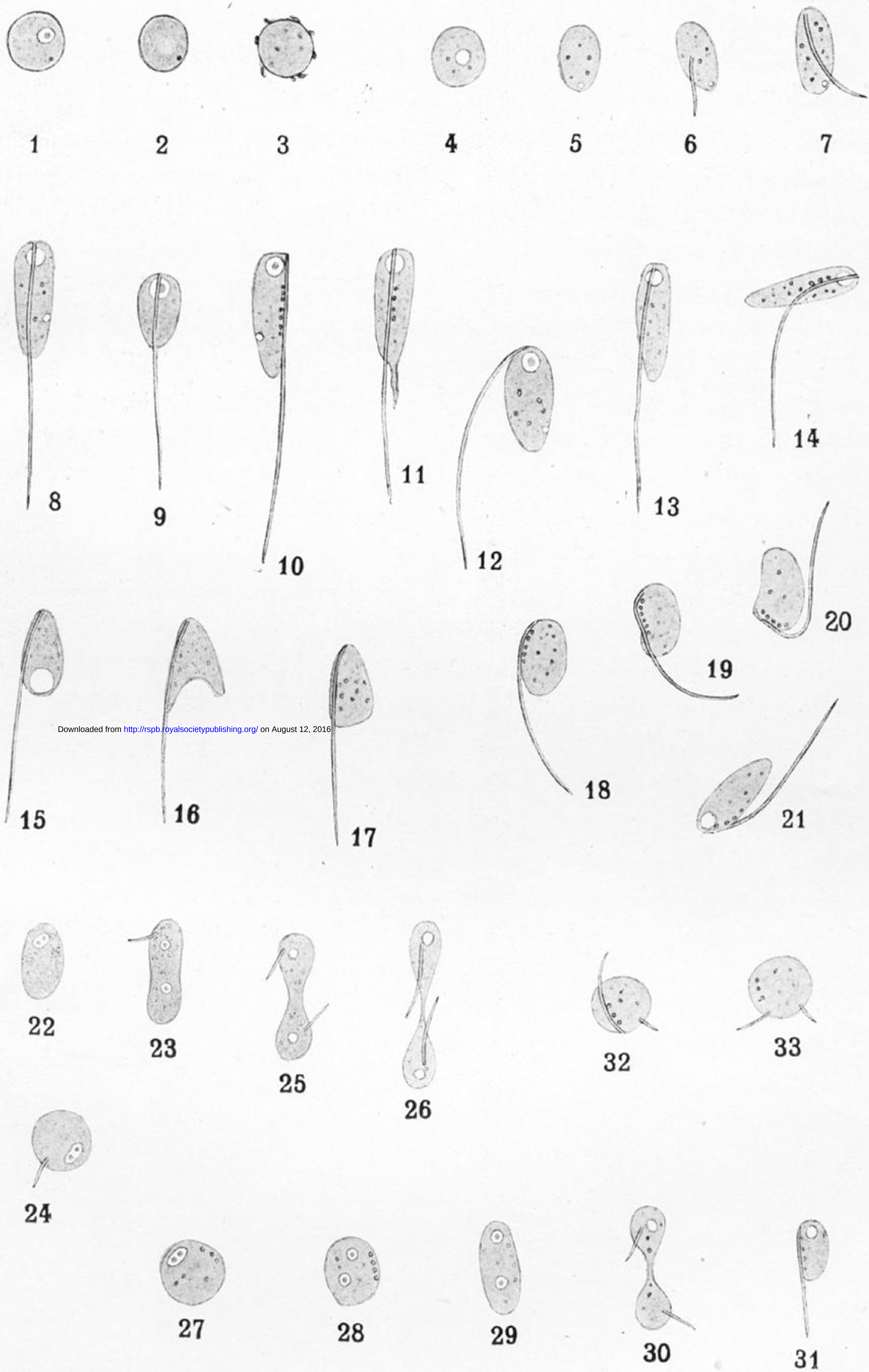
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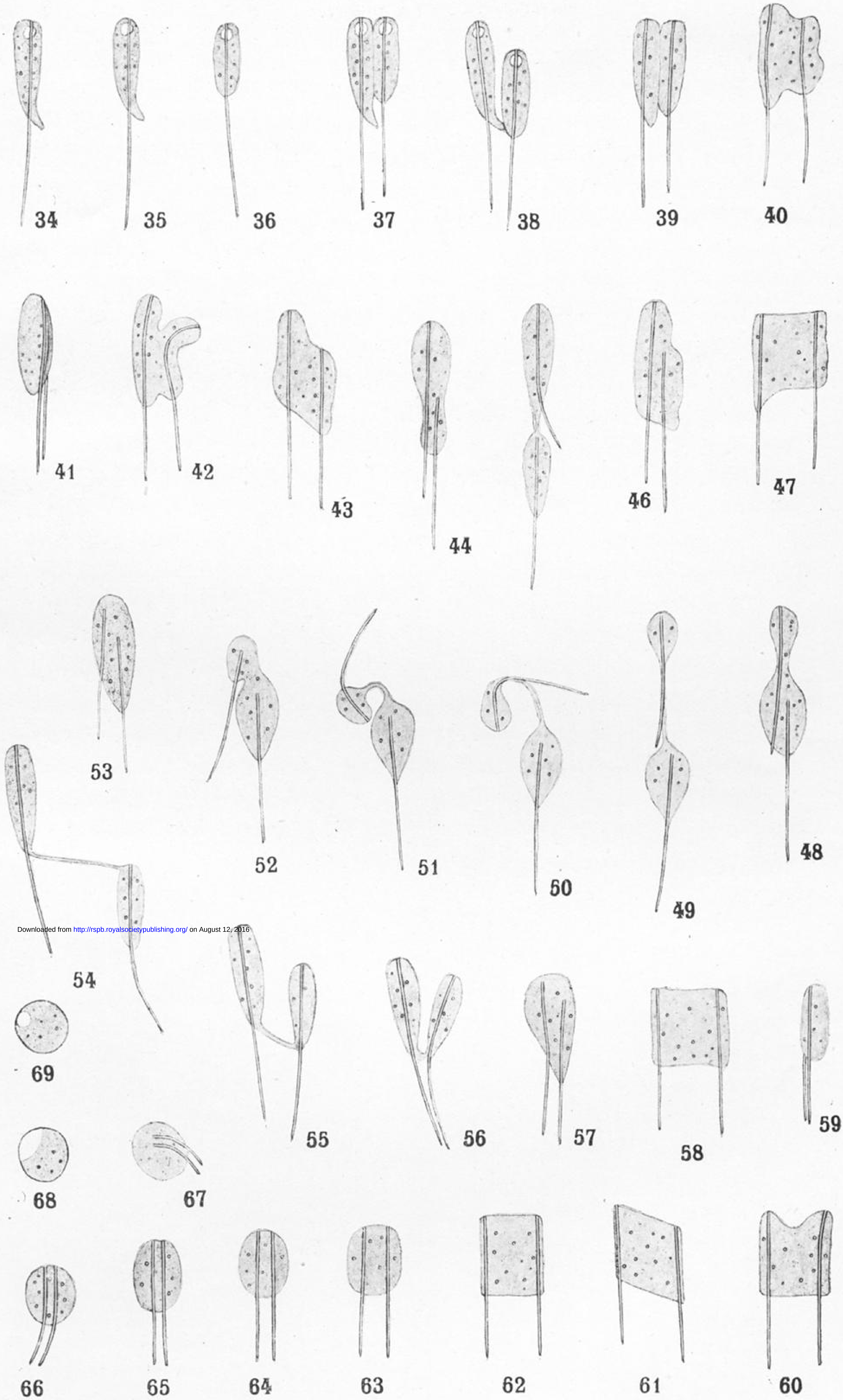
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