The Structure and Life-History of Copromonas subtilis, nov. gen. et nov. spec.: a Contribution to our Knowledge of the Flagellata.

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With Plates 4 and 5, and 3 Text-figures.

Contents.

Introduction.

I believe there is no group of Protozoa which has yielded more interesting results from its investigation than that of the Flagellata. Even since the wonderful work of Dallinger and Drysdale was published, the flagellates have been invested with a fascinating uncertainty regarding their reproductive capacities. And, although this work has never been confirmed, the recent discoveries of Schaudinn and Prowazek have revealed the existence of life-histories which are in no way less remarkable than those described by the English investigators. Yet in spite of all this the flagellates —with the exception of the trypanosomes—are much neglected by protozoologists.

In so far as it indicated the sexuality of the group, the work of Dallinger and Drysdale has received confirmation. But the remarkable multiple fission and sporulation—including the formation of ultra-microscopic spores—which they saw have been seen by no one since. And hence, although we may indeed say that, as the result of recent work, "the pronounced scepticism of Klebs, Senn, and others with regard to the occurrence of a sexual process in this class . . . is now completely refuted," it is by no means legitimate to conclude that "the views of Kent and of Dallinger and Drysdale, are at length vindicated."[1] One form of "reproductive granule" described by Dallinger and Drysdale appears to be in reality a starch grain! And Stein's "endogenous reproduction," from the nucleus in euglenoids turns out to be due to the presence of a parasite. There is still room for much research on the monads. With the possible exception of "Monas dallingeri" (Sav. Kent), no uniflagellate monad has had its life-cycle worked out with any degree of completeness up to the present day.

Having found a monad of this kind which can always be easily obtained, and is very well suited for microscopic

[1] 'Zool. Rec. Protozoa,' 1904.

investigation, I took the opportunity of working out the details of its life-history as far as I was able to do so. The present paper is the outcome of this, and although my work is incomplete it contains some observations which are of sufficient interest, I believe, for publication. I hope to be able at a future date to decide several points which at present remain obscure.

In the later part of my paper I have given a very brief outline of some of the more important work which has been done on flagellate morphology and life-history. My reason for doing so is that it is impossible to discuss many of the problems connected with the flagellates without reference to recent work in this direction. And many important papers are in journals which are not readily accessible to the zoologist, and but few are in the English language. I am fully aware of the many omissions made from the literature of the subject, but my aim has been to contribute something towards our knowledge of the Flagellata, not to write a monograph on them.

Material and Methods.

Whilst I was working with the small parasitic Protozoa, which live in the gut of our common frog (Rana temporaria L.), and toad (Bufo vulgaris L.), I often found it necessary to keep the contents of the alimentary canal for several days, in order to follow the development of the contained animals. A few days after removal from the frog the fæces nearly always contained a small uniflagellate monad in great abundance. It is this monad whose life-history I am about to describe in this paper.

In order to obtain a suitable number of the monads for investigation I find it best to proceed as follows: A frog or toad is killed and its large intestine removed. The contents are then carefully expressed into a small, perfectly clean glass dish and covered over with a glass plate. As the fæcal matter thus obtained is usually too thick for microscopical

examination, it is diluted with a suitable fluid. Water will answer the purpose, or 0·75 per cent. NaCl solution. But in most cases I prefer to use a solution containing albumen, as the fixation of film preparations for staining is very much easier when albumen is present. I have found the solution used by Grassi and Schewiakoff (**23**) for investigating intestinal parasites to be very suitable. It consists of 20 cc. egg albumen, 1 gr. NaCl, and 200 cc. distilled water. Another 10 cc. of albumen improves the solution for films. If some of the diluted fæces be kept for about five days a large number of monads can easily be obtained.

Examination of the living monads was carried out in hanging-drop preparations, waxed round the edges, or in slides upon which a drop of the culture had been placed and covered by a coverslip—also carefully waxed round. I have also used the moist chamber of F. E. Schultze with success. The best results were usually obtained with hanging-drop preparations. These are also the most easy to make into permanent preparations—by removing the coverslip, spreading out the drop, and fixing.

In transferring drops of culture containing the monads to slides, etc., I always used a sterilised platinum loop or needle.

A word must be said about the saline albumen solution which I have just described. It does not keep for more than a week or two, and must always be filtered before use as it harbours numerous micro-organisms. One of the most constant of these is a small Amœba, resembling A. limax, Duj., which lives on the surface, where it forms a slight scum. These organisms crawl actively about for some time, and finally encyst.

Although with the illumination properly arranged, and with good lenses, etc., a very great deal of the structure and development of the monads can be seen in the unstained condition, nevertheless I have found that many of the changes undergone by the monads are more easily watched when they are stained intravitam. The intravitam stains which I have used are neutral red, Brillantkresylblau (Grübler), and

methylene blue. Neutral red has been the most generally useful. It does not stain the living nucleus, but will colour the cytoplasm a faint pink, so that the nucleus appears more distinctly by contrast. The food masses are coloured various shades of red, orange, and yellow (see p. 86). Brillant-kresylblau has also been very useful. It stains the cytoplasm a pale bluish or purplish colour, leaving the nucleus as a very distinct grey globule. Food masses take up the colour very strongly, many of them staining red or purple in a metachromatic manner. Methylene blue has been of but little service.

In making permanent preparations I spread out a small drop of the culture solution on a coverslip and then fix the moist film so made as quickly as possible. The most suitable fixatives are Schaudinn's sublimate-alcohol (2 : 1), used hot, and formalin (Schering—40 per cent. formaldehyde). The former has been especially useful. Osmic vapour is very useful for displaying the flagella, and good preparations can also be made after fixation in Hermann's solution.

By far the most useful stain is Heidenhain's iron hæmatoxylin. I use this alone, or sometimes counterstain with eosin or orange G. Other stains which have occasionally been of use are Delafield's hæmatoxylin, used very dilute, and Giemsa's stain. The latter is difficult to use, and untrustworthy, but it has proved of some service on special occasions —e. g. in cyst formation. Methyl green (used in the manner described on p. 83) has also been useful.

The permanent preparations were always mounted in balsam, except those stained by Giemsa's method, which were mounted in cedar-wood oil.

For investigating the minute anatomy of the animals I have successfully used the method devised by Schewiakoff (**45**) for ciliates. It consists in killing the organisms with osmic vapour and examining them in 10 per cent. soda solution. Many structures are rendered very clear by this method.

In examining the living animals I always used the 2·5 mm. apochromatic water-immersion of Zeiss (apert. 1·25), cor-

recting for the thickness of the coverslip by means of the correction-collar. For permanent preparations I used Zeiss's 3 mm. apochromatic oil-immersion (apert. 1·40), or less frequently the 2 mm. (apert. 1·40). Compensating oculars 2, 6, 12, and 18 were employed. I used a large Zeiss stand and artificial (incandescent) light.

I have always attached the greatest importance to the observations made on the living animal, stained preparations being used to check and amplify these observations.

Culture.—In a state of nature the frog usually deposits its fæces in the water or on damp earth. They must, therefore, be frequently diluted with water, so that a watery solution of the fæces is probably a normal medium for the monad. I attempted, however, to discover whether the monads could live in other culture-media. Unfortunately, I have not made an extended series of experiments in this direction, nor have I been able to discover any other natural habitat of the monad than that already recorded. I have succeeded, nevertheless, in keeping the monads for several days in a state of activity and frequent division in organic infusions of several kinds, and infusions of fæces of several different mammals and cf a snake. An organism described as a "zoospore" was observed in infusions of cow-dung by Cunningham (11) in India. It bears some resemblance to my monad when seen under a low power.

Systematic.

It is not possible at present, owing to our ignorance of the life-histories of most flagellates, to assign this form to any very definite systematic position. Beyond doubt it belongs to the class Mastigophora, Diesing, and to the sub-class Flagellata (Cohn) Bütschli. From the morphology of the adult form it may further be referred to the order Euglenoidina, Klebs. So little is known of the various members of this order that it is difficult to decide upon the right family, sub-family, etc., which should include the form

under consideration. It is, indeed, premature to attempt detailed classification, for there can be no doubt that, with increased knowledge of flagellate life-histories, our present system will have largely to be recast. I would point out, however, that the creature should most probably be referred to the family Peranemida, Klebs, and sub-family Petalomonadina, Bütschli. Its nearest allies appear to be Petalomonas, Stein, and Scytomonas, Stein. Judging from the figures of this latter (S. pusilla, Stein) given by Klebs (**28**), Pl. XIV, fig. 9 a–d, there is considerable similarity between the two organisms. But very little is known of the various species of Petalomonas and Scytomonas, so that it is impossible to make any definite statements on the subject. I must be content, therefore, to leave the monad in its present unsettled position, awaiting the work of future investigators.

I propose to name this monad Copromonas subtilis, nov. gen. et nov. spec. The systematic position may therefore briefly be expressed thus:

Phylum.—Protozoa.
Class.—Mastigophora (Diesing).
Sub-class.—Flagellata (Cohn emend. Bütschli).
Order.—Euglenoidina (Klebs).
(? Family.—Peranemida [Klebs]).
(? Sub-family.—Petalomonadina [Bütschli]).
Genus.—Copromonas (nov. gen.).
Species.—subtilis (nov. spec.)

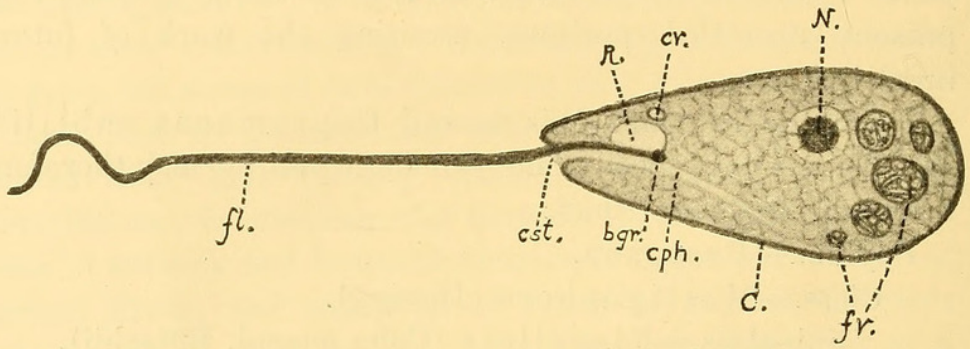
Structure.

The general anatomy of Copromonas is seen in the accompanying diagram (text-fig. A).

Although the organism, when seen under a low power, appears to be of a very simple structure—consisting merely of a globule of protoplasm with an anteriorly-directed flagellum—it possesses, in reality, considerable morphological differentiation. The body is more or less ovoid or pyriform,

and varies considerably in size. About 16 μ is an average length, but individuals are found of all sizes from about 7·5 μ to 20 μ. The breadth averages about 7–8 μ. The anterior ends bears a flagellum (*fl.*), in length usually rather greater than the body. This flagellum arises from a depression at the anterior end; the depression is a cytostome (*cst.*), or cell-mouth, and leads into a longitudinal tube, the cytopharynx (*cph.*). This extends backwards for a variable length, and is somewhat spirally disposed. It can be seen, in carefully stained preparations, that the flagellum runs along the wall of the cytopharynx for a short distance, and takes its origin from a basal granule (*bgr.*), which is strongly coloured by

TEXT-FIG. A.



iron-hæmatoxylin. The base of the flagellum is in intimate relation with a large vacuole-like space (*R.*), the reservoir. Into this latter a minute contractile vacuole (*cv.*) rhythmically discharges its contents. Sometimes two small contractile vacuoles are present, instead of the usual one.

The reservoir itself does not pulsate, but from the fact that it is sometimes absent it may be inferred that it periodically collapses, driving out its contents. No communication with the cytostome can be made out, however.

When the monad is at rest, either during division or when compressed by a coverslip, it can be seen that the contractile vacuole pulsates at the rate of once in about thirty seconds.

It will be seen from the diagram that the reservoir, on its pharyngeal aspect, is related to the root of the flagellar

apparatus. On account of the small size of the animal it is exceedingly difficult to be quite certain of the exact inter-relations of these parts. At times the basal granule seems to be situated on the posterior part of the reservoir, from which the flagellum then arises. This arrangement somewhat resembles that seen in Euglena, as described by Wager (**52**), but in Copromonas no forking of the flagellar insertion can be seen. When such a condition exists the flagellum appears to cross the reservoir, thus lying for a short distance within this structure.

The nucleus (*N.*) lies somewhat posteriorly, and is not connected in any way with the flagellum, as is so often the case in flagellates (cf. Plenge [**32**], etc.). In the living monad it appears as a greyish, spherical vesicle, slightly more refractive than the surrounding cytoplasm. In stained preparations, however, it can be seen to possess the structure shown in the diagram. That is to say, it consists of a central, deeply-staining chromatic mass, surrounded by a clear zone which contains practically no chromatin. Surrounding this is an achromatic nuclear membrane. Achromatic strands unite the membrane with the central portion. I have found that the structure of the nucleus is most easily demonstrated as follows: A small drop of solution containing the monads is placed on a glass slide. A small drop of a solution of methyl green in 1 per cent. acetic acid is then placed in the centre of a coverslip, which is carefully lowered on to the first drop. The coverslip is then pressed firmly down, and the preparation examined with the water immersion. In such a preparation the central chromatic part of the nucleus is coloured green: the nuclear membrane swells out slightly and becomes very distinct. Many other details of the anatomy can be made out in this way.

It will be seen that the nucleus is of the second type distinguished by Prowazek (**36**) in Flagellata (see infra, p. 104).

The posterior region of the body is usually more or less filled with food masses. These are of varying sizes, and formed in the manner described below (p. 86). Outside the

entire body there is a well-developed cuticular layer (*c.*). Its presence is very well demonstrated in degenerating or macerated monads (see p. 103). It does not appear to be composed of cellulose, as it is stained a pale greenish-yellow with Schultze's solution. No striation is visible, in correlation with the non-contractility of this investment (see "Movements," infra).

As I have already remarked, there is no connection between the nucleus and the flagellum. But I may here call attention to the fact that in stained preparations a very distinct dark line is sometimes seen uniting the base of the flagellum to the nucleus. After examining a considerable number of monads which show this I am satisfied that it is really due to the cytopharynx, the animals having rolled over so that the cytopharynx appears to be in line with the flagellum, and to connect it with the nucleus, over which the cytopharynx has come to lie.

I have never seen any appearances which would lead me to suppose that the flagellum is "ciliated" in the manner described by Fischer (**20**) in Monas and Euglena—the so-called "Flimmergeissel" arrangement.[1] The flagellum, on the contrary, appears to be a perfectly regular and undifferentiated filament.

Movements.

Copromonas displays a very characteristic series of movements. Under ordinary conditions it draws itself slowly and evenly along by means of its flagellum. When undisturbed the monad uses only the anterior end of this organella for locomotion; the remainder, comprising about the posterior three quarters, remains rigid. Stimulation, either by shaking or other means, causes a vibration of the whole flagellum, as in the case of Peranema (see Verworn [**51**]). In turning,

[1] I think, with Plenge (**32**), that these appearances are due to foreign bodies adhering to the flagellum.

the flagellum is directed backwards along the body, and by forcible movement of the anterior end the body is tugged round. The only other movements which occur are rolling, to a limited extent, caused apparently by a screw-like movement of the flagellum, and a curious lurching movement which is every now and then observable. It usually occurs when the animal is about to turn, but it is difficult to see how it is brought about. After progressing steadily and gracefully forward for some distance in a straight line, the monad suddenly gives a clumsy lurch sideways; then, appearing to regain its equilibrium, it continues its course, usually in a different direction.

Intrinsic movements do not take place. Owing to the rigidity of the surrounding pellicle no "euglenoid" movements are possible. The pellicle is not contractile, so that the contours of the body remain constant. In contrast with many other flagellates there is never any tendency to become amœboid. Irregularity of shape is only seen in monads undergoing degenerative changes (see infra "Degeneration," p. 102).

Nutrition.

The method of nutrition is holozoic. There can be no doubt that the depression at the anterior end of this organism —the cytostome—is a true cell mouth. Into this structure food particles are being constantly introduced by the forward movement of the monad. Bacilli, micrococci, and minute organic particles of all sorts, which are very plentiful in the medium in which the monad lives, enter and pass along the cytopharynx, and are taken up by the protoplasm at the posterior end. Many of the larger bacilli, etc., which enter the mouth, and even pass along the pharynx, are not ingested but return to the exterior again. If a monad be carefully watched for any length of time it will be seen that most of the larger particles behave in this way, only the smaller being actually ingested. Occasionally an unusually large bacterium

makes its way into the cytostome, and gets its end stuck fast in it. In this case the monad may swim about for a considerable time with the rod sticking out of its mouth, presenting a curious appearance to the observer.

Subsequent changes in the ingested food masses are most easily seen in organisms stained intravitam with neutral red. It can then be seen that the following series of events takes place: At first minute particles are seen in the protoplasm at the bottom of the cytopharynx. They appear to lie freely in the cytoplasm, without any vacuole surrounding them. After a time the particles are found to have agglutinated, and are enclosed in vacuoles. Digestion now takes place, and the agglutinated masses are gradually eroded. By means of neutral red the different stages of digestion are very beautifully demonstrated. Ingested particles at first stain a bright red. In the food vacuoles the food bodies also take up the stain strongly, but they are coloured reddish-orange. Later stages are usually of an orange or yellowish hue; so that the posterior end of the organism may contain food balls of different colours of red, orange and yellow—the colour corresponding with the stage which the digestive process has reached. It may be inferred that the digestive juice is neutral in reaction, but becomes somewhat alkaline as digestion proceeds. The change of colour may also be due partly to reduction. (Neutral red becomes yellow with alkali, to which it is exceedingly sensitive, and is reduced to a colourless leuco-product.)

The whole process, as I have observed it in Copromonas, bears a close resemblance to that described and figured in Paramœcium by Prowazek (**33**).

Stokes (**49**) has described the ingestion of food by means of a "mouth" in Petalomonas, a genus which, as I have already pointed out, is probably closely allied to the form under consideration. The method of feeding seems to be identical in the two genera.

As regards the larger euglenoids, much doubt still exists regarding the function of the so-called "mouth." Although

the experiments of Saville Kent (**24**) appeared to have definitely proved that food was ingested at this aperture in Euglena, the recent work of Wager (**52**) throws some doubt upon the matter again. From Wager's observations it is clear that at least one function of this structure is connected with excretion—that is to say, it serves as a duct for the reservoir. Khawkine (**26**) has made the suggestion that liquid food enters by the mouth, but the evidence in support of this is by no means conclusive.

In many monads (e. g. Oikomonas, etc.) food is ingested at any point on the surface of the body. This, however, never takes place in euglenoids such as Copromonas, which possess an external cuticular covering.

Life-cycle.

The life-cycle may conveniently be considered in two periods—a period of asexual multiplication, and a period of conjugation and encystment. These two periods are not sharply separated from one another, but overlap—that is to say, in any given culture some of the monads will finish conjugation before others begin.

The first period of the life-cycle is of variable length, and is made up of a very variable number of cell generations. It is, therefore, impossible to make any definite statement of the duration of this period. In cultures made in the manner described on p. 77 the following is the course of events frequently pursued, and may be taken as a fair average. But it must be remembered that it is only an approximation to the truth—not an invariable sequence of phenomena.

1st day. Culture made; no monads; a few cysts found after careful examination.
2nd ,, No monads.
3rd ,, No monads.
4th ,, A few very small monads.
5th ,, A good many monads, many dividing.
6th ,, Monads in large numbers, actively dividing.

7th day. Monads plentiful, many dividing and a considerable number conjugating.

8th „ Many monads conjugating, some encysting and some dividing.

9th „
10th „ Conjugation practically finished; a few dividing;
11th „ encystment.
12th „

?

?

&c.

Sometimes the life-cycle extends over about seven days; at other times it may take more than twice as long.

(1) Asexual multiplication.

This takes place by means of longitudinal division, and may easily be observed in the living animal. As a rule division is proceeding most actively in cultures about a week old. By fixing and staining film preparations at this time a large number of specimens showing all stages of division may be obtained with considerable ease.

General account of division (see Pl. 4, figs. 1–10).—The monads grow to a large size before division. The first sign of the onset of the process is observable in the locomotor apparatus. It is seen that the animal (Pl. 4, fig. 1), which was actively swimming about, is becoming sluggish in its movements. After a short time it comes to rest completely, and the flagellum displays slow coiling movements (fig. 2). Gradually the movements become slower and slower, and at the same time the flagellum gets shorter and shorter, and is finally completely drawn in (fig. 3). During this process the nucleus has elongated, and now appears as a bright band stretching across the cell. After watching the motionless monad for a few minutes the observer will see two minute peg-like outgrowths appearing at the anterior end. These are the new flagella, which grow up side by side, and very

soon begin to writhe about (figs. 4 and 5). As they increase in length they exhibit the characteristic movements of these structures, and the dividing organism again becomes motile. A cleft has meanwhile appeared between the bases of the flagella. It extends backwards slowly, cutting the reservoir in two as it does so. The nucleus becomes completely parted into its two daughter-products, and by the cleft gradually extending to the posterior of the body two daughter-individuals are formed, and subsequently break away from one another (see figs. 9 and 10). The whole process lasts twenty minutes or thereabouts.

The foregoing is a general account of the way in which longitudinal division is effected. I will now describe in detail the manner in which the various organellæ of the monad are doubled in the formation of daughter-individuals.

Details of division.—These must be considered in the case of (A) the nucleus, (B) the flagellum, (C) the cytostome, (D) the reservoir or contractile vacuole.

(A) The nucleus.—(Pl. 4, figs. 6–10; Pl. 5, figs. 34–40). Nuclear division is effected by a kind of amitosis. It thus differs from the phenomenon which has been observed in all other members of the order Euglenoidina which have been accurately investigated. (See infra, p. 104).

In the living organism no details of division can be seen in the nucleus. It can be seen to elongate, and become constricted into two—as shown in figs. 1–5, Pl. 4—but beyond this nothing can be made out. However, in preparations stained with iron-hæmatoxylin, and carefully differentiated, the following details of division may be observed (see Pl. 5, figs. 34–40). Before division the nucleus consists—as already noticed—of a central chromatin-containing body, ("Innenkörper") surrounded by a clear, non-stainable zone ("Kernsaftzone") bounded by the nuclear membrane. Achromatic strands cross the clear zone, connecting the nuclear membrane with the central body (fig. 34). In strongly differentiated iron-hæmatoxylin preparations the central body appears to consist of an achromatic substance in which

chromatin is suspended in the form of granules of variable size.

The first phase of division is characterised by the nucleus becoming elongated and somewhat fusiform (fig. 35). The central body appears to throw off granules of chromatin at opposite poles. A little later the fusiform shape gives place to a more or less oblong form, like a short, blunt rod (fig. 36). Enlargement of the ends of the rod next takes place, with the formation of the characteristic dumb-bell figure (fig. 37). The chromatin masses itself at the ends of the dumb-bell, the intermediate portion becoming band-like and staining a lighter tint (fig. 38). Minute chromatin granules may be seen scattered throughout. The two ends of the dumb-bell then undergo increased differentiation and separation, becoming rounded off, and containing the aggregated chromatin elements of the nucleus. For some time these daughter-nuclei remain connected by a fine filament as in fig. 39. Finally the filament disappears, and two completely formed daughter nuclei are left (fig. 40).

A general discussion of the nuclear phenomena will be found on p. 103. I will here merely call attention to the fact that division is amitotic; there are no differentiated chromosomes, no extra-nuclear division centres. In this respect it resembles that of some rhizopods (cf. for example, the Leydenia-phase of Chlamydophrys).

(B) The flagellum.—(Pl. 4, figs. 2–5; Pl. 5, figs. 41–46). A description of the manner in which the new flagella are formed, as seen in the living monad, has already been given (p. 88). The finer details of the process can be made out only in stained preparations. The flagellar insertion is most clearly seen in osmic vapour preparations (fig. 41). In these the basal granule is very distinctly seen, lying in the wall of the cytopharynx against the reservoir. As we have already seen, the flagellum is retracted before division. In stained specimens it can further be seen that it gradually disappears in the direction of the basal granule, until only this structure is left (figs. 42 and 43). The basal granule

then divides (fig. 44), becoming dumb-bell shaped, and finally being constricted into two daughter-granules. From each of these a new daughter-flagellum springs up (fig. 45), and on reaching the surface becomes visible as the little peg-like structure—the shoot which develops into the flagellar stem —which I have already described in fig. 4. A corresponding stained stage is seen in fig. 46.

The manner in which flagella multiply has not been made out in many organisms. Dallinger and Drysdale (**12**), James-Clark, and others were of opinion that new flagella arose from the splitting of the old. On the other hand, Pelletan (in Dinobryon) and Klebs (in Euglena [**27**]) state that the new flagella arise by a new growth—one daughter-cell taking the old, the other the new. The process seen in Copromonas is of interest in connection with the morphology of the basal granule (see infra, p. 106). It is probable, however, that the flagella divide differently in different species.

(c) The cytostome.—The doubling of this structure is exceedingly difficult to observe. Even in the active adult it is often hard to distinguish, although it becomes more evident in monads which have been flattened out. From a number of observations on living animals and permanent preparations I believe that the cytostomes of the daughter-organisms are both new growths, the old cytostome apparatus having degenerated.

Before division the cytostome and cytopharynx are visible as a depression from which a dark line extends backwards (Pl. 4, fig. 1). As division proceeds this line becomes less distinct (figs. 2 and 3), and finally disappears. When the new flagellar rudiments first appear they are seen to be growing out of little pit-like depressions (see figs. 8 and 9, Pl. 4, and fig. 46, Pl. 5), which gradually penetrate the cytoplasm of the daughter-cells. These inpittings are the new cell-mouths and their continuations the new cell-gullets.

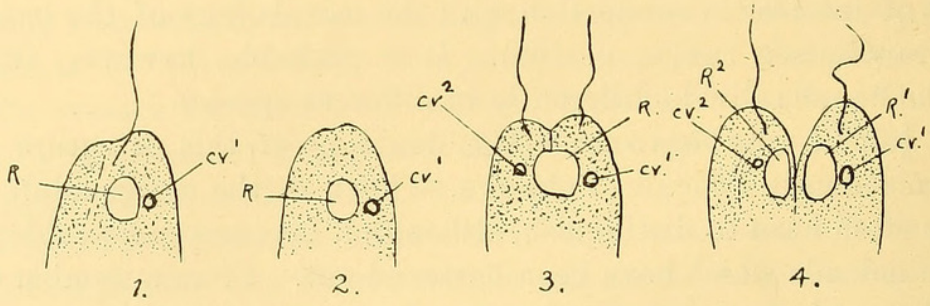
As a rule, it appears to be the case among flagellates that one of the daughter-monads retains the old cytopharynx, etc., while the other develops a new one. This happens, for

instance, in Trichomastix lacertæ, according to Prowazek (**39**). But really very little information is to be gleaned from the literature on the subject.

(D) The reservoir and contractile vacuole.—Owing to the circumstance that the monad is quite motionless during the doubling of these structures I have been able to observe the process with considerable accuracy. It can be most easily described with the aid of a diagram (see text-fig. B).

Before division the monad is seen to possess one reservoir (*R.*) with a single adjacent pulsating vacuole (cv^1). In fig. 2 the flagellum has been drawn in, and the cytopharynx has disappeared. Later (fig. 3) the two new flagella have made

TEXT-FIG. B.



their appearance and a cleft can be seen between them. On the left of the reservoir a new contractile vacuole (cv^2) has suddenly made its appearance, the old one (cv^1) remaining in its original position on the right.

The interflagellar cleft gradually extends backwards (figs. 3 and 4), and as it does so it completely halves the reservoir—one half going to each daughter-monad. At this stage the new cell-mouths are distinctly visible, lying over the vacuole apparatus (fig. 4, and see also Pl. 5, fig. 46).

During the whole of this process the vacuole (or vacuoles) continue to pulsate at a rate of about twice a minute.

I have described this process in some detail, because so little appears to be known about it in most flagellates. Division of the contractile vacuole has been described by some writers, but I think this is very doubtful. For the

majority of Flagellata, we can still say with Senn (**47**): "Über die Art der Vacuolenvermehrung wissen wir nichts näheres."

Transverse or multiple division has never been observed. A large number of cases of the former which have been described among flagellates are almost undoubtedly to be regarded as late stages in longitudinal division. There can be no doubt that it does occur in some forms, however, e. g. in Oxyrrhis. And amongst the Chlamydomonadina, according to Dangeard (**14**), division may be either longitudinal or transverse, the result being dependent upon the position of the achromatic spindle during mitosis. The plane of division is at right angles to this; and the position of the spindle itself is determined by the relative positions of the cell-protoplasm and the chloroleucite.

After longitudinal division has continued for a period varying from about two to six days, a considerable number of the monads will be found to be conjugating. I will therefore now describe this process and its sequelæ.

(2) Conjugation and Encystment.

The conjugating individuals are indistinguishable from their forerunners. Every monad, apparently, is a potential gamete. No difference, therefore, exists between the gametes themselves—that is to say, they are isogamic, displaying no sexual differentiation. It is true that occasionally one of the conjugants appears to be distinctly smaller than the other. But then it must be remembered that in cultures of the monads a great variation in size is often observable, and it is most probable that size-variation in this case is merely an expression of individual differences in rate of growth and food assimilation.

Conjugation may be easily watched in a hanging-drop preparation, though it is difficult to make out anything of the nuclear phenomena by this means, owing to the activity of the monads. The process, as seen in the living animals, may

be most easily described, I think, by recording a typical case (see Pl. 4, figs. 11–16). The first thing to be seen is the approach of two monads to one another. Hitherto they have been swimming about apparently at random, but they now draw near and come in contact by their anterior ends (fig. 11). Each monad appears to be normal, possessing one nucleus, reservoir, etc. For a few moments they swim about merely touching one another, and gliding over one another to some extent. But it is soon seen that an actual adhesion is taking place, so that the monads become firmly united at their anterior ends. Both the flagella continue to move actively, and very often get entangled.

After swimming about for some minutes in this manner it can generally be made out that one of the flagella is becoming shorter. At the end of a quarter of an hour this is usually very evident, and a little later, or perhaps even now, one flagellum is completely drawn in. Fusion is extending backwards, so that the conjugants have the appearance of one large, bilobed monad, rather than of two applied to one another (fig. 12). Active movements still occur, and further fusion is seen slowly to be taking place. In about half an hour more the monads present an appearance like that seen in fig. 13. It appears as though one monad absorbs the other; for a little later they have the appearance, shown in fig. 14, of one monad bearing a projecting process on its side. Still later the fused individuals present the appearance of one somewhat asymmetrical organism (fig. 15), only differing from the ordinary monads in being slightly bilobed at the posterior end. Sooner or later the remodelling is completed, and an organism exactly like an ordinary monad is formed, though it is usually noticeably larger, and sometimes can be seen, after careful examination, to contain two nuclei in place of the usual one.

The subsequent history of these binucleate monads is not always the same. Development may proceed along one of two lines: (1) The animal may encyst, or (2) it may continue to feed and divide longitudinally, just like an ordinary indi-

vidual. In the first case (1), the animal gradually becomes more rounded in shape, and decreases considerably in size. The flagellum is finally drawn in, and a very delicate cystic membrane is formed. A considerable time elapses during these changes, and I have never been fortunate enough to be able to observe the changes which occur in the living animal inside the cyst. These can be very clearly made out in stained specimens, however, and are described below.

The reason why the contents of the cyst are so difficult to distinguish is probably to be sought in the manner in which the reduction in size is brought about. I think this is probably effected by the protoplasm giving up part of its water. By doing so the cytoplasm would, for a time at any rate, become relatively denser, and so render the nucleus less distinct.

It is not easy to see the cyst when first formed. It is only visible as a pale border to the rounded-off cell, and is not easily distinguished from a mere optical effect which makes a halo appear round a small, brightly illuminated object. The cysts appear more distinctly after some hours' time, and seem to be composed of a soft, gelatinous substance. I have found that the most rapid way of demonstrating their presence at this and earlier stages is to proceed as follows : the hanging-drop containing the cysts is carefully spread out, after removing the coverslip, and allowed to dry rapidly. It is then fixed for ten minutes in absolute alcohol, and stained for ten minutes in Giemsa, then rinsed in water, blotted with a cigarette paper, and mounted in cedar oil. By this method the cysts are stained pink, the protoplasm inside dark blue, with the nuclei sometimes visible as dark blue, purple, or red bodies.

The gelatinous cysts, in the normal course of events, become hard and slightly yellowish in colour. They are not uncommonly encrusted more or less completely with the minute organic particles which abound in the fæces cultures. There is a good deal of variation in the shape of the cysts, some of the commonest forms encountered being shown in figs. 17, 18,

and 19, Pl. 4. They are roughly spherical, ovoid, or sometimes of the form shown in fig. 19, with a notch at one end. This notch marks the former position of the cytostome. In fully-formed cysts the nucleus can be clearly seen as a refringent sphere, lying in the middle of the protoplasm.

The second case (2), in which the zygote does not encyst, is of considerable interest. After swimming about for a time, the monad—which, although really a zygote, is indistinguishable from the asexually-reproducing animals—comes to rest and divides in the normal way. It can be seen to contain but one nucleus (which stained preparations show to be derived from the fusion of the two gamete nuclei), although the manner in which this single nucleus arose I have not been able to observe satisfactorily in the living animal. On one occasion when I carefully watched the conjugation of a pair of monads and their subsequent development I found that three hours and ten minutes elapsed from the time when complete fusion of the gametes had taken place until the zygote began to divide longitudinally.

The zygotes and their descendants appear to be able to continue dividing for a considerable period, though I cannot say definitely how long. In cultures in which most of the monads appeared to have conjugated—though one cannot be certain that all have done so—I have often found a few dividing individuals a week or ten days afterwards. In the end, however, the majority of these appear to encyst, the flagellum being drawn in and the cyst wall formed as in the case of the monads which encyst immediately after conjugation. (See Pl. 4, figs. 31–33).

As far as I am aware there is but one flagellate monad which has had ascribed to it the ability of continuing to lead an active life after conjugation. I refer to Dunaliella, one of the Phytomonadina, whose life-history has been investigated by Teodoresco (**50**). He describes the fusion of isogametes. One loses its flagella and is absorbed. Under favourable conditions encystment does not follow fusion, but the monads remain active. The nuclear phenomena have not been

elucidated, and I am unable to find any proof that the zygotes continue to divide like ordinary monads. Teodoresco does not appear to have followed out a pair of conjugants until the zygote which they produced divided, but he is probably correct in supposing that this takes place. "Si cela est vrai," he remarks, "ce serait alors le seul cas connu jusqu' à présent d'une zygote, provenant de l'union de deux gamètes mobiles, qui dans les conditions favourables de vie, ne passerait pas à l'état de repos." That such a condition actually does exist in Copromonas there can be no doubt. For, as I have already said, I have watched the living animals from the beginning of conjugation to the completion of longitudinal division in the resulting zygote.

I have succeeded in following out the nuclear phenomena which occur at conjugation in permanent preparations only. I will now give the results which I have obtained from a study of these preparations, which were made in the manner already described (see Pl. 4, fig. 21–33).

Before conjugation the nucleus appears to be quite normal, like the resting nucleus already described. The nuclei of both conjugants remain in this state until a considerable degree of cytoplasmic fusion has taken place and one flagellum has completely disappeared (figs. 21, 22). Each nucleus now divides, going through all the stages which I have already described in the process of asexual multiplication. The nuclei do not always divide simultaneously (cf. fig. 23). Even before division is complete it can usually be seen that one of the daughter-nuclei stains more palely than the other. This pale nucleus is a reduction nucleus, and it very soon degenerates and breaks up. In fig. 24 a pair of conjugants are seen in which both the nuclei have divided, so that there are now four nuclei present. Of these four, two (the lower two in the figure) are reduction nuclei, and may be recognised by their lighter colour. I should point out that the nuclei here appear rather larger than they really are, as the specimen has been slightly flattened out on the coverslip.

So far as I have been able to make out, only one equal

division of the nucleus takes place in the manner just described. But in monads in a later stage of fusion small granules of chromatin can often be seen apparently emerging from the nucleus. At first these appear as little projections from the central chromatin mass; but later, as they become separated, they are seen as small granules attached by a slender stalk to the central mass (see fig. 25). Finally, they become completely separated, and degenerate in the cytoplasm. This process might also be described as a heteropolar division. It appears to occur in the organisms after the first reduction division has taken place, for the degenerate fragments of the first reduction nucleus are usually to be seen in the cytoplasm (see fig. 25).

It is impossible to say whether a single reduction occurs in this manner, or whether more than one granule is extruded from the nucleus, on account of the fact that small broken-up bits of the first reduction nucleus are usually lying about in the cell. I can only say that at least one small nuclear mass is eliminated in this way. Personally I believe that only one granule is formed in each case, but I cannot prove that this is so, as I have never succeeded in watching the process in the living monad.

By the time that the nuclear reductions are completed the two conjugants have become very largely fused with one another as regards the cytoplasm (fig. 26). The two reduced nuclei now approach one another, and the nuclear membranes come in contact. If the fate of the zygote is immediate encystment the flagellum is drawn in (fig. 28) and a cyst wall formed in the manner already described. Inside the cyst, therefore, one sees two nuclei in contact with one another, as shown in fig. 29. A process of fusion between the nuclei then takes place in the cyst (fig. 30), so that ultimately the cyst contains but a single nucleus (fig. 33). The nuclear membranes first break down at the point of contact, and the central chromatin masses finally fuse. When spherical the cysts have an average diameter of 7 μ–8 μ.

The nuclei come together and fuse in the same way in those

zygotes which continue to lead an active life instead of encysting. But in this case, of course, the fusion of the nuclei is accompanied by an external remodelling of the cytoplasm (see fig. 27).

It is a matter of some difficulty to make out the fate of cell-organs other than the nucleus during conjugation. From its behaviour during division the basal granule of the flagellum might be thought to play some part in the process of conjugation. But although I have devoted a good deal of time and care in endeavouring to discover whether the basal granules fuse during conjugation I am still quite ignorant on the subject. Appearances suggesting a fusion are sometimes seen, but it is impossible to attach much importance to them on account of their very small size and the numerous other granules which often fill the cytoplasm. In many stages, indeed, I have not been able even to distinguish the basal granule of the flagellum which has been retained.

Regarding the reservoir and cytostome I am still uncertain. It appears to me that in both these structures one is absorbed during conjugation, whilst the other is retained and forms the permanent organella in the active zygote. Fusion does not appear to take place between the reservoirs, but one apparently collapses, leaving the other one functional. Two reservoirs may sometimes be seen until quite a late stage in conjugation (cf. Pl. 4, fig. 26).

The food masses of both the conjugants get mixed up in the zygote. They are all absorbed before encystment, so that the cysts have clear protoplasmic contents. I have not been able to follow out the fate of the basal granule in the cyst.

I have observed an abnormal fusion of three monads, but have never succeeded in tracing out the result of such a union. This condition is depicted in Pl. 5, fig. 47. It can be seen that two of the flagella have disappeared, only the basal granules remaining. There is no reason to suppose that complete conjugation ever occurs between more than two monads. But the observation is of some interest from the fact that a similar "dreifache monströse Kopulation" has been seen in

Polytoma by Prowazek (**36**). Dallinger and Drysdale (**12**) also stated that more than two monads might unite, previous to sporulation, in Bodo, although this has never been confirmed.

The points of special interest in the conjugation and consequent events in Copromonas are the nuclear reduction and the fate of the zygote. Some general remarks on the former subject will be found on p. 111. I will now pass on to the later history of the cysts.

Development from the Cysts.

Cysts formed in either of the ways described above appear to have exactly the same destiny. They are able to withstand drying for a considerable length of time, and do not liberate their contents until they reach a suitable medium—in this case the fæces of a frog or toad.

When the contents of the large intestine of a frog or toad is examined, as a rule no monads of this species can be discovered. After a long and careful search a few cysts are usually—though not always—found to be present. Cysts are practically always present, probably, although they cannot always be found. For if some of the fæcal matter be placed in a carefully cleaned watch-glass and diluted (see p. 77) a favourable medium for the development of the cysts is formed. In the course of a few days—probably in about three days—the first monads will be found. When first liberated they differ from the adults in their small size, globular shape, and simple structure. They possess no reservoir or cytopharynx, and no food bodies. The protoplasm is peculiarly pale and transparent, not at all like the granular protoplasm of the adult. These monads develop gradually into the adult form, and then begin to divide in the usual way.

I have not been able to watch the liberation of the monad from the cyst, in spite of repeated efforts to do so. I think it highly probable that the animal is set free by the cyst dissolving, not by its bursting; for I have never seen any signs

of ruptured cysts at this time which would suggest that a monad had emerged. All the cysts do not dissolve at the same time. I have sometimes, after carefully watching cysts on a slide which contained no free monads, found monads just liberated on another part of the slide at the end of the observation. After the cyst has dissolved the flagellum probably grows up from the basal granule, but this again I have not been able to watch.

The cysts must reach the frog's rectum by entering at the mouth and traversing the alimentary canal. They are probably ingested by the frog with its food, or in water, and must be very widely disseminated in nature, as they appear to be present in practically all frogs and toads.

A similar kind of life is led by the shelled rhizopod Chlamydophrys stercorea, Cienk., whose life-history has been elucidated by Schaudinn (**44**). It lives in the fæces of various animals, undergoing a remarkable development there. It is to be found sometimes in the fæces of frogs and toads, living side by side with Copromonas. Its presence here has not been previously recorded, I believe.

Durable cysts of considerable thickness are formed by this organism, and it was proved by Schaudinn that it was necessary for these cysts to traverse the alimentary canal before they could develop in the excreta. Simply placing the cysts in the fæces does not suffice to open them. In Copromonas, however, this is not the case. As long as the cysts are allowed to remain in the fæces for a day or two they can undergo development. It is not necessary to pass through the frog. This can be shown in the following manner: A small drop of an old culture which contains many cysts is allowed to dry on a coverslip. This kills any free monads which may be present, but does not injure the cysts. The contents of the large intestine of a frog are taken and diluted with water or salt-solution. This is then boiled for some minutes, in order to kill any organisms which may be present, filtered, and boiled again. The resulting liquid is quite free from monads, but forms an excellent culture medium for

them. A drop of this is placed on the dried-up cysts on the coverslip, and a hanging-drop preparation made on a hollow-ground slide. The coverslip is carefully waxed round the edges, and examined from time to time. In three or four days monads are usually to be found swimming about and dividing. In preparations made in this way a large number of cysts never develop, and many perish by bacterial invasion. The first monads to appear are similar to those which first appear in the ordinary course in the fæces.

Occasionally the cysts open in the rectum of the frog, instead of waiting until they leave it in the fæces. In this case the monads become parasitic for a period. Division may take place inside the frog under such conditions, but I have never seen any signs of conjugation in this situation. This is due, perhaps, to the inhibitory effect of the numerous other Protozoa which live in the frog's intestine. Most of these die in the course of a day or two after removal from the frog.

Degeneration.

Even in my most healthy cultures a number of monads always underwent degenerative changes and died. Degeneration was also brought about by keeping the monads for many days in hanging-drop preparations. A want of oxygen has probably much to do with this, for much less degeneration occurs in the Schultze chambers—where oxygen is supplied by the presence of green algal filaments—during an equal length of time.

Degenerating monads sometimes lose their regular contours, and become irregular and lumpy looking. Owing to the stoutness of the cuticular covering they do not become amœboid, as is so often the case in degenerating flagellates. Not uncommonly individuals grow abnormally large, being apparently unable to divide when they reach a certain size. These large and irregular forms present a very different appearance from that of the ordinary individuals.

One of the first signs of degeneration is the vacuolation of

the cytoplasm. Large clear vacuoles appear at any point in the creature's body, and the reservoir often reaches enormous dimensions—sometimes completely filling the anterior half of the animal.

The nucleus increases in size at first, but later breaks up into small fragments of various sizes. The cytopharynx appears to dissolve. As the animal dies it comes to rest, and the flagellum ceases to move. If it be watched at this stage it is seen to gradually fade away—apparently dissolving.

The dead monad is permeable to bacteria at only one spot—the cytostome. Through this bacterial invasion comes. First, the minute bacilli enter, but later, as the breach is widened, hordes of large bacilli and spirilla force an entry. At this stage the corpse is a mere bag containing a seething mass of microbes. As the nutritive remains of the monad get exhausted the bacteria gradually forsake their prey, until finally nothing but the skeleton of the monad—consisting of the cuticle—is left. The cuticle is thick and very resistant. It persists for a very long time in the form of an open sack, the opening marking the site of the former cell-mouth.

It is worthy of note that one can often observe degenerating monads side by side in the same drop of fluid with perfectly healthy individuals in active division or conjugation. The bacteria are usually most dense during the second day and thereabouts, but degenerating monads are not usually seen until several days later, when the number of bacteria has very greatly decreased.

General Discussion.

(1) Nucleus, Flagellum, and Basal Granule.

I propose to say in the following pages a few words about some of the more interesting points which a study of the morphology and life-cycle of Copromonas raises. In the first place, I must say something about the morphology of

the nucleus, flagellum, and basal granule—three structures which are probably connected phylogenetically.

Prowazek (**36**) distinguishes four different types of nucleus among the Flagellata :

(1) Simple nuclei, with an evenly-distributed chromatic network, and no internal structures (karyosome, division centre, etc.), e.g. Herpetomonas.

(2) Vesicular nuclei, with direct division; with central chromatin mass surrounded by a clear zone, across which a more or less distinct network extends outwards to the nuclear membrane. Such a nucleus may be seen in some species of Bodo, and is well seen in Copromonas.

(3) Centro-nuclei[1] containing a "neucleolo-centrosome" (Keuten) and separate chromatin masses. This type of nucleus is characteristic of Euglena and its allies.

(4) Vesicular nuclei with karyokinetic division: e.g. Polytoma, Chlamydomonas, etc.

To these four categories we may make the addition of a fifth, for the reception of the kind of nucleus found in Tetramitus (Calkins [**7**]).

(5) Nuclei in which the achromatic division-centre lies freely in the cell, whilst the chromatin is diffuse in the form of chromidia.

The nuclei will be seen to be of very different structure throughout the group. This difference is no less marked in the method of division. All stages, from amitotic constriction into two to karyokinesis with chromosomes, achromatic spindle and division-centres are to be met.

Fisch (**19**) described mitosis in Codosiga in 1885. In 1894 Blochmann recorded its occurrence in Polytoma and Monas vivipara (**2**) and in Euglena (**3**). His pupil Keuten carefully worked out the nuclear division in this last form in 1895 (**25**), describing the nucleolo-centrosome and a longitudinal splitting of the chromosomes. Schaudinn next year

[1] The centronucleus, as defined by Boveri, is a nucleus which contains a cytocentre—either in a consolidated or diffuse form. In the case of Euglena, etc., the cytocentre is the neucleolo-centrosome, i.e. is of the consolidated type.

(1896) brought forward some interesting observations on Oxyrrhis (42). He discovered that the intra-nuclear division-centre—the nucleolo-centrosome—could be made to leave the nucleus and enter the cytoplasm by placing the animal in diluted sea-water. In the cytoplasm the division-centre might grow in size and even divide. These observations are of great interest when considered in relation to the nuclear origin of the centrosome in Actinosphærium and Acanthocystis, as demonstrated by Hertwig and Schaudinn.

Mitosis was demonstrated in various Chlamydomonadina by Dangeard in 1898 (15). In the same year Calkins' account (7) of the remarkable nuclear phenomena in Tetramitus appeared. In this animal the chromatic and achromatic parts of the nucleus are completely separate, except during division. The former is in the form of chromidia, the latter in the form of a large consolidated division-centre. Prowazek (35) and Dangeard (16) again worked out mitotic division in Polytoma in 1901. The former described the presence of about eight chromosomes, the latter found not more than six. Prowazek also described the formation from the nucleus of a minute body—the "entosome"—which appears to act as a division-centre. Various euglenoids were investigated by Dangeard (17) in the following year, and one (Entosiphon) was described by Prowazek (37). It appears to have a method of division similar to Euglena, but more primitive. Dangeard (18) also described mitosis in Monas vulgaris. In 1904 Steuer gave us a description of mitosis in the euglenoid Eutreptia (48), and Awerinzew has just described anew (1) the nucleus and its division in Chilomonas.

Amitosis is found in some forms. It was recorded in Monas guttula by Prowazek (1903 [36]), and by the same writer in Bodo lacertæ in 1904 (39). From his description of the division—apparently amitotic—of the nucleus of Trichomastix it would seem that the axial rod plays the part of a directive centre.

The various kinds of division, whose history I have just

imperfectly sketched, can all be brought into a more or less perfect series with one another. Beginning with Copromonas, we have a nucleus in which chromatic and achromatic elements are united in the resting nucleus, and do not separate at division. A step further we find the nucleus of Entosiphon, which, although apparently identical with that of Copromonas when in the resting state, shows some separation of achromatic spindle and chromosomes during division. In the Chlamydomonadina also a similar condition is found, but here the chromosomes can be clearly counted. A stage further we come to the nucleus of Euglena, in which achromatic division-centre and chromatic elements are distinct from one another even in the resting nucleus. In Eutreptia the chromatin seems, in the resting state, to be less closely aggregated round the division-centre, and the outline of the nucleus is irregular. Chilomonas (see Awerinzew [1]), shows a nucleus (consisting of the neucleolo-centrosome and part of the chromatin ?) surrounded by chromidia. Such a condition may be derived from that seen in Eutreptia by the loss of the nuclear membrane. Finally, we reach the nucleus of Tetramitus, in which all the chromatin has left the division-centre and lies freely in the cytoplasm in the form of chromidia.

That this is a progressive phylogenetic series I do not believe. I think it probable that the Copromonas type of nucleus is the most primitive, and the Euglena type the most highly evolved. From the Euglena type the Tetramitus type may have arisen by regressive changes. I mean that I believe the inter-relationships should be expressed in the form of a V with Euglena at the angle and Copromonas and Tetramitus at the ends rather than as a straight line. These points will have to be taken into account when the Flagellata come to be re-classified.

Let us now leave the nucleus and consider the flagellum and its connections. The flagellum has been classified under three headings by Prowazek (36), according to its relations to the nucleus. These three classes are :

(1) Flagella arising directly from the nucleus, as in Mastigamœba, etc.

(2) Flagella united to the nucleus by means of an intermediate link, the "zygoplast"—e.g. species of Monas, Chlamydomonadina, etc.

(3) Flagella arising from a basal granule, independent of the nucleus, a condition seen in Copromonas.

And to complete the list I will add:

(4) Flagella arising from a special nucleus—the kinetonucleus—as in the trypanosomes.

An intermediate condition between (2) and (4) may be seen in Hæmoproteus noctuæ, where kinetonucleus and trophonucleus remain united by an achromatic thread. Many varieties of (2) are to be seen. In Chlamydomonas, for example, the flagella are attached to a basal granule from which a strand—the rhizoplast—runs to the nucleus, on the membrane of which it ends in a knot-like enlargement.

The first arrangement occurs also in the swarm-cells of Mycetozoa (Plenge [**32**]). It was first described in Mastigamœba aspera by F. E. Schultze (**46**) in 1875, and has since been observed in allied forms by various writers (cf. Goldschmidt [**22**]). It may be compared with the attachment of the axopodial rays to the nucleus in Heliozoa—e.g. in Camptonema (Schaudinn [**41**]), also with the axial rod and nucleus of Trichomastix (Prowazek [**39**]). Plenge has suggested that the flagellum in soft-bodied organisms like Mastigamœba is attached to the nucleus because it happens to be a relatively fixed point.

The homology of the basal granule still remains obscure. It has been thought to be homologous with the basal granule (end knob) of the tail filament of a spermatozoon. And the end knob has been shown from the work of Moore, Meves, Paulmier, Hermann, Lenhossék, Benda, Korff, and many others to be the centrosome.[1] The axial filament is an outgrowth of it.

[1] It is beyond the scope of the present paper to enter in detail into the enormous literature on this aspect of the centrosome. The reader who seeks further information on the subject will find an excellent account in Maier (**30**), and also much that is of importance in Wilson (**53**).

This led to the formulation of the well-known Lenhossék-Henneguy hypothesis, which states that the centrosomes form the basal granules of flagella and cilia. The work of Gurwitsch, Maier, Henry and others on ciliated epithelium and infusoria clearly shows that this generalisation cannot be made. The flagella certainly arise from centrosomes in sperms and in the so-called "Centralgeissel" arrangement, but not as a rule in other cases. The basal granules of flagella and cilia appear to be merely cytoplasmic thickenings in most cases (Henry, Gurwitsch, etc.).

The possible homology of the basal granule with the trypanosome blepharoplast[1] must not be forgotten. For a long time the nature of this structure remained doubtful. Wasielewsky and Senn regarded it as an ectoplasmic thickening, a kinoplasmic differentiation unrelated to the nucleus. Rabinowitsch and Kempner thought it was a nucleolus. And whilst Laveran and Mesnil regarded it, from a Lenhossék-Henneguy point of view, as a centrosome, Bradford and Plimmer compared it with the infusorian micronucleus. Its real nature was revealed by Schaudinn's study of Hæmoproteus noctuæ (**43**). He showed that it was a separate nucleus specially concerned with the locomotory functions of the cell, a kinetonucleus, taking origin from the original compound nucleus. The origin of the blepharoplast from the synkaryon has since been observed by Bradford and Plimmer in Trypanosoma brucii.

From the kinetonucleus the flagellum takes origin. It is, however, a true nucleus, undergoing reduction and fusion at conjugation. A similar reduction, etc., occurs in Herpetomonas, according to Prowazek (**38**).

My observations on the basal granule of Copromonas and the part it plays in division (see p. 90) suggest that it may indeed be homologous with the kinetonucleus. An interesting comparison may be made with the flagellar apparatus in some Rhizomastigina. In Mastigamœba Prowazek (**36**) states that the flagellum is retracted into the nucleus before

[1] For the more important literature see Woodcock (**54**).

this divides. Presumably new flagella subsequently grow from the daughter-nuclei, as from the basal granules of Copromonas. A similar condition appears to exist in Mastigina, according to a recent paper by Goldschmidt (**22**). In some Bodos the basal body is as large as the nucleus, and stains very strongly with nuclear stains. It divides in division of the monad.

Unfortunately, although many interesting comparisons can be made, the problem of the homologies of these various structures must still be left unsolved. It can be settled by further research only. At present it is not possible to make any definite pronouncement on the subject.

Conjugation.

Much controversy has taken place on the subject of conjugation in the Flagellata. Only a few years ago it was denied that any conjugation existed in the group. But now, owing largely to the labours of Prowazek, it can be definitely stated that conjugation occurs here as in all the other chief subdivisions of the Protozoa.

Early observers—Cienkowski, Stein, and others—described fusions between two monads in various species, interpreting them as processes of conjugation. In the years 1873 to 1875 the remarkable observations of Dallinger and Drysdale (**12**) appeared, and gave rise to much dispute. Processes which, if they really exist, must be described as conjugation, were recorded in many monads, which have subsequently been referred to the genera Polytoma, Bodo, Monas, Cercomonas, etc. Following conjugation, some remarkable methods of sporulation, including the formation of ultramicroscopic "microspores," were claimed to have been seen. Saville Kent (**24**) accepted the work of these two observers, but Bütschli (**6**) regarded it with great scepticism, admitting that conjugation takes place in the Phytomonadina only. A similar attitude was adopted by Klebs (**28**) in 1892, and by Blochmann (**4**) three years later. In the latest systematic

account of the Flagellata which has appeared (**47**), Senn (1900) regards conjugation as still unproven in the group.

Apart from the Phytomonadina all accurate knowledge of the matter has been acquired during the last half-dozen years. The most important researches are those of Prowazek and Schaudinn, the latter throwing much light upon the trypanosomes and their allies. In the Phytomonadina conjugation has been demonstrated in many genera: e. g. in Polytoma (by Krassilstschik [**29**], Dangeard [**16**], Prowazek [**36**], and others); in Chlamydomonas, etc., by Dangeard (**14**, **15**); in Dunaliella (Teodoresco [**50**]), Volvox, Pandorina, and others.

Goldschmidt (**22**) has just worked out a complete life-history in the Rhizomastigina—in Mastigella. It would appear from his account that the mastigamœbæ are closely allied to the Rhizopoda, for he describes karyokinesis, formation of gamete-nuclei from chromidia, differentiated micro- and macro-gametes, etc., which strongly recall the conditions seen in rhizopods.[1]

Apart from these two groups—the Phytomonadina and Rhizomastigina—conjugation has been shown to occur in Monas vivipara (**36**) and Bodo lacertæ (**39**) among the Protomonadina, and in the following members of the Polymastigina: Trichomonas intestinalis (**44**, **39**), Hexamitus intestinalis (**39**), Lamblia intestinalis (**44**). It has also been proved to take place in Trypanosoma lewisi, Hæmoproteus (Halteridium, Trypanosoma) noctuæ (**43**), and in Herpetomonas muscæ-domesticæ (**38**). In all these forms heterogamy is found. But autogamy takes place in some flagellates, e. g. in Trichomastix lacertæ (**39**), Herpetomonas muscæ-domesticæ (**38**), Bodo lacertæ (**39**), etc. Sexual differences between the

[1] Perhaps Pseudospora should be considered in the same category. It certainly shows affinities with the Rhizopoda, but from Miss Robertson's recent paper, 'Quart. Journ. Micros. Sci.,' vol. 49, 1906, p. 213, it is not clear to me how far gamete-formation in this animal corresponds with that of the Mastigamœbæ.

conjugants exist in a few forms—in trypanosomes and their allies, and in Bodo lacertæ.

Conjugation has not been proved to occur in the Chromomonadina, and no proof of its occurrence in the Euglenoidina has been given before. Indeed, Copromonas is the only uniflagellate monad—with the doubtful exception of the "uniflagellate monad" of Dallinger and Drysdale, the "Monas dallingeri" of Kent—which has had its life-history worked out. And even in this I cannot claim that it has been done with completeness. There can be little doubt that there is still much more to be discovered of the ways of life of Euglena and its allies. The observations of Entz, Zacharias, or others have certainly not proved that conjugation takes place in this order.

Nuclear Reduction.

A reduction of the nuclear chromatin is a phenomenon which is usually met with in connection with conjugation. Leaving the Phytomonadina out of the question for the moment, it may be stated that nuclear reduction occurs in all flagellates as a preliminary to karyogamy. It has been observed in every case where accurate investigation has been made.

The most common method by which this is affected is by two divisions of the gamete nucleus, resulting in the formation of two reduction nuclei ("polar bodies," "Richtungskörper") and a reduced gamete nucleus. The reduction nuclei degenerate, and the reduced nuclei approach one another and fuse. Such phenomena occur in Trichomonas intestinalis (**39**, **44**), in Bodo lacertæ (**39**), in Hexamitus intestinalis (**39**) and in the autogamy of Trichomastix lacertæ (**39**). Reduction divisions of both trophonucleus and kinetonucleus are found in trypanosomes and allied forms. And, as I have already shown, a nuclear reduction consisting probably in two reduction divisions (see p. 97) takes place in Copromonas. In Monas vivipara reduction appears to be brought about by the expulsion of chromidia from the nucleus (**36**).

In spite of all the attention which has been bestowed upon the Phytomonadina, no one has yet succeeded in demonstrating that a nuclear reduction takes place in the gametes before conjugation. Even in Polytoma, which has received the attention of so many investigators, no division of the gamete nuclei before fusion has been seen (cf. **16**, **21**, **29**, **36**). Prowazek [**36**] suggests that the formation of dwarfs ("Zwergindividuen") may have some significance in this respect. In Chlamydomonas also, Dangeard [**14**] finds that no nuclear reduction takes place before conjugation, the conjugants having the same number of chromosomes as the ordinary individuals. He suggests that reduction occurs during germination.

Now in the lower plants reduction does not seem always to occur just before conjugation. For instance, it occurs at the beginning, not at the end, of the life-cycle in the Desmidiaceæ, Spirogyra, etc., with the first division of the fertilised oosphere. That a similar condition obtains in Polytoma is possibly indicated by the fact (observed first by Krassilstschik, and since confirmed by Prowazek) that the first division after leaving the cyst is into eight, whilst subsequent divisions are into four daughter-cells.

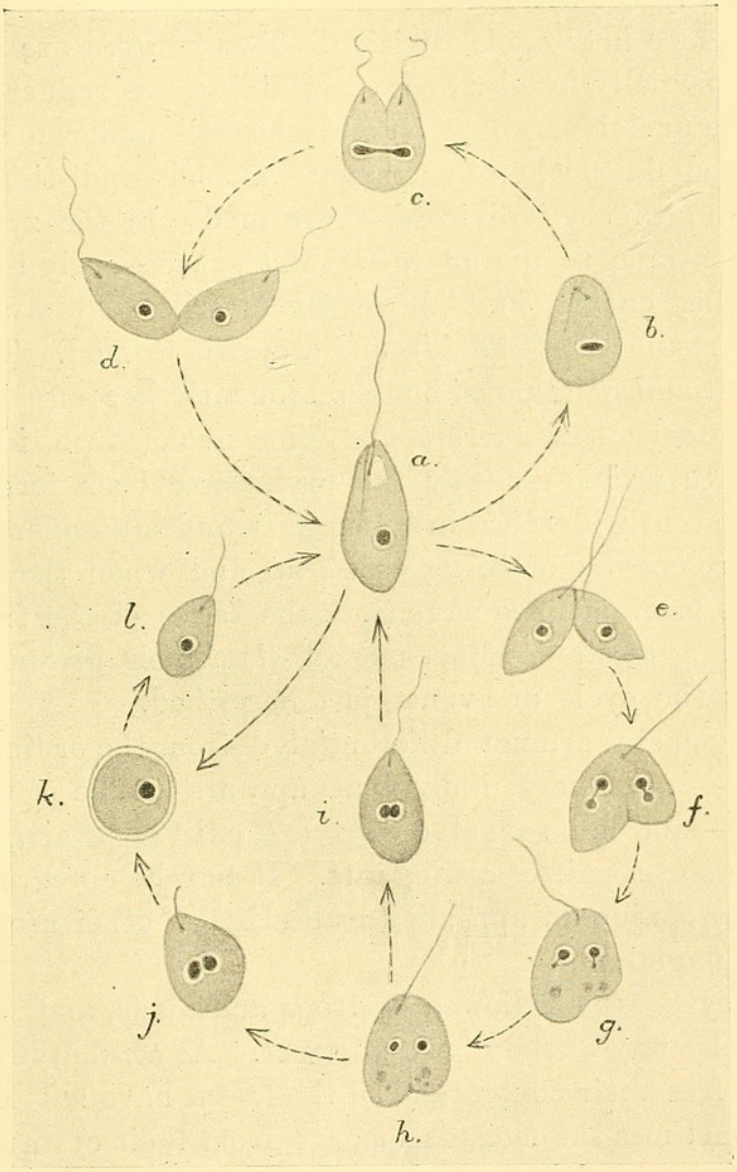
I believe the foregoing facts furnish further evidence of the plant affinities of the Phytomonadina, and consequently for their sharp demarcation from the rest of the Flagellata.

Summary of Life-cycle of Copromonas subtilis.

In conclusion I will give a brief synopsis of the life-cycle of Copromonas. This can be most easily done by means of a diagram (see text-fig. C). Starting with the adult monad, *a*, it will be seen that the life-history comprises two different cycles—an asexual (upper cycle, figs. *b–d*), and a sexual (lower cycle, figs. *e–l*). This latter is intimately connected with the formation of resting cysts. During the asexual cycle multiplication takes place by means of longitudinal division. The organism comes to rest, and the flagellum is drawn in.

The nucleus divides, as does the basal granule of the flagellum. Two new flagella are formed, and two new cytostomes. One of the daughter individuals takes the contractile vacuole, the

TEXT-FIG. C.



other develops a new one. The reservoir is halved, each daughter-cell taking half. The nucleus divides amitotically (figs. *b*, *c*, *d*).

After a time the monads conjugate in pairs (*e*). The

anterior ends come in contact and fuse. One flagellum is lost. By means of the remaining one the conjugants swim about during conjugation. Each nucleus divides once and one of the daughter-nuclei at once degenerates (*f*). A second nuclear reduction occurs in which a chromatin granule (or granules ?) is extruded (*g*). This process might also be described as a heteropole division. Two courses are now open to the partially fused monads containing two reduced nuclei: (1) The nuclei may fuse (*i*) and the cell undergo a process of remodelling, whereby a zygote monad—indistinguishable from an ordinary individual—is formed (*a*) ; the zygote may then continue to divide (*a–d*), and subsequently its descendants may encyst (*k*). (2) Or the flagellum is drawn in, the zygote rounds itself off (*j*), and a cyst is formed. This is at first soft and gelatinous, but subsequently becomes hardened and more or less encrusted. The nuclei fuse inside the cyst. In whichever way the cysts have been formed they are apparently identical in appearance and subsequent history. They are capable of being dried up, and when they reach a suitable medium once more (i. e. the fæces of the frog) they liberate a small hyaline monad (*l*) which grows up and repeats the cycle of events just described.

The gametes are not differentiated from the ordinary individuals—i. e. every individual appears to be a potential gamete—and no sexual differences exist between the two members of a pair of conjugants. It may be noted, however, that one conjugant appears to absorb the other after it has lost its flagellum.

The cysts are swallowed by frogs and toads, and reach the rectum by way of the digestive tract. As a rule the cysts do not set free their contents until the fæces have left the frog. But sometimes the monads emerge from their cysts and lead a semi-parasitic life in the large intestine. Development does not appear ever to be completed inside the frog.

Note on Copromonas sp., from the Fæces of the Newt.

In searching through the intestinal contents of the common newt (Molge vulgaris) I came across a few small monads very clearly resembling C. subtilis. I was therefore led to make some culture experiments with the fæces, in the manner adopted in the case of frogs and toads. As a result I found that a very similar organism exists in this situation, only differing from C. subtilis in its smaller size. It is possible, indeed, that it is the same species, which does not attain its full size in the fæces of the newt.

The size of this monad is approximately 7 μ–10 μ in length by 3 μ–4 μ in breadth. It reproduces in the fæces by longitudinal division in exactly the same manner as C. subtilis. Conjugation and encystment have never been observed.

In view of the fact that so little of the life-history has been investigated, I think it best not to bestow a specific name upon this animal. Nevertheless, I believe it will be found to be specifically distinct.

A figure of the organism is given on Pl. 5 (fig. 48).

Zoological Laboratory, Cambridge,
August, 1907.

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DESCRIPTION OF PLATES 4 & 5.

Illustrating Mr. C. Clifford Dobell's paper on "The Structure and Life-History of Copromonas subtilis, nov. gen. et nov. spec.: a Contribution to our Knowledge of the Flagellata."

PLATE 4.

Figs. 1–5 and 11–20 are drawn from living specimens, under a 2·5 mm. apochromatic water-immersion, with compensating ocular 12. The remainder are from permanent preparations—fixed hot sublimate-alcohol, and stained

Heidenhain's iron hæmatoxylin; drawn under a 3 mm. apochromatic oil-immersion, compensating ocular 18.

Fig. 1.—Copromonas subtilis—adult individual before division.

Fig. 2.—Same individual, drawing in flagellum preparatory to division.

Fig. 3.—The same; flagellum completely drawn in, and nucleus dividing.

Fig. 4.—Still later; two new flagella growing up side by side, nucleus in later stage of division, etc.

Fig. 5.—Later; nucleus and reservoir divided; growth of new flagella, etc.

Fig. 6.—Stained preparation, showing chief anatomical features.

Figs. 7–10.—Various consecutive stages in division.

Figs. 11–16.—Conjugation.

Fig. 11.—Attachment of two monads by their anterior ends.

Fig. 12.—The same, twenty-five minutes later. One flagellum has been drawn in.

Fig. 13.—The same, thirty minutes later.

Fig. 14.—Fifteen minutes later.

Fig. 15.—Twenty minutes later; monads almost completely fused.

Fig. 16.—Fifteen minutes later. Complete fusion has taken place, and the resulting zygote has become re-modelled to the form of an ordinary individual.

Figs. 17–19.—Various forms of cyst met with in cultures.

Fig. 20.—Monad shortly after liberation from cyst.

Figs. 21–33.—Conjugation and encystment.

Fig. 21.—Fusion of two individuals; one flagellum is being drawn in.

Fig. 22.—Further fusion has taken place; only one flagellum present.

Fig. 23.—First nuclear reduction. On the left, nucleus in an early stage of division (cf., Plate 5, fig. 35); on the right, nuclei almost divided.

Fig. 24.—The reduction nuclei have been formed, and are degenerating. They are pale and situated below the gamete nuclei. (This specimen is slightly flattened.)

Fig. 25.—Further nuclear reduction by heteropole division (granule extrusion). Parts of the first reduction nuclei are seen degenerating below.

Fig. 26.—Later stage, containing two reduced gamete nuclei.

Fig. 27.—Nuclei fusing; zygote assuming motile monad form.

Figs. 28–30.—Fusion of nuclei and encystment.

Fig. 31.—Descendant of zygote monad before encystment.

Fig. 32.—A similar organism at a later stage.

Fig. 33.—Resting cyst; formed from either 30 or 32.

PLATE 5.

All drawings are made from permanent preparations, stained Heidenhain's iron hæmatoxylin. Fixation: Fig. 41, osmic vapour; remainder, sublimate alcohol. Figs. 34–40 drawn under a 2 mm. apochromatic oil-immersion (apert. 1·40), compensating ocular 12. The others under the 3 mm. apochromatic, compensating ocular 18.

Figs. 34–40.—Nuclear division.

Fig. 34.—Nucleus before division.

Fig. 35.—First stage in division; somewhat fusiform; chromatin granules extruded at opposite poles.

Fig. 36.—Nucleus now in the form of a short rod.

Fig. 37.—Nucleus dumb-bell shaped.

Fig. 38.—Chromatin aggregating at the ends of the dumb-bell, so as to give rise to two daughter-nuclei connected by a broad band.

Fig. 39.—Daughter-nuclei still connected by a filament.

Fig. 40.—Two fully-formed daughter-nuclei.

Figs. 41–46.—Flagellum and its method of multiplication. The drawings are of the anterior end of the organism.

Fig. 41.—Ordinary individual before division, showing basal granule, etc.

Fig. 42.—Flagellum being drawn in.

Fig. 43.—Flagellum completely drawn in, only basal granule left.

Fig. 44.—Division of basal granule. The daughter granules lie one on either side of the reservoir, and are still connected.

Fig. 45.—Growth of new flagella from the basal granules. The latter are still seen to be connected by a fine filament.

Fig. 46.—Later stage. The flagella are longer, and are seen growing out of the depressions caused by the formation of the new cell-mouths. The reservoir is cleft in two.

Fig. 47.—Abnormal union of three monads.

Fig. 48.—Copromonas sp. from the fæces of the newt.