The Golgi Apparatus of Copromonas subtilis, and Euglena sp.

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With Plates 46-48 and 9 Text-figures.

INTRODUCTION.

The problems surrounding the morphology of the cytoplasmic inclusions of the germ-cells and gland-cells have largely been solved in recent years. The discovery of a Golgi apparatus in Sporozoa (Hirschler, 1914) and the papers of Nassonov (1924, 1925) on the possible connexion between Golgi material and contractile vacuole in various Protozoa have served, so far as our present work is concerned, to shift the search mainly to that part of the field associated with unicellular organisms. Recently Miss M. Daniels (1938) has successfully investigated the cytoplasmic inclusions of three species of Gregarina by means of the ultra-centrifuge. The present paper has arisen out of the work of Mrs. Lamont and one of the present authors (B. N. S.) on Nebela collaris and Amoeba proteus, respectively; in neither organism has a true Golgi apparatus been found, either by the use of the centrifuge, or by any recognized staining method. Our attention has therefore turned to the flagellates, on which Nassonov, Grassé, and Duboscq and others have already done interesting work in this particular field. We began by examining Copromonas, because we believed that it was one of the simplest monads known. Unfortunately we found that it was more complicated than the previous monograph of Dobell (1908) had led us to believe, and, because of some special problems which arose, we had to turn our attention to a larger organism which was better known. Euglena had already been studied in this laboratory by Miss Patten and Beams (1936), who had given a very satisfactory account of the
staining properties and relative specific gravities of the various granules. We had the opportunity of going over most of the material which had been used for their work. According to Wenyon (1926), Euglena and Copromonas are allied genera, and we certainly obtained considerable light on the conditions in Copromonas by studying Euglena as well.

PREVIOUS WORK ON COPROMONAS.¹

The genus Copromonas was established by Dobell (1908). Later on several species of this genus were described by various workers.

According to Dobell's description, Copromonas subtilis is a simple monad provided with a single flagellum arising from a depression at the anterior end. This depression is the cytostome, which leads into a longitudinal tube, the cytopharynx. The flagellum runs along the wall of the cytopharynx for a short distance and originates from a basal granule. The base of the flagellum is usually associated with the reservoir, and at times the basal granule seems to be situated on the posterior part of the reservoir. There are one or two small contractile vacuoles which discharge their fluid into the reservoir. The latter does not pulsate, but sometimes it is absent, and thus it may be that it periodically collapses, driving out its contents.

In the nucleus is a central deeply staining mass surrounded by a clear zone which contains practically no chromatin. The nuclear membrane, which is achromatic in nature, is united to the central portion by achromatic strands. Dobell says, 'The nucleus lies somewhat posteriorly, and is not connected in any way with the flagellum, as is so often the case in flagellates. But I may 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

¹ According to Wenyon (1926), Dobell's genus Copromonas is probably Stein's genus, Scytonomas. In 1878, Stein recognized a family, Scytonomonadina, with a number of genera, Scytonomas, Petalomonas, Menoidium, Sphenomonas, etc. In his text, Dobell does not refer to Stein's genus. See, 'Der Organismus der Infusionsthiere', III Abt., page x, by F. R. von Stein, 1878.
number of monads which show this I am satisfied that it is really due to the cytopharynx, the animal 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.'

In asexual multiplication by longitudinal division, the animal becomes gradually motionless and the flagellum is completely drawn in. During this process the nucleus becomes elongated. Later on the basal granule divides into two, and from each of these a new flagellum is developed. Meanwhile a cleft appears between the bases of the flagella; this cleft, while extending backwards, cuts the reservoir into two. Further extension of the cleft divides the animal into two daughter individuals. One contractile vacuole persists in one of the daughter individuals, while a new one arises in the second.

Dobell has described the process of conjugation and encystment in *Copromonas subtilis*, giving a detailed account of what happens to the nucleus during these phases of the lifecycle. He was not able to shed much light on other structures (basal granules, reservoir, contractile vacuoles, &c.). On conjugation, one reservoir apparently collapses and the other one remains functioning. Sometimes both the reservoirs remain functional up to a quite late stage. Cysts, when they are first liberated from the large intestine of a frog or toad, have no reservoir, cytopharynx, or food-bodies.

Dobell believes that the *Copromonas* type of nucleus is the most primitive type, and the *Euglena* type of nucleus the most highly evolved. In the latter type a 'nucleolo-centrosome' (Keuten) is present which is absent in the former type, according to Dobell.

The description given by Wenyon, of the same species of *Copromonas* cultured from the pig's faeces, differs in some respects from that of Dobell. Wenyon has described an intra-nuclear centrosome or central granule, which has a definite function in connexion with the division of the nucleus. According to him, the flagellum runs a longer way inside the animal than has been indicated by Dobell, before it ends in a blepharoplast. During the division of the animal by longitudinal fission
the two daughter centrosomes are connected by a fibre-like structure.

**Material and Methods.**

The material for the present investigation was obtained by making cultures of *C*opromonas *s*ubtilis from the faeces of frogs, by the method described by Grassi and Scheviakoff (1888). We used a few c.c. more egg albumen than was used by Dobell (1908). The culture solution consisted of 40 c.c. of egg albumen, 1 gram NaCl, and 200 c.c. of distilled water. This gave better results in making smear preparations. It is not possible to say definitely in how many days a really good culture can be obtained. Sometimes it took more than two weeks before a good yield of *C*opromonas could be got for our work, and in such cultures dividing, conjugating, and encysted forms could be seen.

The methods used were those that have been described in 'Microtomist's Vade Mecum' (1907 ed.) and 'Biological Laboratory Technique' (Gatenby, 1987). Both silver and osmic methods were tried, but it was found that Weigl osmic technique was most satisfactory in demonstrating osmiophilic material during different phases in the life-cycle of *C*opromonas. Silver preparations were not very satisfactory for showing Golgi bodies, as is generally the case in other Protozoa, but they showed mitochondria, axostyle, and rhizoplast quite clearly. Other fixatives were also used—Bouin, Schaudinn, Hermann, Champy, and Champy-Nassonov, &c.

Tests for fat (Sudan IV method), volutin, and glycogen were also used. Neutral red was used in various dilutions—1/10,000 to 1/30,000 in normal salt solution. The stains mostly used were iron alum haematoxylin, acid fuchsin and methyl green, thionin, gentian violet, neutral red acetic, and Mann's methyl blue cosin. For the demonstration of the Golgi apparatus animals were fixed and osmicated in tubes. The hanging drop or coverslip preparations were not very satisfactory.

**Euglena sp.**

The arrangement of the cytoplasmic bodies in *Euglena*, according to the views which have been taught for generations, is shown in Text-fig. 2, after Borradaile (1938). There is a gullet,
immediately below which lies a delicate vesicle, the reservoir
(r.), abutting against the wall of which is a second and smaller
vesicle, the contractile vacuole (c.v.), which discharges periodi-
cally into the reservoir, which itself discharges at longer periods
into the gullet. Around the contractile vacuole are accessory
contractile vacuoles (A.V.) which surround the main vacuole and
re-form it. These parts can all be seen in the living organism.

In 1931, André Sigot of Strasbourg demonstrated what he
called ‘plaquettes osmiophiles autour du reservoir’, as shown
in Text-fig. 1. Sigot, who is a follower of the late Dr. Parat’s
nomenclature, describes a ‘vacuome’ in the form of small
neutral red stainable bodies, which, according to Miss R. Patten
and H. W. Beams, are probably the volutin granules of other
protozoologists. These granules are marked v in Text-fig. 1.
Miss Patten and Beams recognize plastids, paramylum, and
mitochondria as well. The manner in which all these bodies
become layered on ultra-centrifuging is shown in Text-fig. 3,
mitochondria (M.) being the heaviest, volutin and paramylum
the lightest, the chloroplasts (c.p.) coming in between.

Miss Patten’s material, which had been deposited in this
Department, has been re-examined by the senior author. In
figs. 1 and 2, Pl. 46, are two examples of Euglena prepared
by the Weigl (Mann-Kopsch) method and bleached. Similar
organisms are shown in Text-fig. 1 of Miss Patten and Beams’s
paper (1936). We find, however, contrary to Miss Patten and
Beams, that in many of the organisms there is an additional
vesicle in front of what Sigot, and Miss Patten and Beams, have
called the reservoir. More recently we have investigated further
material of Euglena, and find that in the viridis type the
osmiophil material lies at the lower end of the reservoir, where
it forms a separate vacuole, whereas in the gracilis type the
osmiophil material is intimately related to the whole wall of
the reservoir. Sigot is therefore quite correct in his statement; in
Miss Patten’s slides both viridis and gracilis types of vacuole
systems can be found. This matter is further discussed in a
forthcoming paper in “La Cellule.”

In Miss Patten’s material all sorts of conditions of the osmiophil
accessory contractile vacuoles may be found, varying
from the appearance shown in Borradaile's figure (Text-fig. 2), where they are uniform in size and applied to the wall of the large contractile vacuole (shown partly in fig. 2, Pl. 46), to a condition where this uniformity is interrupted by the swelling up of a few, or sometimes almost all of the accessory contractile vacuoles. These swollen bodies are well shown in the three figures in Pl. 46. Occasionally, as in fig. 1, Pl. 46, they almost completely embrace the contractile vesicle, but in the majority of cases the ventral side of the vesicle has attached to it a large
bladder-like structure (c.x.) in direct contact with the contractile vacuole. We believe that the body marked c.x., in figs. 2 and 3, Pl. 46, is swollen with water and is ready to discharge into the contractile vacuole. It is, as has been assumed by previous workers who have carefully observed the living organisms, merely an accessory contractile vacuole. Probably in the enlarged condition it is more difficult to observe in the living state. We do not know for certain whether this is so.

It will be seen, therefore, that our conception of the contractile vacuole and reservoir apparatus of *Euglena* agrees in all
essentials with the conventional account found in the textbooks. There is one point more we wish to emphasize—in the ultra-centrifuged Euglena the reservoir cannot be found immediately after centrifuging, and the contractile vacuole and its parts may be made to drift away from the anterior end of the organism, as shown in fig. 3, Pl. 46.

We now come to the question of the homology of the parts which are osmiophil. According to our view, the osmiophil substance represents the Golgi material of higher forms, and with the contractile vacuole constitutes a compound structure.

Copromonas.

In the photograph on fig. 16, Pl. 48, (a) is a typical example of the osmiophil structure in Copromonas. Here is a very large, extremely osmiophil body, which, excepting the basal body of the flagellum, in nearly 90 per cent. of specimens is the only structure which becomes jet black after a few days’ treatment in osmium tetroxide. Knowing Dobell’s work on Copromonas, and bearing in mind the position and size of the reservoir of Copromonas, one naturally assumes at once that the osmiophil body is the blackened wall of the reservoir. This, however, is not the whole story. It is, indeed, true that in many cases the blackened vesicle is the only vacuole in this region of the organism, but in other cases a separate reservoir without osmiophil walls and abutting against the osmiophil vesicle may be seen, as, for example, in figs. 5 and 9, Pl. 46, and figs. 13–15, Pl. 47.

Now in the first place it must be mentioned that the amount and arrangement of the osmiophil substance may be very variable. Compare, for example, figs. 11 and 13, Pl. 47, with figs. 5 and 8, Pl. 46. In some specimens, as in fig. 7, Pl. 46, and fig. 10, Pl. 47, the osmiophil material may be scanty, while in all preparations monads can be found in which no blackening may have taken place. We have found examples in division in which one side contained osmiophil material, the other none whatever. We are certain, however, from our knowledge of the technique, that in the majority of cases, if not in all cases, where no blackening has taken place, the fault has been due to the fact that the
reduction of the osmium tetroxide has been prevented by the particular surroundings of the organism on the smear. For, where monads are osmicated in a tube, and not on a smear, the percentage of examples containing blackened vacuoles or granules approaches 100 per cent., whereas in thick smears many more individuals have failed to blacken. This matter is referred to below, p. 586. At this juncture we may answer a question which will possibly have presented itself to the mind of the reader—namely, what proof is there that the blackening is not an artefact? Firstly, in individuals kept in osmic solution in a hanging drop the blackening may be seen to be appearing in the small vesicles previously known to be contractile vacuoles or around previously identified reservoirs, and, secondly and more cogently, the blackened material undergoes definite division and sorting out during the division of the organism. See the photographs in figs. 16-19, Pl. 48, and the drawings in figs. 11, 13, 14, and 15, Pl. 47. Lastly, in ultra-centrifuged individuals the mitochondria pass centrifugally, whereas the lipid or osmiophil material passes up centripetally against the cell-wall. It will therefore be unnecessary to labour the point further.

It has been mentioned that a continually occurring form of osmiophil body is the structure shown in fig. 4, Pl. 46, and in fig. 16, Pl. 48. This perfectly spherical body is a phase of the cycle which we believe to be taking place throughout the life of the organism. In fig. 9, Pl. 46, is another phase. Here there is a distinct non-osmiophil reservoir (a.), partly embraced by a group of granules (a.), which are energetically osmiophil. In fig. 6, Pl. 46, is still another phase, in which there is a space or reservoir (a.) surrounded by blebbed structures strongly recalling the condition in *Euglena* (fig. 2, Pl. 46). Now in *Chilomonas*, Nassonov recognizes the spherical phase (diastole) and a granular\(^1\) or collapsed phase (systole). We feel that the case of *Chilomonas* might bear further examination, though we recognize the possibility of such a simple condition.

Now Dobell describes from his observation of the living organisms a simple reservoir and one or two contractile vacuoles

\(^1\) Presumably, as in *Copromonas*, the condition in which the osmiophil material divides during cell division in *Chilomonas*. 
which are supposed by him to discharge periodically into the reservoir. Such a condition of affairs is shown presumably by our fig. 7, Pl. 46, where the reservoir (r.g.) has one satellite contractile vacuole (a.). Likewise in fig. 10, Pl. 47, there are two reservoirs and two contractile vacuoles. But our observations lead us to believe that this condition is not any commoner than the other types already mentioned, and again can only be regarded as one possible phase of the cycle.

From Nassonov's description of Chilomonas, we naturally began by looking for the type shown in fig. 4, Pl. 46, where there is a single osmiophil vacuole. For a time we believed that this was always the reservoir, and that where the granular condition existed as in fig. 9, Pl. 46, it represented a systolic phase. But the discovery of specimens such as figs. 5, 9, Pl. 46, 13, 14, and 15, Pl. 47, where a non-osmiophil reservoir (r.) could be quite clearly seen, showed that the matter was more complicated. Furthermore, such examples as that of fig. 5, Pl. 46, where two equal osmiophil spheres (a.) as well as a reservoir (r.) existed side by side just before the onset of division suggested that in such cases the osmiophil spheres were preparing for the division. Both of us have found many stages like those in figs. 5 and 8, Pl. 46, but they cannot be regarded as the only, or commonest, type of prophase of division.

Division.

Before continuing with a description of the resting phases and endeavouring to interpret them, it will be advantageous to examine some division stages. In the photographs on Pl. 48, it will be seen that the osmiophil material in fig. 16, Pl. 48, leaves its position, and, becoming more irregular in outline, drifts down to the upper middle line of the dividing individual, fig. 17, Pl. 48, and splits into two parts, figs. 18 and 19, Pl. 48. Comparable stages are drawn on figs. 13, 11, 14, and 15, Pl. 47, in order of division. Fig. 19, Pl. 48, shows a phenomenon which we have often noted, namely, that when the demarcation between the two attached individuals reaches the region wherein lie the food vacuoles, the osmiophil material may break up or bodily drift quite far down into the middle of the cell. This is
also noteworthy in fig. 15, Pl. 47, where in the right-hand organism a large vesicle has drifted below the nucleus.

Now a stage intermediate between that in figs. 17 and 18, Pl. 48, is shown in fig. 11, Pl. 47. This is very common, and is undoubtedly the usual method. The stage before it, in which the two nuclei are still in the dumb-bell stage, is drawn in fig. 13, Pl. 47. Here we have two reservoirs, and the osmiophil granules in much the same position as in the photographed organism in fig. 17, Pl. 48. In figs. 12, 14, and 15, Pl. 47, later stages are shown, the nucleus having divided completely. In fig. 14, Pl. 47, one reservoir is in diastole, the other partially in systole, and in fig. 15, Pl. 47, the two reservoirs, comparatively very large, are in diastole. In fig. 12, Pl. 47, the two organisms contain a good deal of osmiophil material, as in the photographs on Pl. 48.

**Relationship and Homology of Reservoir and Contractile Vacuole.**

Having shown that the osmiophil material is divided into two parts between the daughter organisms, it is now necessary to examine the questions surrounding the homology of the osmiophil and apparently non-osmiophil vacuoles. Examination of figs. 5, 7, Pl. 46, 10, 14, and 15, Pl. 47, shows that the degree of osmiophility of the vacuoles may vary considerably. In fact, it is impossible sometimes, as in figs. 7 and 10, to say whether the single vacuole present is a reservoir or a swollen contractile vacuole. In figs. 14 and 15, Pl. 47, the structures marked G are presumably contractile vacuoles, for there is a clearly marked reservoir as well. Likewise in fig. 5, Pl. 46, the bodies marked G are contractile vacuoles and not reservoirs, because a true reservoir is present. This homology has puzzled us considerably, but we believe that swollen osmiophil vacuoles (contractile vacuoles as in figs. 5 and 8, Pl. 46) can take the place of the true reservoir; and actually, in some specimens which in the living state show no reservoir, the clear space which presently appears is not necessarily re-formed in the exact site of the old reservoir, but is an enlarged osmiophil vacuole taking its place. In fig. 15, Pl. 47, for example, it is possible that in the right-hand organism the old reservoir, on collapse, will be
replaced by the growing osmiophil vacuole (g). The degree of osmiophilia can then be a function of the amount of stretching of the osmiophil wall. In Pl. 47 some of the osmiophil vacuoles which are possibly in process of forming new reservoirs are marked rg.

**Examples in Division with Apparently a Blackened Reservoir or Contractile Vacuole in Only One of the Daughter Cells.**

It has already been mentioned that in smears the osmication of the Golgi material may be, in some cases, markedly capricious. In monads osmicated in a tube the number of dividing individuals in which one daughter organism contains an osmiophil structure, the other organism none, is greatly reduced. Even so, allowing for the well-known fact that isolated cells such as leucocytes or protozoa may not osmicate as evenly as occurs in pieces of metazoan tissue treated with a favourable specimen of commercial osmium tetroxide, it seems certain that in a small percentage of cases no osmiophil material does exist in one daughter cell. This must be due either to non-division of the existing osmiophil material or to some change in the chemical nature of the osmiophil material in one of the individuals. Further than to remark that the conditions depicted in the photomicrographs on figs. 17–19, Pl. 48, are the usual ones, and that such division stages as are shown on Pls. 46 and 47 among figs. 4–15 hold for most cells examined, we cannot go at present.

**Note on Bodies Other Than the Osmiophil Material.**

Our observations on *Copromonas subtilis* revealed structures which were overlooked by Dobell (1908) and Wenyon (1926). There is a stiff rod of apparently proteid nature which runs from the basal granule to the posterior end of the animal. We have identified this as the axostyle, figs. 4, Pl. 46, and 18, Pl. 47, and various Text-figs. In addition to this there is a rhizoplast connecting the basal granule with the nucleus, fig. 8, Pl. 46. We were able to see this structure in stained silver and osmic preparations. In properly differentiated Schaudinn preparations, stained with iron alum haematoxylin, both rhizoplast
and axostyle are very clearly seen, and we have no doubt that these structures are quite distinct and separate from the cytopharynx. We do not believe the statement of Dobell (1908), as quoted in the paragraph dealing with previous work, that the cytopharynx sometimes seems to have a connexion with the nucleus, and that there is no connexion between the nucleus and the basal granule. Dobell actually figured the axostyle in his Pl. 4, figs. 1, 2, 16, &c.

In the early stages of division, by longitudinal fission, in Copromonas, the basal granule is the first structure to divide, Text-figs. 4 and 5, and the daughter granules are always connected by a fibre or thread-like structure. Text-fig. 4 is a much earlier stage in the division of this body than has been indicated by Wenyon (1926). At this stage the nucleus is slightly elongated. The rhizoplast and axostyle are not seen after the division of the basal granule into two. We believe that both these structures probably disappear or degenerate during the early stages of division. The flagellum persists, although it is withdrawn considerably during the division of the basal granule. The second flagellum is developed from the second basal granule. The two axostyles are visible in fairly late stages in the division, when the nucleus has nearly divided into two, fig. 13, Pl. 47.

There is a considerable amount of disagreement regarding the question of the axostyle during the division of an individual. According to Wenyon (1907) and Kofoid and Swezy (1915), the axostyle splits longitudinally into two. Dobell (1909) and others hold the view that the axostyles arise from the paradesmose. Kuczynski (1914), Wenrich (1921), &c., do not agree with the former views, and claim that the old axostyle disappears and new ones are formed as outgrowths from the blepharoplasts. We, as a result of our investigation in Copromonas, agree with the last group of workers, although we are not very definite on this problem. We have come to this conclusion from the fact that we could not see the axostyle, even in properly stained and differentiated preparations, during the division of the basal granule.

1 See Wenyon's (1926) 'Protozoology', vols. 1 and 2, and Hirschler (1932), 'Zeits. für Zell. und mikros. Anat.', 15 B., 4 H.
Text-Figs. 4-7.

**Copromonas.**

Figs. 4 and 5.—Stages in division showing division of basal body (centriole).

Fig. 6.—Volutin granules (methyl blue).

Fig. 7.—Sudanophil fat (Sudan IV).

A., axostyle; B.B., basal body (centriole); F., sudanophil fat; F.V., food vacuole; N.C., nucleolo-centrosome; V.L., volutin.
granule, and the movement of these granules to the anterior end of the individuals. Fig. 13, Pl. 47, is the stage when two axostyles are very clearly seen. We have seen two axostyles in later stages than those indicated in fig. 13, Pl. 47.

Our preparations do not lead us to agree with Wenyon (1926) that basal granules go as far down, during division, as figured by him. In undividing monads we have always seen the basal granule at the anterior end of the organism, as we have shown in our diagrams.

Text-fig. 7 shows the distribution of the sudanophil fatty substances in Copromonas subtilis. They are generally irregular lumps distributed throughout the cytoplasm. Volutin granules often lie in the posterior region of the organism (Text-fig. 6) and are stained in neutral red, as has been claimed by Miss Patten and Beams (1936) and others in different flagellates. Mitochondria are generally elongated in shape and are beautifully revealed by both silver and osmic methods (Text-fig. 8 x). It is interesting to note that in the preparations that were made to study fat and volutin granules the axostyle is a much more prominent structure than by the routine fixatives.

Structure of Copromonas.

In Text-fig. 8 we have given our conception of the structure of Copromonas subtilis. The flagellum passes into the organism ending in a basal granule B.B. (blepharoplast, centriole, &c.), from which pass down two other structures, a finer, the rhizoplast (R.P.) which forms a connexion with the nucleo-centrosome (N.C.) of the nucleus. The coarser filament (A) stretches the whole length of the organism and is known as the axostyle. From the gullet or cytostome (c.y.), a canal, the cytopharynx (c.v.p.), passes into the upper region of the animal, and often has an ovoid food vacuole (f.v.) attached to it. When the food vacuole becomes detached from the cytopharynx it assumes a spherical shape. The lower region of the monad is usually crammed with food vacuoles, bacteria, &c., in various stages of digestion. Storage bodies are found throughout the organism in the form of sudanophil fat (f.), shown in black. In addition, so-called volutin granules (v.l.) are found in the
lower region of the cell, and these may be made to stain vitally in neutral red.

The osmo-regulatory mechanism of the monad consists of a reservoir (n.) usually but not always with non-osmiophil walls,
and a number of contractile vacuoles (c.v₁, c.v₂) which arise from and inside granules of osmiophil material (o.m, Golgi bodies) which lie in this region, and which are carefully divided between the daughter monads during binary fission. There are many mitochondria (M.) lying principally in the lower region of the organism.

**Summary of the Life-cycle of Copromonas subtillis, partly after Dobell (Text-fig. 9).**

The adult monad (A) in the upper circle is undergoing asexual multiplication by binary fission, in (B) two reservoirs having arisen probably by swelling up one or two contractile vacuoles. The osmiophil material (g.) forms a mass above the dividing nucleus, which, as the new cell-wall is formed (C), is forced down in front of it and finally divided into two groups (g.), which scatter around the reservoirs as in (D). In stage B, the basal granule has divided into two, the rhizoplast and axostyle have disappeared, and in stage C a new flagellum has begun to grow out of the basal body (b.b.) on the right. New axostyles have grown down from the basal bodies in each individual, and in stage (D) the two daughter monads are ready to separate.

The sexual cycle is shown in the lower circle. Two individuals ready to associate come together by their anterior ends, their osmiophil material (Golgi bodies, g.) fuse, and their nuclei give off polar bodies (reduction bodies) as in (F), which degenerate; one flagellum is withdrawn, and the individuals cease to feed but continue motile. In the next stage another reduction body is given off, and the number of food vacuoles is much reduced. According to Dobell, after the conjugation of the matured nuclei the new individual may either pass back to stage (A) or may pass on to encystment as in stages (I), (J), and (H). In both (I) and (H) the nuclei are fusing, and in stage (J) the cyst-wall has formed. The osmiophil material (g.) and nucleus (n.) are shown.

**Discussion.**

Elsewhere (1988) it has been pointed out that in the choano-flagellate the posteriorly situated contractile vacuoles are quite
Life-cycle of Copromonas, showing nucleus and Golgi apparatus, &c. For explanation see text.

\(a\), axostyle; \(cv\), contractile vacuoles arising within osmiophil (Golgi) material; \(bb\), basal body of flagellum; \(g\), osmiophil material (Golgi apparatus); \(fv\), food vacuole; \(n\), nucleus.

TEXT-FIG. 9.
separate from the parabasal (Golgi apparatus) which lies below the flagellum. It is certain, too, that in a ciliate like Spirostomum the contractile vacuole wall is not osmiophil, and true Golgi bodies are scattered in the ground cytoplasm. The same appears to apply to Blepharisma, which has been studied by Miss I. Moore (1934). It seems certain that among some Protozoa contractile vacuoles are not necessarily associated with osmiophil material. In Paramoecium, Chilomonas, Nassula, Dogielella, &c., there can be no doubt that osmiophil material is an intimate part of the osmo-regulatory pump (Kitching). In Chilomonas, Nassonov (1924) was the first to show that the contractile vacuole wall blackened densely after treatment in osmium tetroxide solution. In the present paper we have shown that the osmiophil material is divided between the two daughter flagellates and forms a remarkable picture at the telophase of division (fig. 18 c, Pl. 48). Sigot (1931), working on Euglena, states that, 'au moment de la division du Flagellé, l’ensemble de l’appareil (his “plaquettes osmiophiles”) se divise, cette division se produit en même temps que celle de la cinétide et avant celle du noyau, sans que nous puissions préciser si chaque élément se clive à ce moment ou s’il se produit simplement un partage des corps osmiophiles existants entre deux cellules filles. Nous avons vainement essayé de colorer ces éléments au rouge neutre et au vert Janus.'

We agree with Sigot that what we call the Golgi apparatus of flagellates does not colour in neutral red, is divided between the daughter cells in the same manner as the Golgi apparatus of higher forms, and is as characteristically osmiophil as the Golgi bodies of Metazoa. There is however no reason for supposing that the neutral red staining volutin granules have any connexion or homology with the metazoon Golgi apparatus.

We have not had the opportunity of examining the division stages of the Golgi apparatus of Euglena, but in Copromonas the ultimate separation of the osmiophil material takes place usually after the daughter nuclei have completely separated. We believe that the basal granule of the flagellum
(centriole) takes no part in dictyokinesis (division of the Golgi apparatus).

There is a tendency, less noticeable in recent years, for those who have not utilized current Golgi apparatus methods to decry all work done by osmic acid and silver. As we have pointed out above, the osmiophil material is developed on the site of the contractile vacuoles or reservoir, and moves down the cell and becomes divided between the daughter organisms. There can be no doubt that the blackening by osmium tetroxide marks clearly the presence of a definite amount of lipoid substance, which undergoes definite changes during the life of the organism and which is identical with the osmiophil material found in all metazoon cells.

Regarding the relationship between the reservoir and contractile vacuoles, we have already expressed our views (p. 574). The senior author finds it difficult to accept Dobell's (1908) account of the division of the reservoir into two parts prior to the division of the organism. It is quite true that we have found many examples where two vacuoles of reservoir size are present just before the onset of division (figs. 5, 8, Pl. 46, and 10, Pl. 47), but these appear to have arisen by the sudden growth of contractile vacuoles and not by division of a pre-existing reservoir. It is difficult to understand how a thin-walled vesicle filled with water could divide into two. We admit that this point needs further elucidation, and one of us is at present engaged on this problem in *Euglena*. Sigot throws no light on the point.

Regarding the nature of the euglenoid accessory contractile vacuoles (Text-fig. 2) of Borradaile and other well-known teachers, and the osmiophil canals of *Paramoecium* (Nassonov), we believe that if the accessory vacuoles were drawn out around stiff canals as in the ciliate we should have an exact homology and resemblance between the two. Thus we feel that the osmiophil canals of *Paramoecium* are drawn out contractile vacuoles which empty into a reservoir (contractile vacuoles of *Paramoecium*).
**Nassonov's Homology of Contractile Vacuole and Golgi Apparatus.**

Nassonov (1924) says, 'The primitive form of the excretion apparatus of Protozoa resembles a bladder with osmiophil walls, whereas this structure in more highly organized Protozoa can assume a more complicated form. The simplest form of the Golgi apparatus must be assumed to be a bladder (vacuole) with osmiophil walls, or a scale (as in germ and some somatic cells of the lower animals); this primitive form can become more complicated in the somatic cells of higher Metazoa, and assume the form of a net.'

Recently it has been shown by one of us (B. N. S.) in *Amoeba proteus*, and by Mrs. Lamont in *Nebela collaris*, that no Golgi apparatus is present in these forms. Moreover, the contractile vacuoles of these and many other Protozoa have no osmiophil walls. In the choanoflagellates (Saedeleer, 1930), it seems that an osmiophil Golgi apparatus (parabasal) is present at the base of the flagellum, and true contractile vacuoles may be found far removed at the posterior end of the organism. We believe that the true Golgi apparatus first arose in the flagellates in connexion with the base of the flagellum, and in such forms as *Copromonas* has secondarily become associated with the contractile vacuole (Gatenby, 1930). We find it impossible to accept Nassonov's homology in the form in which he has stated it, not only because contractile vacuoles can exist without osmiophil Golgi material, but because in such forms as *Spirostomum* a true Golgi apparatus exists quite separately from the contractile vacuole, which itself has non-osmiophil walls.

We should note that the Golgi apparatus of the trophozoites of various Sporozoa bears a striking resemblance to that of *Copromonas* under certain conditions, a fact which seems to support the view which has in the past been held by some protozoologists, that the Sporozoa are derived from the Flagellata.

In the choanocyte or choanoflagellate, the Golgi apparatus is a bead lying near the basal granule of the flagellum, and the
reason for its constant position here, and its exact function, are quite unknown. It might be suggested that it either produced by segregation from the ground protoplasm substances necessary for the continual operation of the flagellum, or that it in some way assisted in the elimination of the waste products produced by the violent movements of the flagellum. The latter suggestion would seem more likely, as the subsequent association between osmiophil substance and contractile vacuole would naturally assist in the process. This association we believe has already taken place in Copromonas, but not in the choanoflagellata. In the case of the sponge, we do not yet know whether such fresh-water forms as Spongilla have contractile vacuoles in their choanocytes. From this hypothesis of the osmiophil material becoming associated with the osmo-regulatory mechanism of the cell, one of us (Gatenby, 1938) has suggested that the function of the Golgi apparatus of metazoon cells is that of dehydrating secretory products.

SUMMARY.

1. In Copromonas subtilis, Dobell, and Euglena sp. there is a Golgi apparatus consisting of osmiophil material in the form of granules, which are associated with the osmo-regulatory mechanism of the cell.

2. Inside the granules, water collects, so that they become spherical vacuoles, identical with what have in the past been called contractile vacuoles (Copromonas) or accessory contractile vacuoles (Euglena viridis).

3. In Euglena viridis, the Golgi apparatus is closely applied to the so-called contractile vacuole, and consists of numerous loaf-shaped osmiophil bodies which undergo a regular series of changes from systole to diastole, and vice versa.

4. In Copromonas, the osmiophil material may form a thick cortex surrounding what has been called the reservoir, it may be attached to the reservoir in fairly regular loaf-shaped bodies as in Euglena, or it may be completely detached from the reservoir.

5. The so-called contractile vacuoles of Copromonas are
vesicles containing water, which are formed on the site of the osmiophil granules.

6. As far as we are able to say at present, the reservoir of Copromonas is indistinguishable from an enlarged contractile vacuole, and new reservoirs probably arise from swollen contractile vacuoles. It is difficult to believe that the reservoir divides into two, as has been claimed by Dobell.

7. During division of Copromonas, two reservoirs can nearly always be found in the early stages before the nucleus becomes dumb-bell shaped. These seem to have originated from the osmiophil vacuoles.

8. The remaining osmiophil material, when present, moves slightly down the cell, occupying a place in the mid-line. When the new cell-wall between the two organisms has passed down, about one-third the length of the dividing monad, the osmiophil material splits into two sub-equal groups and is so divided between the two organisms. There is therefore a definite dictyokinesis to be found in Copromonas.

9. Just at or after this period, the osmiophil material may become scattered about the upper middle and upper region of the dividing monads, but finally becomes situated in the region of the reservoir.

10. The osmiophil material (Golgi apparatus) persists throughout conjugation and encystment, even when a reservoir cannot be found.

11. There is a rhizoplast joining the basal granule of the flagellum with the intra-nuclear nucleolo-centrosome, and an axostyle is present, passing from the basal granule to the posterior end of the organism.

12. During cell division, the basal granule divides into two and appears to lose its connexion with the two nucleolo-centrosomes of the dividing nucleus. The axostyle appears to be absorbed in the early stages of division and cannot be stained at this epoch, but reappears in each moiety of the dividing organism, when the nucleus is dumb-bell shaped. It appears to reform when the two basal granules have taken their definitive position at the anterior end of the cells.

13. We agree with Wenyon that one flagellum passes over
intact to one of the daughter cells at division, the other flagellum arises from the other basal granule.

14. Numerous fat granules are found throughout the organism; what have been called volutin granules in other Protozoa are present in Copromonas, and stain in neutral red.

15. Mitochondria are present mainly in the posterior region of the organism.

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DESCRIPTION OF PLATES 46–48.

LETTERING.

A., axostyle; B., basal granule (blepharoplast); c.v., contractile vacuole; e., food vacuole forming; G., Golgi apparatus (osmiophil substance); G.X., special part of Golgi apparatus referred to in text; N., nucleus; R., reservoir; R.G., reservoir with thick osmiophil cortex.

Figs. 1–3.—Upper region of Euglena sp. prepared by Weigl method and slightly bleached (preparation by Miss R. Patten (1936)). Fig. 3 has been ultra-centrifuged, the reservoir being absent.

Figs. 4–15.—Copromonas subtilis Dobell, prepared by an osmic method (Weigl or Kolatchew). Figs. 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, and 15 are division stages.

Figs. 16–19.—Photomicrographs of Copromonas at rest (fig. 16) and in stages of division (figs. 17–19). In fig. 19 the left-hand Golgi apparatus has floated down below that in the right-hand daughter organism (Kolatchew method).