Morphology of the Osmiophil Material of Rhabdomonas costata, and its Behaviour during Division.

By

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With 10 Text-figures.

Introduction.

The problem of the Golgi apparatus in Protozoa has now been investigated in numerous species, and many convincing data have been accumulated. Nassonow (1924, 1925), applying the metazoan Golgi techniques to ciliates and flagellates, demonstrated the presence of an osmiophilic cortex surrounding the contractile vacuoles in many of these organisms. He likewise showed that the vacuolar canals of *Paramecium* were also osmiophilic in nature. Since the osmiophil material in these forms was apparently secretory and lipoidal in nature, he put forward the now well-known hypothesis that the osmiophil material, together with the contractile vacuole, was homologous with the Golgi apparatus of higher forms.

Later workers accepted this hypothesis only in part, as it did not include organisms which are lacking in a contractile vacuole. Gatenby (1938) modified this thesis, and suggested that the osmiophil material *alone* was the protozoan homologue of the metazoan Golgi substance. There is much evidence in support of this view. Daniels (1938), working on *Gregarina* spp., has demonstrated that the osmiophil bodies of these sporozoans, after ultra-centrifuging, occupied the same position relative to the other inclusions as did the Golgi bodies in the ultra-centrifuged metazoan cell. Other workers have shown that the protozoan osmiophil material, during division, behaves in a manner similar to the Golgi apparatus of higher forms.

Thus Gatenby and Singh (1938) in Scytomonas (Copromonas), and Gatenby and Smyth (1940) in Chilomonas, have shown that the osmiophil material breaks in two in most dividing forms, and becomes separated evenly between the two daughter cells.

In a recent account (Smyth, 1943, 1944 a, in press) a form of osmiophil material previously unknown in Protozoa was described in the euglenoid flagellate Astasia harrisii; this material was considered to represent the Golgi apparatus in this form. It is found as a mass of osmiophil substance lying below the reservoir to which it is connected by an osmiophilic canal-like structure. Previous to this, such canals were known only in Paramecium (Nassonow, 1924), though many other Protozoa possessing a canicular system had been investigated (Moore, 1934; Brown, 1938; Turner, 1940). In the present paper a brief account of the osmiophilic inclusions of Rhabdomonas costata will be given, and it will be shown that this flagellate possesses an arrangement comparable to that previously described in Astasia.

Previous Work.

Apart from work on Rhabdomonas, the vast amount of controversial literature on the Golgi apparatus in Protozoa will not be reviewed here. The subject has recently been reviewed by the writer (1944 b) and a detailed account has been published elsewhere.

Rhabdomonas is a form on which very little work has been carried out. R. P. Hall (1931) has given an account of the cytoplasmic inclusions of Menoidium incurvum, a flagellate which is either identical with, or closely allied to, the organism under discussion. He describes the Golgi bodies as scattered granules which blacken on exposure to osmic acid fumes after preliminary treatment with neutral red. The results of this worker have been contested by many cytologists (Gatenby, 1941; Smyth, 1941; et alii), as the technique of neutral red prior to osmication has long since been abandoned by modern workers in this field as being non-specific for the Golgi material. The blackening has been shown to be due to chemical

action between osmium tetroxide and neutral red. Patten and Beams (1936) have given a brief account of the cytology of Menoidium sp. Using the Weigl and Kolatchew techniques they failed to impregnate any structures in the normal or ultra-centrifuged organism. Since these workers likewise failed to impregnate the cortex of Chilomonas in the same culture (which several cytologists have shown to be osmiophilic), it is questionable whether much weight can be given to their results.

Material and Methods.

The organism was easily identified as Rhabdomonas costata Pringsheim from the data given by Pringsheim (1942) in his reclassification of some of these flagellates. It is probably identical with Menoidium incurvum figured by Pascher (1914); this is confirmed by the figures of Patten and Beams (1936) who have used the older generic name.

The Rhabdomonas were obtained from a stream in University College Gardens, and grown easily in large wide dishes of tap water to which had been added a few boiled barley seeds. The flagellates may also be grown in pure culture using the technique of Pringsheim (1942).

Equal quantities of starch, chalk, and soil are mixed together and placed in the bottom of a test-tube which is half-filled with tap water. The tubes are then plugged, autoclaved for about thirty minutes, and inoculated with the organisms a few days later. This is the method used for Astasia.

Preparations were made by the same techniques used for other Protozoa (Smyth, 1940, 1941, 1944a), namely, the Weigl and Kolatchew osmic methods. In the former the organisms were carefully centrifuged by means of a hand centrifuge, the water drawn off, and the concentrated flagellates allowed to stand for some minutes to counteract any disturbance caused by centrifuging. They were then fixed in Mann's fluid for thirty minutes, washed in several changes of distilled water, and placed in pure 2 per cent. osmic acid in a small tube and incubated at 32° C. for several days. When the osmiophil material was suitably blackened, the osmic solution was pipetted off, the

organisms placed in distilled water and kept overnight in the oven. Bleaching was then carried out with 4 per cent. hydrogen peroxide for a few minutes, and the organisms brought up to 70 per cent. alcohol, stained in neutral red acetic, dehydrated, cleared, and mounted by the albumen film technique employed for Astasia (Smyth, 1944a). The details of the flagellar apparatus were studied by staining in iron alum haematoxylin after Champy fixation. The Kolatchew osmic method was also used with some success, but the Weigl is the more satisfactory method, and it is on this technique that most of the following observations on the osmiophil material are based.

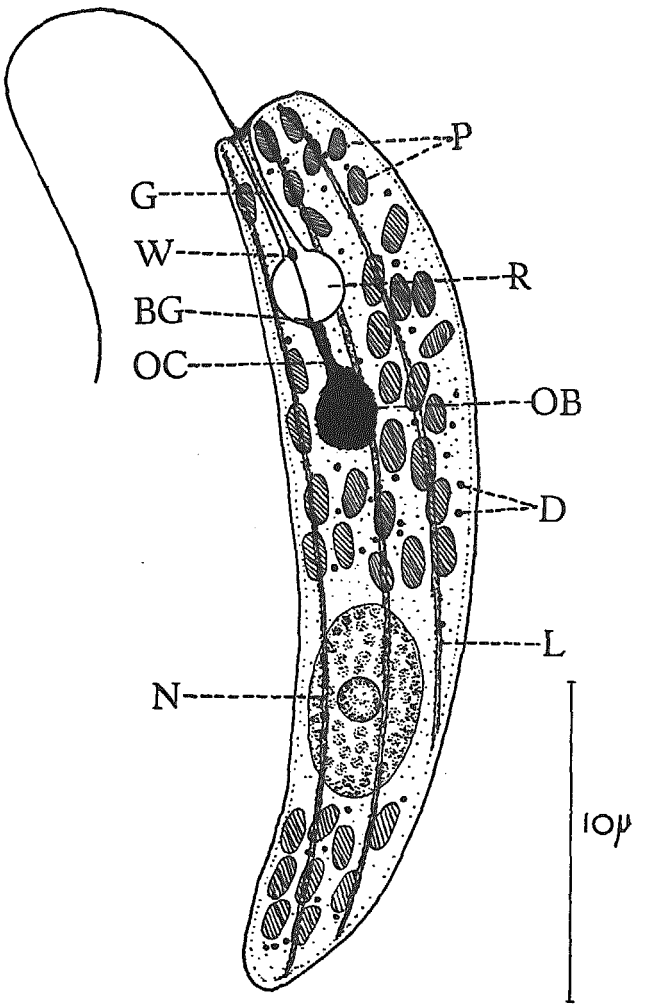
Morphology of Rhabdomonas.

Text-fig. 1 gives my conception of the morphology of Rhabdomonas costata. The body is about 30μ long, lunate and persistent in form, and sculptured by longitudinal striations (L) easily visible in both living and fixed conditions. The gullet or cytostome (G) leads into an almost spherical reservoir (R). The single terminal flagellum passes down the gullet and ends in a single basal granule (BG), heavily stainable in iron alum haematoxylin, at the base of the reservoir (R). A swelling (W) is just visible on the flagellum at the point where it enters the reservoir. The osmiophil material (OB, OC) will be described in detail later.

The cytoplasm contains numerous paramylum grains (P) of nearly uniform size. In a well-fed culture the bodies are very numerous, but fall off in size and number as the culture becomes older and poorer in food material. Also scattered throughout the cytoplasm, in between the paramylum grains, are small osmiophil granules (D) similar to those described in Astasia and other Protozoa (Smyth, 1941–4). The nucleus (N) is large and oval, and lies towards the posterior end of the organism; it moves to the anterior end prior to division.

Osmiophil Material.

The osmiophil material was easily seen in Weigl preparations counterstained in neutral red acetic. The blackening of the



TEXT-FIG. 1.

Morphology of Rhabdomonas costata. (Slightly diagrammatic.) BG, basal granule; D, diffuse osmiophil granules; G, gullet; L, longitudinal striations; N, nucleus; OB, osmiophil body; OC, osmiophil 'canal'; P, paramylum grains; R, reservoir; W, swelling on flagellum.

cortex in Chilomonas was used as a control, for the contractile vacuole of this flagellate has been shown to possess an osmiophil ring (Nassonow, 1924; Gatenby and Smyth, 1940). If then in a mixed culture of Chilomonas and Rhabdomonas the cortex of the vacuole blackens successfully in Chilomonas, it is reasonable to infer that the impregnated structures in Rhabdomonas are truly osmiophilic and not precipitation artifacts, as is often the case with careless technique or the use of impure osmic acid. Without such a control results are liable to be uncertain, and this may account for the discrepancies between the results of different workers. For example, Patten and Beams (1936), referred to above, failed to impregnate the vacuolar cortex in Chilomonas, thus leaving their results in Menoidium open to question.

In this organism it must be emphasized that, like some other forms such as Syctomonas (Gatenby and Singh, 1938), the amount and arrangement of the osmiophil material varies very considerably. In general the whole osmiophilic apparatus bears a striking resemblance to the type described in Astasia harrisii. That shown in Text-fig. 2 is most commonly found in Weigl preparations. It consists of a large osmiophil body (OB), somewhat oval in shape, and lying a short distance below the reservoir. This body is connected to the base of the latter by a short curving canal-like structure (OC), which is frequently so densely osmiophilic that it appears solid, and its exact nature is uncertain. In some of its forms it recalls the condition found in the radiating canals of Paramecium, which have been shown by Nassonow (1924) to possess osmiophilic walls. The osmiophil body usually takes the form of a solid mass of material which blackens densely in Weigl preparations and resists bleaching by hydrogen peroxide or turpentine. Its shape varies considerably, being oval or round in the majority of organisms studied, but often is very irregular in form, especially in dividing organisms (Text-figs. 3, 7).

The canal-like portion (OC) is sometimes lacking as shown in Text-fig. 10; and when this condition occurs, the osmiophil body is often found in a position nearer the reservoir than it normally occupies when the canal is present. At first it was

thought that this condition might be due to failure to impregnate it, caused by under-osmication, but individuals lacking this canal were found in preparations in which the osmication period had been considerably extended. It seems likely then that in a number of organisms, during some period in their life-cycle at any rate, the 'canal' is either lacking altogether or possibly osmiophobic in nature.

In Text-fig. 10 the osmiophil mass is present in the form of a ring of material, instead of the solid type of body so frequently found. This ring has a very thick cortex which is osmiophilic, and a small central osmiophobic region, resembling the condition found in organisms such as Chilomonas, &c. In Text-fig. 9 the solid osmiophil mass is closely applied to the lateral wall of the reservoir, and the 'canal' is absent. This arrangement has many variations, and in rare instances the mass is even found above the reservoir.

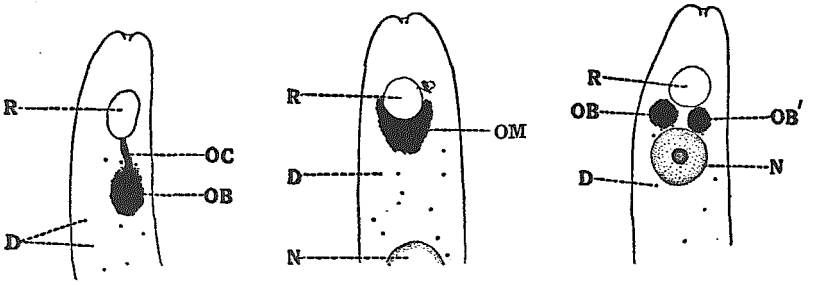
Text-fig. 3 shows what is possibly the first step in the division of the organism; the osmiophil material is apparently spreading laterally in preparation for division. In Text-fig. 4 the osmiophil substance has become almost evenly divided into nearly spherical masses lying just below the reservoir, and above the nucleus. The latter has moved from its normal position in the cell, thus following the usual course of division in these flagellates.

A still later stage in the division is shown in Text-fig. 5, in which the flagellate has divided about half-way down its length, and an equal amount of the osmiophil substance has become separated out into each monad. In one of these the material has broken up into several pieces, with aggregations of smaller blebs distributed nearby. Text-fig. 6 is a figure of the last stage of the division, when the two flagellates are almost completely divided, and ready to separate; reservoirs are not visible in either individual, and the two osmiophil masses (OB, OB') now occupy a definite position in each organism. In all the late dividing stages such as those just described above, the osmiophilic 'canals' were not present. It is not possible to say whether this is due to the disappearance of the material of the 'canal' or to a reduction in its osmiophility.

TEXT-FIGS. 2–10.

D, diffuse osmiophil granules; N, nucleus; OB, osmiophil body; OC, osmiophilic 'canal'; OM, osmiophil mass; R, reservoir.

All figures are from Weigl preparations, counterstained in neutral red acetic, and drawn with the aid of a camera lucida. Bauch and Lomb binocular microscope. Text-figs. 2, 3, 4, 7, 8, 9, 10, ×5000. Text-figs. 5, 6, ×3500.

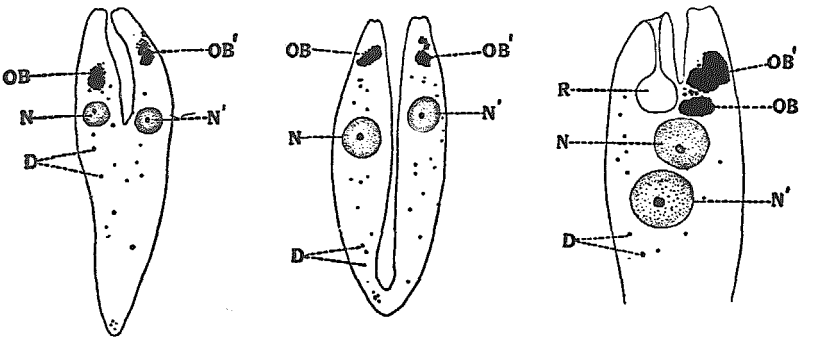


2 3 4

Fig. 2.—Normal resting stage.

Fig. 3.—Possible early division stage, with the osmiophil material spreading along the lower wall of the reservoir.

Fig. 4.—Later stage. The material has separated into two distinct bodies (OB, OB').

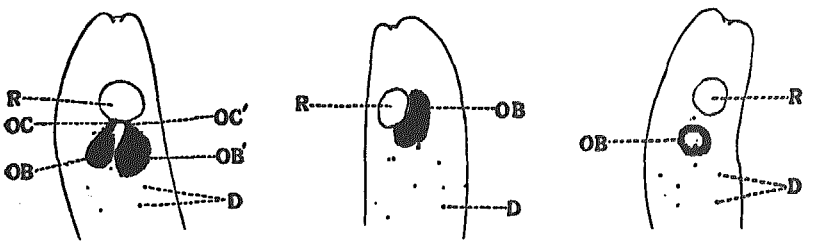


5 6 7

Fig. 5.—Still later stage. Nucleus has become divided into two; an equal amount of osmiophil substance is contained in each daughter cell.

Fig. 6.—Individuals preparing to separate. Division almost complete.

Fig. 7.—Division stage showing uneven distribution of the osmiophilic material.



8 9 10

Fig. 8.—Resting individual showing two osmiophil bodies and their 'canals'.
Fig. 9.—Resting stage; material applied to wall of reservoir.
Fig. 10.—Resting stage showing ring of osmiophil material.

The sequence of events described above is undoubtedly that which takes place in the majority of dividing organisms, but in a few cases when they divide the osmiophil material may be carried over completely to one daughter individual; Text-fig. 7 illustrates such an arrangement. The flagellate has divided for some little distance at its anterior end. The reservoir has been carried over whole to one individual, and the original nucleus has completed its separation into two daughter nuclei (N, N'). The distribution of the osmiophil material is asymmetrical, since all of it lies in the same individual; and it would appear that at division the other daughter cell would be deprived of its osmiophilic material. It may be possible, of course, that this organism contains material which has lost its osmicating power, but this seems unlikely in view of the fact that in non-dividing individuals impregnation is very consistent; it seems more likely that uneven distribution of the substance has caused it all to pass into one monad. Other unusual types also occur; for example, in Text-fig. 8 two bodies with their respective canals are present; although this condition is sometimes found in early dividing organisms, it is figured in the present case in an individual which shows no signs of division. It is believed that this probably represents either an unusually early division stage, or else is the product of an uneven division which results in the production of one organism containing two osmiophil systems. In all osmicated specimens there are always scattered

osmiophil granules present in the cytoplasm. These are scattered throughout the cell in the spaces between the paramylum granules. They are round, small, and deeply impregnated with osmic acid after the Golgi fixatives, and are undoubtedly made up of the same material as the osmiophilic bodies and their related 'canals' described above.

Discussion.

The arrangement of the osmiophil material, although variable in form, in general resembles the type described by the writer (1944a) in Astasia, except that in the latter the 'canal' is longer and more frequently present in Weigl preparations. In Astasia, too, there are small osmiophil vacuoles at the anterior end of the 'canal' just below the reservoir; these vacuoles have never been observed in Rhabdomonas.

The exact nature of the osmiophil body is difficult to determine; if a contractile vacuole was present at the base of the reservoir, this body would undoubtedly represent a thickened cortex surrounding such a structure, and forming with the 'canal' a complex vacuolar system such as is found in Paramecium. Pringsheim (1942) states that a contractile vacuole is absent in this organism, and from numerous observations on living material I have arrived at the same conclusion. It seems likely that the osmiophil material is merely an aggregation of some viscid substance, possibly lipoidal in nature, which can sometimes form a tube-like structure connecting it to the reservoir. This conclusion is based on careful study of numerous Weigl preparations, and explains to some degree the great variation in form of the osmiophil complex. If this material was aggregated on the wall of a contractile vacuole it could be compared with the arrangement in such forms as Chilomonas or Euglena.

The process of division and distribution of the osmiophil substance appears normal enough, at least in most organisms, where a true dictyokinesis takes place. In a few instances abnormal conditions occur, such as have been already described in Text-fig. 7. Here undoubtedly a division is about to occur,

in which one organism will be separated off from its partner without receiving its share of the osmiophil substance. Cases such as this have been also described in other Protozoa. In Syctomonas, Gatenby and Singh (1938) were the first to observe this phenomenon; they mentioned that in a few dividing individuals no osmiophil substance was found in one of the pair of daughter cells. Gatenby and Smyth (1940) noted similar instances in Chilomonas, where in about 3 per cent. of the dividing organisms the entire contractile vacuole and its cortex moved over whole to one individual. Later, in Vorticella, Gatenby (1941) showed that in this ciliate the osmiophil cortex never divides, but the organism that retains the stalk after division also retains the whole osmiophil vacuole; a new vacuole appears before the organisms separate. This writer also puts forward some evidence that the new osmiophil cortex is probably formed by aggregation of the scattered osmiophil granules present in this form.

The diffuse osmiophil granules found in Rhabdomonas are very similar both in size and distribution to those found in Astasia and many other flagellates previously described. There is no evidence as yet to show that these granules take part in the re-formation of the osmiophil complex where this is lacking after division, but further results may prove this to be the case.

Nassonow's Homology.

The evidence in support of Gatenby's modification of Nassonow's Homology has been given earlier in this paper. In Astasia the writer put forward some evidence that the osmiophil material represented the homologue of the Golgi apparatus in Metazoa. In Rhabdomonas costata, which possesses a very similar osmiophil apparatus, the fact that the material in question undergoes a definite dictyokinesis gives even stronger support to this view. In addition, the osmiophil substance bears the following resemblances to the metazoan Golgi apparatus: it is demonstrable after treatment by the standard metazoan Weigl osmic technique; it resists bleaching by turpentine or hydrogen peroxide; its shape and arrangement

vary considerably as though taking part in some metabolic cycle; it is polarized, i.e. it occupies a definite position in every organism, being always found between the nucleus and the reservoir system.

Without the application of the ultra-centrifuge it is not possible to conclude definitely that the osmiophil material does represent true Golgi substance in Rhabdomonas, but the fact that it answers most of the other criteria for the identification of the Golgi apparatus in metazoan cells is a strong indication that this is probably the case.

Summary.

1. In Rhabdomonas costata Pringsheim the osmiophil material, as shown in Weigl preparations, is very variable in amount and arrangement.

2. In the majority of organisms it takes the form of a large osmiophil body lying beneath the reservoir, to which it appears to be connected by a short osmiophilic canal-like structure. Small scattered osmiophil granules are aggregated in the region of the reservoir and sparsely distributed throughout the cytoplasm.

3. In some individuals the osmiophil 'canal' is absent.

4. The osmiophil body may also be present as a ring with a thickened osmiophil cortex and an osmiophobe centre.

5. The behaviour of the osmiophil substance during division is as follows: the body loses its canal and spreads along the lower wall of the reservoir; as division proceeds it becomes divided into two masses, and when the organism separates into daughter individuals each monad contains nearly equal quantities of the osmiophil substance. There is thus a definite dictyokinesis in Rhabdomonas.

6. In a few instances the osmiophil material is carried over whole to one of the two organisms formed by division.

7. In some normal (non-dividing) individuals two osmiophil bodies and their respective canals are found.

8. From the fact that the osmiophil material in Rhabdomonas is demonstrable after the metazoan Golgi Weigl

technique, is resistant to bleaching, and is separated out evenly in most cases during division, it is believed to represent the Golgi apparatus in this organism.

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