The Excretory Organs of Opalina.

Part II.

By

Maynard M. Metcalf,
Professor of Zoology Oberlin, Ohio, U. S. A.
(From the Zoological Institute, Würzburg.)

(With 15 figures in text.)

Since writing Part I of this paper I have found specimens of the *Opalina* for which ZELLER figured structures which DELAGE and HEROUARD assumed to be excretory vacuoles. I have also found excretory vacuoles in *O. dimidiata* and remarkably well developed excretory organs in the gametes and other minute spring forms of *O. intestinalis*, *O. caudata* and *O. dimidiata*. I will add here a few words about the phenomena observed.

That which ZELLER described in his large plump *Opalina* from *Rana esculenta* is not, nor does his description imply that it is, an excretory organ. His drawing (Fig. 19 of Part I of this paper), which shows what might very naturally be so interpreted, is evidently meant to show merely superficial ridges with their intermediate valleys converging into a hemispherical depression at the posterior end of the body. Like DELAGE and HEROUARD, I was at first misled into interpreting this figure as showing a group of tubules with a common external aperture. Since I have seen the animal itself the interpretation of ZELLER's figure is clear. ZELLER's description („eine recht eigentümliche in Falten gelegte Einziehung des hinteren Körperendes") does not imply that the structures are internal, and they are not so. They are merely superficial furrows between the spirally longitudinal ridges of the body. The posterior end of the body shows a considerable hemispherical depression and into this depression run the furrows mentioned.

I have found no excretory vacuoles in this very large *Opalina*. It is too thick and opaque for satisfactory study of such structures in total preparations, and I have not as yet found sufficient material to enable me to make sections. If I find more material I will make sections and endeavor to see if this species does have any excretory vacuoles.

In another paper, which I am now preparing, I will describe somewhat more fully this *Opalina* which seems to be a distinct species, and which it is but natural to call *Opalina zelleri* after its discoverer.

In full grown *Opalina dimidiata* it is difficult to observe the excretory vacuoles, for the animals are not flat and thin as is *O. obtrigona* and they are usually quite opaque. In smaller individuals one often sees clearly an axial series of vacuoles in the posterior end of the body (Fig. A). These may form either a continuous or an interrupted series. They reach back to the pointed posterior end of the body and are sometimes found to open there to the exterior. In the multinucleated *Opalinae* one frequently, especially in the spring, finds many of the nuclei each completely enclosed in a vacuole. In small *Opalinae dimidiatae* I have some times found these perinuclear vacuoles connected with the axial series of large vacuoles.


Fig. A.

One frequently sees the *Opalinae dimidiatae* trailing behind them a mass of granules which comparison with other species shows have been in all probability extruded from the vacuole aperture. In two instances in living *Opalinae*, once in *O. intestinalis* and once in *O. caudata*, I have observed the extrusion of these granules, which are of about the same size and appearance as the microsomes at the nodes of the cytoplasmic reticulum. They are figured within the body in Fig. 14 of Part I of this paper, and Fig. B shows them both outside and inside the body of a small individual of *O. intestinalis*.

The macrogametes of *Opalina intestinalis*, *O. caudata* and *O. dimidiata*, and also the other minute individuals of these species found in the spring,[1]) have remarkably highly developed excretory vacuoles. Especially in *O. intestinalis* I find these organs larger in comparison with the size of the body than in any other protozoan I know, for the numerous vacuoles in the *Radiolaria* and *Heliozoa* can hardly be compared with these structures in *Opalina*. Observation of Figs. B to K will show the character of these organs in *O. in-*

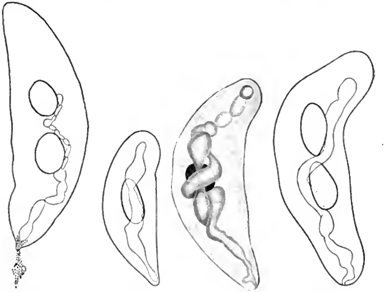

Fig. B. Fig. C. Fig. D. Fig. E.

testinalis. One finds an elongated axial vacuole stretching from the posterior end of the body almost or quite to the anterior end. In its course it comes into close relation to the nucleus or nuclei. As in Fig. C it may merely run alongside the single nucleus. When the nucleus is in division and is dumbbell-shaped, the vacuole usually coils spirally around it (Figs. D and E). When two nuclei are present the vacuole generally runs between them (Fig. F). In small individuals the nucleus may crowd the large vacuole to one side (Fig. G). The close relation between nucleus and vacuole in this species recalls the perinuclear vacuoles of the multinucleated

[1]) In a brief note now in press with the Zoologischer Anzeiger, I have described the processes of reproduction and the heterogamous gametes of these three species. A fuller description will soon follow.

Opalinae. One often finds the excretory organs interrupted into a series of vacuoles (Figs. B, H and J. Compare also Fig. L), and in many cases the continuous or interrupted organ is branched (Fig. J. Compare also Figs. K and L), the branching occurring generally either near the posterior end of the body, or near the posterior nucleus, or near the posterior end of the single nucleus, when but one is present.

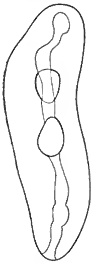

Fig. G.

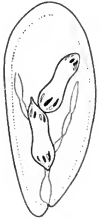

Fig. F. Fig. H. Fig. J.

Generally the posterior end of the excretory organ is found ending blindly, near, usually very near, the posterior tip of the body (Figs. C, D, G and H). In some cases it seems to come actually to the limiting membrane of the body, but without any visible external aperture (Figs. E and F). Occassionally, both in living animals (Fig. J) and in acetic-carmine preparations (Compare Figs. K and L) the external aperture itself is very clearly seen. Sometimes the position of the aperture is indicated by the trail of extruded granules which the animals carry behind them. Generally these are merely entangled in the posterior cilia, but occassionally they are seen in contact with the body surface. In the latter cases I think they are freshly extruded and that their position indicates the position of the potential aperture (Fig. B). In those cases where the opening itself has been clearly seen, I do not remember to have found the extruded granules present. Doubtless, with good fortune, one could so find them.

Often the posterior end of the excretory organ is considerably enlarged (Figs. G and J). Frequently this part is much more clearly seen even when the anterior portions are present. In some instances one finds the vacuole visible only in the posterior end Fig. K). In many cases no such elongated vacuole or system of vacuoles is seen at all.

In *Opalina caudata* almost identical conditions are often found. In many cases in the macrogametes of this species I have found what seems to be a branch of the excretory organ near its posterior end, lying between ectosarc and endosarc (Fig. L). The presence of groups of refractive spherules, unstained by acetic carmine, in the ectosarc makes it difficult to observe this part of the excretory organ. I am, however, fully convinced of its presence, for, in those cases in which it consists of a series of more or less distinct vacuoles, it is often clearly visible and not to be confounded with groups of ectosarc spherules.


Fig. K.


Fig. L.


Fig. M.

In the minute macrogametes of *Opalina dimidiata* one finds very similar excretory vacuoles, generally elongated and often branched, also often interrupted into a series of more or less distinct vacuoles (Fig. M). In many individuals, as in *O. intestinalis* and *O. caudata*, one fails to demonstrate any excretory vacuole. Sometimes in these macrogametes one finds one or both of the nuclei surrounded by a narrow perinuclear vacuole. I believe that, in some cases at least, this is in connection with the axial vacuole, but with such minute forms it is difficult to be certain.

The microgametes of these three species are very minute. Their protoplasm shows a general, foam-like vacuolization, with the vacuoles larger in comparison with the size of the body than is true with other individuals. That these vacuoles are united in any significant way, I have not observed.

The real nature of the excretory vacuoles of these species of *Opalina* is best shown from sections (Fig. N). They do not have a clear and definite limiting membrane such as is seen, for example,

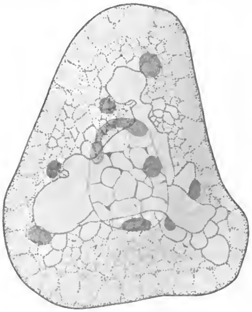

Fig. N.

around the vacuole of *Hoplitophrya uncinata.* The axial vacuoles seem to be nothing more than enlarged vacuoles of the general cytoplasmic foam. The cytoplasmic structure in the *Opalinae* is clearly that of a foam, and the axial vacuoles may be in connection with the smaller vacuoles of the body. The visible presence of the excretory vacuoles in some individuals studied, and their apparent absence in others, seems to indicate that they enlarge at times, probably by fusion, and again return to their condition of ordinary cytoplasmic vacuoles.

Snch an excretory system of vacuoles formed apparently by a more or less temporary enlargement and fusion of the general cytoplasmic vacnoles, seems very primitive and suggests that, if *Opalina* be a true ciliate protozoan, it may be a very primitive member of the group. Its reproduction and the character of its nuclei support such an interpretation.

The intimate association of the excretory vacuoles with the nucleus is noteworthy. In the spring, when this system of enlarged vacuoles is most clearly seen, the nuclei are in very active division. Division of the cells at this time is very much more rapid than their growth, so that the animals become smaller and smaller before encystment. After the cysts are eaten by the tadpoles the little *Opalinae* work their way out of the cysts in the small intestine or rectum of the tadpole. Nuclear division then continues, also certain other nuclear activities, consisting in part of extruding into the cytoplasm a large portion of their chromatin. Through all the period in which the excretory vacuoles are enlarged these activities are emphasized. Possibly during this period the nutritive activity of the nuclei is also increased, this activity, and the growth attending it, being hidden by the fact that division is so much more rapid than growth, the size of the individuals found thus continually decreasing.

The little *Opalinae* that hatch from the cysts are tremendously active before and just after they emerge from the cysts. Their rapidity of motion in the cysts makes the observer fairly dizzy. When they emerge they dart away with a speed that is utterly different from the rather slow movements of ordinary *Opalinae*. This great activity does not continne many minutes, bnt all these minnte individnals in the tadpoles rectum, whether recently freed from the cysts or not, are somewhat more active than the ordinary large individuals. This greater activity may help a little to explain the great development of the excretory vacnoles, but does not seem sufficient to completely account for it.

I have called the organs described excretory vacuoles. That they do subserve excretion seems altogether probable from what we know of other Protozoa. The posterior part of the organ is occassionally contracted and its contents, granules and liquid, extruded. This contraction must be infrequent or I wonld more often have seen it. It is probably so infrequent and irregular that one could hardly with propriety speak of its rythm.

The material extruded is quite sticky. It clings for a long time to the posterior cilia: it collects bacteria and minute particles of debris: often it attaches the animals to one another, or to particles of dirt, and holds them so strongly that they are for a long time unable to swim free. Animals freshly drawn from the rectum of the host into salt solution or Locke's fluid are often seen trailing these masses of sticky material behind them, but, after they have been sometime in these fluids, more individuals show the extruded material and its amount averages more.

Fig. O.

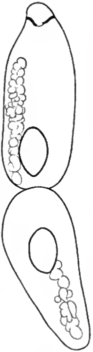

Fig. P.

When feeding *Opalina* cysts to tadpoles, one has usually all sizes of free swimming *Opalinae* in the dish with the cysts. The tadpoles eat the free swimming individuals as well as the cysts and many of the former pass apparently uninjured through the intestine to the rectum. One sees, in these individuals that have thus passed unencysted through the alimentary canal of the tadpole, that the excretory vacuoles ore unusually well developed. It is from such forms that Figs. B, D, E, F, J and K were drawn.

The absence of an excretory vacuole in *Opalina* is one of the diagnostic characters always emphasized. Though we now find that it has in reality a well developed system of excretory vacuoles, I cannot see that this fact gives any reason for changing our opinions as to its relationships. The only other member of the family *Opalinidae* I have been able to study is *Hoplitophrya uncinata*, parasitic in the proboscis sheath and in the alimentary canal of the Turbellarian *Gunda segmentata.*[1]) There is a somewhat striking resemblance between the ex-

[1]) Through the courtesy of Dr. J. Wilhelmi I obtained at Naples abundant material of this species. I also had the privilege of studying many of his slides showing the parasites within their hosts. I have since received additional material

cretory organs in this species and in the binucleated *Opalinae*. Fig. O shows the excretory organ of *Hoplitophrya* in its usual condition. It is elongated, stretching nearly the whole length of the body. Its external opening, when present, is posterior. It frequently sends a branch to the nucleus, or rather the nuclei, for micro- and macronuclei lie together. Frequently, instead of a continuous, even, elongated vacuole, one finds a much lobulated structure, or even a series of almost distinct vacuoles, in a corresponding position (Fig. P). The organ in *Hoplitophrya* is never, I think, axial, but always peripheral. Possibly one might attempt to homologize the whole organ in *Hoplitophrya* with the posterior, more superficial branch of the organ, sometimes found in *Opalina* (Compare Fig. L), but any such attempt would seem to me fantastic. The points of resemblance worth emphasis are the elongated form of the organs, their frequently consisting of a series of vacuoles, their posterior openings, and their usual close connection with the nucleus. So far, then, as the character of its excretory organs has bearing on the question of the relationship of *Opalina*, it emphasizes its resemblance to other members of the family *Opalinidae*. The same cannot be said of the results reached by NERESHEIMER and myself in the study of its reproductive processes, or of the results of my study of the nuclear phenomena, both sets of phenomena seeming more primitive than anything we know in other Ciliata.

Würzburg, July 6[th], 1907.

Explanation of Figures.

Fig. A. A very small *Opalina dimidiata*, showing an axial series of three connected vacuoles, and two of the three nuclei with perinuclear vacuoles apparently in connection with the posterior vacuoles. Acetic-carmine. × 620 diameters.

Fig. B. A small *Opalina caudata* with the excretory organ interrupted into a series of vacuoles, which, however, are apparently all connected. Posteriorly is a mass of granules partly inside and partly outside the body. Acetic-carmine. × 620 diameters.

Fig. C. A macrogamete of *Opalina intestinalis*, showing the axial excretory vacuole passing along one side of the single nucleus. Acetic-carmine. × 620 diameters.

---

from the Naples Zoological Station. I am glad here to express my thanks to the Smithsonian Institution whose table at the Zoological Station I occupied for two months.

Fig. D. A small *Opalina intestinalis*. In the middle is the dumbbell-shaped nucleus and below it a disc-shaped, darkly staining mass, probably extended from the nucleus before division. The excretory vacuole extends through the whole length of the body, in its course coiling once and a half around the nucleus. In this acetic-carmine preparation the outlines of the vacuoles were as clear and sharp as those of the nucleus. × 620 diameters.

Fig. E. A small *Opalina intestinalis* from a tadpole of *Bombinator pachypus*. The excretory vacuole bends around the posterior nucleus and then runs between the two nuclei. Acetic-carmine. × 620 diameters.

Fig. F. A small *Opalina intestinalis* in which the axial excretory vacuole passes between the two nuclei. Acetic-carmine. × 620 diameters.

Fig. G. A macrogamete of *Opalina intestinalis*. The excretory vacuole sends a branch toward the nucleus. Acetic-carmine. × 620 diameters.

Fig. H. A macrogamete of *Opalina intestinalis* in which the excretory organ is more or less interrupted into a series of vacuoles. Acetic-carmine. × 620 diameters

Fig. J. A small *Opalina intestinalis*, showing a branched vacuole, varicose, with a posterior enlargement and an external aperture. From the living animal. × 620 diameters.

Fig. K. *Opalina caudata*. A small individual in which the condition of the excretory vacuole resembles that usually seen in the large individuals. The external aperture showed very clearly. Acetic-carmine. × 620 diameters.

Fig. L. A macrogamete of *Opalina intestinalis*, showing the branched excretory vacuole and its external opening. Acetic-carmine. × 620 diameters.

Fig. M. A macrogamete of *Opalina dimidiata*, showing the branched excretory vacuole, with the posterior nucleus between the two branches. Acetic-carmine. × 620 diameters.

Fig. N. A cross section of a small *Opalina intestinalis* from the rectum of a tadpole of *Bombinator pachypus*. The nuclear net and membrane are shown in pencil, also the chromosomes and the refractive spherules of the endosarc. The outlines of the excretory vacuoles are shown in dotted ink lines, as are also the outlines of the general vacuoles of the cytoplasmic foam. The latter are not perfectly accurately drawn. The excretory vacuoles lie over the whole upper surface of the nucleus and enfold it on three sides. In the adjacent two sections the axial group of excretory vacuoles could be traced to the posterior end of the body. Corosive-sublimate-acetic acid, Safranin and Lichtgrün. × 3633 diameters.

Fig. O. *Hoplitophrya uncinata*. The excretory vacuole sends a branch to the nucleus. Corosive-sublimate-acetic acid, Delafield's haematoxylin. × 435 diameters.

Fig. P. *Hoplitophrya uncinata*, in division. The excretory vacuole is lobulated. Corosive-sublimate-acetic acid, Delafield's haematoxylin. × 435 diameters.