Opalina.

Its Anatomy and Reproduction, with a Description of Infection Experiments and a Chronological Review of the Literature.

By

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(With Plate XIV—XXVIII and 17 Textfigures.)

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Acknowledgements.

The work upon which this paper is based has been done in the Zoological Institute in Würzburg. Professor Boveri directed my attention to the favorable character of *Opalina caudata* for cytological study, and suggested also that it would be interesting to compare the reproductive processes in this binucleated species with the phenomena of reproduction which Neresheimer had then recently described for *O. ranarum* and *O. dimidiata*, both multinucleated forms. I cannot adequately express my thanks to Professor Boveri for these suggestions and for the constant advice he has given during the course of the work. I am especially indebted to him for suggestions in regard to the comparative cytological part of this paper.

Professor Spemann kindly shared with me larvae of *Rana esculenta* which he had reared for experimental study. To Dr. Lo Bianco and the Director of the Zoological Station in Naples I am much indebted for material preserved and sent me. Dr. J. Wilhelmi gave me material of *Gunda segmentata* infected with *Hoplitophrya uncinata* which I desired to compare with its reputed relative *Opalina*. He also most kindly lent me many of his series of sections of this Turbellarian, that I might study the parasites. Dr. F. Balzer preserved material of *Opalina* for me during two months in the early spring when I was away from Würzburg, and, during my absence in the summer, Mr. E. Schneidt did me a similar service. Mr. W. B. von Baehr kindly lent me a fine preparation of sections of *O. ranarum* in the rectum of a tadpole of *Rana temporaria*, the only preparation I have had showing zygotes of this species. To all of these, who have so greatly helped me in obtaining material for study, I wish to express my most hearty thanks.

I desire to thank Geheimrath Prof. Dr. F. E. Schulze, and especially Dr. Max Hartmann for assistance in obtaining numerous books. I also wish to thank most cordially the authorities of the Smithsonian Institution, through whose kindness I was enabled to spend two months at the Zoological Station in Naples. During this time I did but little upon *Opalina*, but had the opportunity to study *Hoplitophrya*, which I was very glad to compare with *Opalina*. It is a pleasure to express my appreciation of the assistance and many courtesies received from the Director and Staff of the Zoological Station.

In the spring of 1900 Mr. Ernst Teichmann, at Prof. Boveri's suggestion, began a study of the cytology and reproduction of *Opalina caudata*. The study, however, was never completed. Most of his preparations are mislaid and cannot be found, but I have had the use of one series of his sections, and more recently Prof. Boveri obtained from Mr. Teichmann his drawings and lent them to me. I found in these drawings interesting observations most of which my study had already confirmed, but, as Mr. Teichmann's results were never published, I cannot well refer to them, since, in attempting to do so, I would be in danger of falsely interpreting his drawings.

Material and methods.

In my study of the cytology of *Opalina* I have used chiefly the binucleated species *O. intestinalis* and *O. caudata*, both of which are found in the recta of *Bombinator pachypus* and *B. igneus*. The nuclei in these species are much larger and more satisfactory for study than are those of the multinucleated species. *Opalina intestinalis* is especially good, its nuclei being a little larger than those of *O. caudata*. I have also studied *O. ranarum*, *O. obtrigona*, *O. dimidiata* and *O. zelleri*, using all the methods that were applied to the binucleated species.

For the study of the processes of reproduction I have used *O. intestinalis*, *O. caudata* and *O. dimidiata*, that is — two binucleated and one multinucleated species.

Infection experiments were made with the cysts of these three Opalinas.

The study of the living animals has given valuable results, confirming almost in detail results obtained from the study of

preserved material. These parasitic animals do not live long outside the host. In water they live usually about one day; in water containing some of the rectal contents and part of the rectum of the host they may live two or three times as long. In 0.6 % Sodium chloride solution they live generally about two days. If part of the rectum of the host and a little of the rectal contents be added to the salt solution the animals live longer, from three to nine days. LOCKE's fluid [1]) seems about as favorable a medium as physiological salt solution.[2]) *Opalina obtrigona* lived longest in my cultures. *Opalina caudata* seemed generally the most delicate, though I have several times kept it seven days. Occasionally I have had all the animals in a culture die in less than a day, some change in the rectal contents doubtless occurring which poisoned the *Opalinae*. Often some individuals in a culture will live after many others have died. Generally, for a day or two before the *Opalinae* in a culture die, they will show gradually slower and slower movements. Abnormal nuclear conditions are found in these dying animals, as will be described in the chapter on abnormalities.

It is interesting to note that keeping the animals outside the host tends to cause division, perhaps through the unfavorable environmental conditions.

Large watch-glasses were used to contain the cultures of adult *Opalinae*, these glasses bring covered to prevent evaporation. Attempts to rear isolated adults in microscopic aquaria (hollow-ground slides) were not made; such attempts with the gametes and zygotes were unsuccessful. These are more delicate than the large forms, so that very likely the latter could be kept alive a couple of days or so in such microscopic aquaria.

For the study of living gametes and other minute forms from the tadpoles, slide cultures were used. The intestine of the tadpole would be placed upon a slide with a drop or two of 0.6 % NaCl

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[1]) Calcium chloride (anhydrous) . . . . . 0.07 %
Potassium chloride . . . . . 0.01 %
Sodium chloride . . . . . 0.06 %
Sodium bicarbonate . . . . . 0.01—0.03 %.
From Journ. of the Boston Soc. of Med. Sc. 1896.

[2]) PÜTTER (1905) says that the best culture medium for *Opalina* is made of
sodium chloride 0.8 % . . . . . 100 parts
sodium and potassium tartrate 30 %. . . . . 5 „
distilled water . . . . . 400 „
and that in this fluid, *when it contains no free oxygen*, *Opalina*, if fed, will live three weeks. I have not tried this fluid, nor used any oxygen-free culture media.

solution, or LOCKE's fluid, and be opened under a ZEISS binocular dissecting microscope (magnification fifty diameters) and rapid observation be made of the forms found, all especially interesting phenomena bring noted. This preliminary survey is important for comparison with later appearances which may be suspected of being abnormal. The intestinal wall and contents would then be separated from the *Opalinae* by pushing the former to one side with dissecting needles. The *Opalinae* would then be covered with a thin cover-glass and, after a few moments waiting to allow the edges to dry, the culture would be sealed with Cheeseborough Manufacturing Company's white vaseline. Wax is not a satisfactory sealing for slide cultures which are to be studied with an immersion lens, as pressure upon the cover-glass tends to cause leaks in the wax sealing. These slide cultures live sometimes as much as two days, but often die within eight to twelve hours. Similar slide cultures were often used for studying the adult *Opalinae*, the cover being supported by a couple of very fine hairs. The slide cultures of adult *Opalinae* may live three days, though more die the first or second day.

For studying the finer structure of living *Opalina* the binucleated species are, as already said, by far the better, but not all individuals, even of *O. intestinalis*, are equally clear. Sometimes one finds nuclei in which while alive one can observe with remarkable clearness the chromosomes, the spindle fibres, and the achromatic granules. It is certain that the structures described in the dividing nuclei are not artifacts, for they have been observed not only in preserved material, but in the living animal as well. Probably no one really gives much weight to the sweeping objections that have been made to cytological studies as dealing largely with artifacts, yet many reagents do undoubtedly produce artifacts which are likely to be misleading; it is therefore a satisfaction to be confident that one is describing natural structures and not things that have been produced by manipulation.

Not only does one find many living individuals which do not show their nuclear structure clearly; occasionally one is even unable to distinguish the nuclei at all. One must usually carefully observe a good many individuals before finding one in which the nuclear structures are very clearly seen. It is interesting to note that the posterior nucleus is often clearer than the anterior. At first I thought this was due to the fact that the protoplasm of the anterior end of the body is more dense than that of the rest of the body, but there is a further and even more important reason for this

difference in clearness in the two nuclei. In individuals in which the system of excretory vacuoles[1]) is well developed, one sees that these vacuoles usually lie close along one side of the posterior nucleus (Fig. 1, Pl. XIV, 248, Pl. XXVI). They may extend also alongside of the anterior nucleus, though this is less usual. When such a vacuole is large and lies above the nucleus under observation, one is likely to see the nuclear structures very clearly. If, as sometimes occurs, the system of vacuoles divides, sending also a branch along the opposite side of the posterior nucleus, one has his best opportunity to observe this nucleus, if only the animal is so oriented that one vacuole lies above and the other below the nucleus. In this case the refractive bodies in the cytoplasm (to be discribed later) lie so far above or below the focal plane of the objective of the microscope that they distort the image but little. It is therefore well to search for the most favorable individuals before settling down to careful study of the nucleus. The anterior part of the excretory organ is seldom well seen in the living animal. It is chiefly through the study of stained preparations that one reaches this explanation of the remarkable clearness of some living nuclei.

In studying the reproductive processes in the spring, it is often valuable first to use living animals and later to treat the same animals with acetic acid or acetic carmine. For example, one can thus allow copulation to proceed to a particular point, and can then confirm his observations of nuclear and other phenomena in the living animals by studying the same animals treated with one of these reagents. It is often well in such cases to use first acetic acid and, after study, to follow with acetic carmine. Some structures, the nucleolus for example, show far better with acetic acid than with acetic carmine. Often the whole structure of cytoplasm and nucleus comes out with remarkable clearness with acetic acid.

Acetic carmine used upon fresh material is very satisfactory for the study of the outlines of the excretory organs and is invaluable in the study of the minute forms in the spring, for, while it is not a sharply definitive stain and while its results, even in the same slide, are often very uneven, yet it is so simple in its application and so prompt in giving its results, that with it one can examine a very great amount of material, and this is essential to the proper understanding of the reproductive processes.

Intra vitam staining with all the usual dyes was tried upon all

[1]) Metcalf, 1907 *b* and *c*.

the species at my disposal execpt *O. zelleri*. The results will be given in the proper connections. They are also shown in a table in the appendix.

For fixing I used chiefly Schaudinn's alcoholic-corosive-sublimate, corosive-sublimate-acetic acid, picro-acetic acid, Flemming's fluid, formol, and absolute alcohol. Of these corosive-sublimate-acetic acid (20 minutes to 36 hours) gave the best results and in the later work was chiefly used.

For staining *in toto* I used principally Grenacher's borax carmine, Mayer's paracarmine, Mayer's haemalum, and Delafield's haematoxylin. Paracarmine did not give very good results. Borax carmine gives a good general stain, but does not show the finest details with sufficient clearness. If a thin sheet of green gelatine be placed on the table of the microscope beneath the slide, the definition of detail is much improved,[1]) but even then the borax carmine preparations are not the best. No satisfactory stains were obtained with Mayer's haemalum, except for protoplasmic structure. Delafield's haematoxylin far outclasses all the other stains used for total objets. It is best to stain as darkly as possible (12 to 24 hours in $^1/_3$ strength, $^1/_2$ strength, or even full strength stain) and then to decolorize with exceedingly dilute hydrochloric acid. The decolorization should be watched under the microscope and when it has reached the right point it can at once be stopped by adding a drop of weak ammonium hydrate. The decolorization should be carried to a point that seems extreme, for the objects become much darker upon adding the ammonia. A little experience enables one to regulate the stain very accurately. If upon adding the ammonia the objects are found to be too dark, most of the liquid can be drawn off and acid again added, the decolorization being carried to the desired point. It should, however, be noted that, upon adding acid after ammonia has been used, the decolorization is much more rapid than before the objects were treated with ammonia. With this stain used in this way preparations of total objects can be obtained which rival for clearness the best sections.

The animals when stained were run through graded alcohols to cedar oil and were mounted in balsam. As Delafield's haematoxylin is exceedingly sensitive to the presence of the least acid, readily fading when in balsam, if this be in the least degree acid, it is

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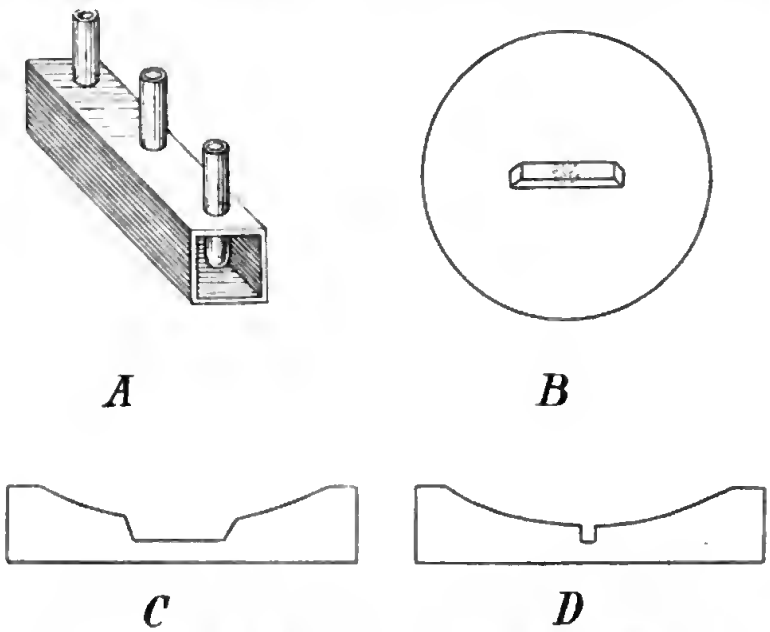
[1]) For suggesting this method, which is a very useful one, I am indebted to Mr. W. Freytag of Würzburg.

well before covering to hold the slide, with the animals in cedar oil upon it, upside down for a few moments over the top of an ammonium hydrate bottle, and to do the same with the balsam on the cover-glass. My preparations so treated have not faded in ten months except near the edges of the cover-glass. Apparently the carbon dioxide of the atmosphere causes decolorization of the objects near the edge of the cover-glass. The objects can be kept from running out from the center toward the edge of the cover-glass by the simple but effective device of placing the balsam before covering in a complete circle just inside the outer edge of the cover.

For sectioning single individuals in predetermined planes Yatsu's (1904) *Ulva* leaf method was used. For imbedding large numbers of animals together I used either Boveri's method of wrapping the animals in a bit of the sloughed skin of a large amphibian (*Cryptobranchus*), or a method which combines suggestions from Lefevre (1903) and from Paul Mayer (1907). In the latter method the animals are carried up to absolute alcohol in ordinary embryo glasses. After dehydration all but a few drops of the alcohol is drawn off. Then with a fine pipette the remaining alcohol, with the animals, is removed and placed in a small gelatine capsule (20 mm by 5 mm) which is set upright in a hole in a pasteboard box (Text Fig. I, *A*). The ends of the box should be removed so that one can look through and see the objects in the bottom of the capsule. After the animals have settled to the bottom of the capsule, the supernatant alcohol is drawn off and xylol added. It is well to change the xylol once or twice to remove all trace of alcohol. After sufficient time, the xylol is removed, melted paraffin in added, and the capsule is set into the warm chamber. The paraffin must be changed to remove all xylol. With care this may be done with a warm pipette, but I find it much easier to effect the change in another way. After the animals have become well infiltrated with the paraffin, the capsule may be removed from its supporting box and its contained paraffin cooled by placing the capsule in cold water. After a few minutes the gelatine capsule will be softened and swollen by the water and the cylinder of paraffin can be easily removed. A second capsule should then be nearly filled with melted paraffin and the tip of the cooled paraffin cylinder, with the contained objects, be cut off and placed in the top of the capsule of melted paraffin and the capsule placed in the warm chamber. As the paraffin cylinder tip melts, the objects sink through the whole length of the capsule, losing *en route* whatever xylol they may

still have had. This capsule may now be taken out of the warm chamber and its contents be cooled in water as before.

A paraffin cylinder with rounded tip is not easy to cut. This difficulty can readily be avoided by using a Lefevre watch glass for reimbedding (Text Fig. I, *C* and *D*). The tip of the cylinder, containing all the objects, is cut off and is placed in the *center* of the groove of an unwarmed Lefevre watch-glass, which has previously been lightly smeared with glycerine. With a hot pipette, melted paraffin in added on each side of the cool paraffin block, care being taken to leave this block with its contained objects still in the center of the groove. The watch-glass is now placed in the warm chamber until all is melted. It is then removed, without jarring, and placed in water, or alcohol (Lefevre), to cool. The resulting block of paraffin is of a shape convenient for sectioning (Text Fig. I, *B*).



Text Fig. I. Illustrating the method of imbedding small objects. *A*, a box containing three gelatine capsules; *B*, the block of paraffin taken from a Lefevre watch-glass; *C* and *D*, sections of a Lefevre watch glass. (*B*, *C* and *D* from Mayer after Lefevre.)

Since the objects to be sectioned are all in the center of the projecting ridge, the ends of the ridge may be cut away and a compact series of sections be obtained. This method is not tedious. It requires no watching.[1])

[1]) I am greatly indebted to Professor Paul Mayer for suggesting the use of gelatine capsules in imbedding. His further suggestion that they might well

To obtain sections of the gametes, whole recta of infected tadpoles were cut. The dirt in the rectnm usually prevents cutting sections thinner than 3, 4, or 5 *micra*, bnt these suffice.

Sections were stained with DELAFIELD's haematoxylin, HEIDENHAIN's iron-haematoxylin, GRENACHER's borax carmine, safranin, safranin and light green (*Lichtgrün*), thionin, gentian violet, methyl violet, methylen blue, methyl green, eosin, dahlia, orange G, fuchsin, magenta, *Kernschwarz*, BIONDI-EHRLICH-HEIDENHAIN's mixture, EHRLICH's triacid mixture and EHRLICH's indulin-aurantia-eosin mixture. All gave results of some value and the comparison of the results obtained from different stains was important, especially in studying the refractive spherules. DELAFIELD's haematoxylin and HEIDENHAIN's iron-haematoxylin were the most generally useful stains. The most differential stain was obtained with safranin and light green (safranin 12 to 24 honrs, light green in 95 % alcohol $^1/_2$ to $^3/_4$ of a minute). The results with all of the stains used are shown in a table in the appendix.

For illumination the light from a Welsbach gas mantel was used, daylight not being strong enough.

The degree of accuracy of the figures is told in each instance in the explanation of plates, any figures, or parts of figures, schematically drawn being so indicated.

Since writing the major part of this paper I have found that smear preparations of undiluted rectal contents of *Rana temporaria* give fine resnlts with cysts and free swimming individuals of *O. ranarum* and I do not doubt that eqnally good results would be obtained with other species. The method should be of especial value with the minute forms in the recta of tadpoles. The smear preparations should not be allowed to dry, but should at once, while moist, be fixed a moment in a hot fixing fluid and then be trans-

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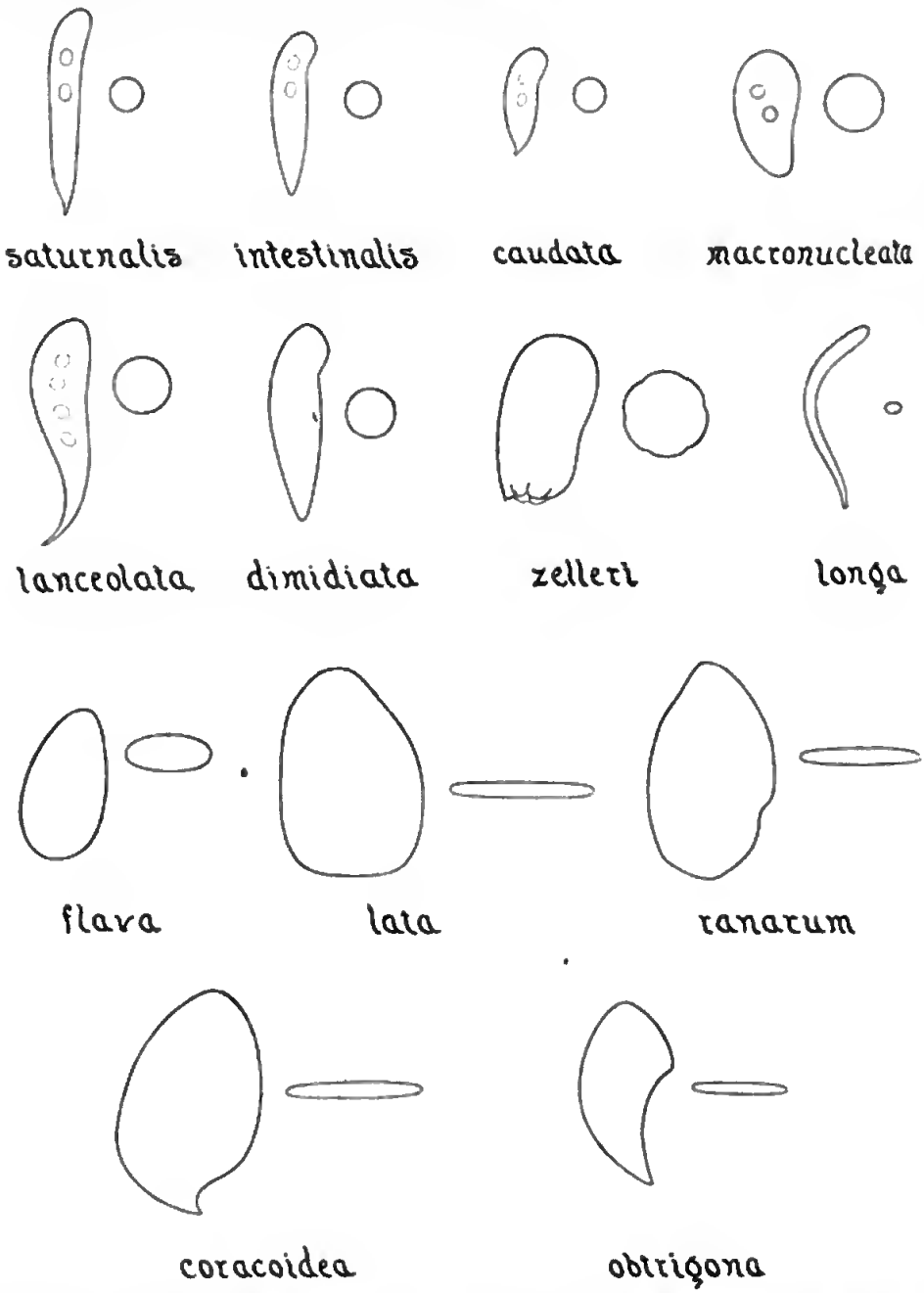
be used for storing small objects in alcohol has also proven very usefnl. Mnch of my *Opalina* material has been kept in gelatine capsules in a jar of 95 % Alcohol. (Alcohol weaker than 80 % softens the capsnles.) A further snggestion, from Dr. R. DOHRN and Dr. GAST, that the lower half of the capsnle be sealed with celloidin before covering with the lid is important, since with very small objects there is danger that some may get between the capsule and its lid and be crushed. Before adding the film of celloidin, it is well, as Doctors DOHRN and GAST suggested, to puncture the capsnle in several places just below its upper edge with a needle, so that the celloidin film will hold firmly. Minute objects so stored transport without danger of breakage and the capsules require mnch less room than the glass tubes ordinarily used. Furthermore there is no cotton plug in which any of the objects may be lost.

fered to cold fluid of the same sort. The heat is valuable since it makes caagulation instantaneous and the objects hold firmly to the cover-glass upon which they are spread.



Textfig. II. Outline drawings of surface views and cross sections of the known species of *Opalina*. The nuclei are indicated only for those forms that have few nuclei. The relative size of the different species is not shown. In each case the anterior end is above and the bend of the anterior end of the longitudinal axis is toward the right.

Occurrence of the species of Opalina.

We now know thirteen species of *Opalina*, twelve parasitic in tailless *Batrachia* (one of these also in *Triton*) and one in a Mediterranean fish, *Box boops*. Their occurrence is as follows (cf. Text-fig. II):

Species with two nuclei, bodies circular in cross section.

O. saturnalis, LEGER & DUBOSCQ,	in	*Box boops*, LAUR.* [1])
O. intestinalis, STEIN (BLOCH?)	„	*Bombinator pachypus*, BON.*
		Bombinator igneus, LAUR. [9])
		Discoglossus pictus, OTTH. [8])
		Pelobates fuscus, WAGL.
		Rana esculenta, L. [3])
		Triton taeniatus, SCHN. [3])
O. caudata, ZELLER	„	*Bombinator pachypus*, BON.*
		Bombinator igneus, LAUR.* [9])
		Bufo variabilis, PALL. [4])
O. macronucleata, BEZZENB.	„	*Bufo melanostichus*, SCHN. [5])

Species with four to five nuclei, body circular in cross section.

O. lanceolata, BEZZENB.	in	*Rana esculenta*, L., var. *chinensis*, OSB. [5])

Species with many nuclei, body circular or broadly oval in cross section.

O. dimidiata, STEIN	in	*Rana esculenta*, L.* [9])
		Bufo vulgaris, LAUR (*cinereus*, L.).
		Bufo variabilis, PALL.
O. zelleri, NERESHEIMER	„	*Rana esculenta*,* [6])
O. longa, BEZZENB.	„	*Rana limnocharis*, WIGM. [5])

Species with many nuclei, body flattened.

O. flava, STOKES	in	*Scaphiopus holbrookii*, HARLAN. [7])
O. lata, BEZZENB.	„	*Rana limnocharis*, WIGM. [5])
O. ranarum, EHRB.	„	*Rana temporaria*, L.* (*fusca* RÖSEL).
		Bufo vulgaris, LAUR.
		Bufo variabilis, PALL.*

O. coracoidea, BEZZENB. in *Rana cyanophlyctis*,[5])
O. obtrigona, STEIN „ *Hyla arborea*, L.* [9]) (*viridis* LAUR.).

*) Confirmed by my own observations.
[1]) LÉGER & DUBOSCQ (1904 *b*).
[2]) STEIN (1867), ZELLER (1877), very rare in this host.
[3]) CONTE & VANEY (1902).
[4]) I found in Naples one toad of this species whose rectum contained a few dozen *Opalinae caudatae*.
[5]) BEZZENBERGER (1904).
[6]) ZELLER (1877), NERESHEIMER (1907), METCALF (1907 *a*).
[7]) STOKES (1884).
[8]) COHN (1904).
[9]) ZELLER (1877).

All the species of *Opalina* which I have studied live chiefly at the upper end of the rectum of the host. A few individuals may be found scattered through the contents of the whole upper half of the rectum (this is especially true of *O. obtrigona* in *Hyla arborea*), but generally the parasites lie in one or more masses between the rectal contents on the one hand and rectal wall on the other. In frogs or toads which have been dead for some hours, the *Opalinae* are often found also in the lower part of the intestine, and occasionally, in frogs that were evidently diseased, I have found the posterior part of the intestine to contain some *Opalinae*. Several species of *Opalina* have been reported from the intestines, as well as the recta, of their hosts. It is possible that these reports are based on observations upon diseased frogs and toads, or upon those that were dead some time before they were examined. LÉGER & DUBOSCQ (1904 *b*) report their new *Opalina saturnalis* as occurring in the whole intestine of *Box boops*. It would be of some interest to know if the whole intestine of normal, freshly killed fish of this species contains the parasites.

Opalina caudata and *O. intestinalis* are rarely, if ever, found in the same individual host. In the two instances in which I have found *O. zelleri*, *O. dimidiata* was also present. ZELLER also found these two forms together. NERESHEIMER (1907), the only other student who has recorded the occurrence of *O. zelleri*, does not say whether he found it with *O. dimidiata* or not. There is a little doubt of the independence of the two species.

The frequency of infection of the several hosts by the several species is shown for the animals I examined in the following table.[1])

[1]) Records were not kept of a number of the hosts which were killed early in the fall of 1906, or of most killed in Naples.

	Number examined	Number containing *Opalinae*
Bombinator pachypus	105	*O. caudata* 61 *O. intestinalis* 34 Both *O. caudata* and *O. intestinalis* 1 [1]) Uncertain, either *O. caudata* or *O. intestinalis* No *Opalinae* 8 [2])
Bombinator igneus	63	*O. caudata* 25 *O. intestinalis* 15 No *Opalinae* 23 [3])
Hyla arborea	49	*O. obtrigona* 21 No *Opalinae* 28
Rana esculenta	77	*O. dimidiata* 59 *O. zelleri* and *O. dimidiata* 2 No *Opalinae* 16
Rana temporaria	15	*O. ranarum* 10 No *Opalinae* 5 (One had been starved a long time)
Bufo variabilis	4	*O. ranarum* 1 *O. caudata* 1 No *Opalinae* 2
Bufo vulgaris	1	No *Opalinae* 1

[1]) This frog was sick, the stomach being greatly distended by a very acid fluid, and the whole intestine being full of gas. The *Opalinae* in the rectum were shrunken, twisted and distorted, and in consequence the identification of the two species of parasites is not reliable. ZELLER does not say whither he found these two species of parasites in the same individual host, though he describes them both from *Bombinator igneus*.

[2]) Two of these were unusually large individuals, obtained in Berlin in the spring of 1908. In one the spots on the ventral surface were of the orange color typical of *B. igneus*. In the other the color was paler orange, more nearly approaching the lemon yellow characteristic of *B. pachypus*. The size and the character of the dorsal surface showed that these animals belonged to the species *B. pachypus*.

[3]) Some of these Bombinator had been kept several months in the laboratory and were very thin.

The structure of Opalina and the phenomena of mitosis.

In the description of the structure of *Opalina*, I will begin for each organ with the conditions in *O. intestinalis* and will then compare with the other species studied.

Cilia.

I have little to add to or to modify in Maier's (1903) discription of the cilia in *Opalina ranarum*. In all species studied the conditions of the cilia are similar. It is easy to cause the disintegration of specimens of *O. intestinalis* and *O. caudata* by pressing intermittently upon the cover-glass above them. One then sees many of the cilia, with their basal granules attached, floating freely in the salt solution. Occasionally such isolated cilia may show a few faint contractions after their complete separation from the body. Similar phenomena have been observed in ciliated cells by Klebs (1883), Bütschli (1885 *a*), Fischer (1895) and Peter (1899), and for the isolated tails of spermatozoa by numerous students (*cf.* Meves 1899 *b*).

In tangential sections of the body of any species of *Opalina*, one sees a network of fibres beneath the pellicula (Fig. 2). The spiral-longitudinal rows of basal granules lie just below the larger fibrils, the course of the two exactly coinciding. The much more delicate transverse fibrils stretch between the longitudinal fibrils, each end coinciding in position with a basal granule. In this regard it is seen that my study confirms Tönniges (1898) rather than Maier (1903). The observation of contraction in isolated cilia shows that their movements are automatic, as Maier claims in opposition to Tönniges, but it seems probable that the coordination of the movements of the cilia may be connected with the presence of this network. The transverse fibrils of this network lie beneath and not in the pellicula, as accurate focussing clearly shows. The longitudinal fibrils are a little more superficial, lying apparently at the level of the outer ends of the basal granules.

Nether *O. intestinalis* nor *O. caudata* have any tuft of longer cilia at the anterior end of the body, such as Léger & Duboscq (1904 *b*) discribe for *O. saturnalis*. In *O. saturnalis* the anterior tuft of cilia is not very distinct from the adjacent cilia.

There is no posterior zone, as in *O. saturnalis*, from which the cilia are absent.

Pellicula.

All species studied have a pellicula of appreciable thickness which is quite distinct from the subjacent cytoplasm (Figs. 3, 6, 7, 8, Pl. XIV). With many stains it colors differently from the ectoplasm. Some of these differentiating stains are gentian violet, methyl violet, methylen blue, thionin, fuchsin, dahlia, EHRLICH's triacid mixture, and DELAFIELD's haematoxylin. With methylen blue the whole ectoplasm is stained green while the pellicula is pale blue (Fig. 18, Pl. XV); with methyl violet the pellicula has a lighter shade than the ectoplasm; with thionin the pellicula is unstained while the ectosarc is green; with fuchsin the light red pellicula is readily distinguished from the more faintly colored ectoplasm. Dahlia gives perhaps the clearest picture, the pellicula being a very faint purplish gray, while the ectoplasma is purple. The contrast between the green pellicula and the blue ectoplasma after staining with EHRLICH's triacid mixture is also very marked. With all stains used the pellicula seems homogeneous.

I have not been able to demonstrate with entire clearness the minute longitudinal ridges which MAIER describes as present on the outer surface of the pellicula. The longitudinal striae are very clear in surface views of tangential sections, but none of my cross sections give satisfactory views of the ridges. In some sections stained with DELAFIELD's haematoxylin they are faintly seem. One cannot, however, doubt the accuracy of MAIER's description, for his work is clearly very careful.

ZELLER figures the outer surface of the body as breaking into thin narrow strips after treatement with acetic acid. He called these strips muscle threads. From my own preparations it seems probable that what he described were strands of pellicula which had separated along the course of the lines of cilia (cf. MAIER, 1903, p. 80).

Ectosarc.

Sub-pellicular layer.

Immediately beneath the pellicula, between it and the usually large alveoles which fill the greater part of the ectoplasm, there is a thin layer of finely alveolar protoplasm (Fig. 8, Pl. XIV). In the outer part of this layer lie the basal granules of the cilia. Other granules, similar in size, lie more internally in the same layer. They resemble in size the granules that lie at the nodes and along the

course of the films of the endoplasmic web, but in their staining reactions they resemble more the ectoplasmic spherules soon to be described.

Alveolar layer[1]) (Figs. 4, 7, 8, Pl. XIV).

Beneath the sub-pellicular layer the ectoplasma shows many alveoles always larger, usually very much larger, than those of either the sub-pellicular layer, or the endoplasma. Methyl violet most sharply defines all the structures of the alveolar layer (Fig. 8). In sections stained with this reagent one sees that there is usnally an onter irregular row of moderately large alveoles and that within this is a second irregular row of huge alveoles. It is not difficult to understand how Maier failed to observe these alveoles in *O. ranarum*, if he used only iron-haematoxylin in staining his sections, for this reagent often gives very unsatisfactory pictures of the structure in this region (*cf.* Fig. 5). The walls of all these alveoles are delicate films along which lie scattered granules. Each alveolus, whether large or small, contains a body which, following the nomenclature of my preliminary paper (Metcalf 1907 *a*) may be called an ectoplasmic spherule. Sections stained with most reagents fail to show a fact which methyl violet clearly demonstrates, namely, that each alveolns, whether large or small, contains only one sphernle.

The spherules as seen in sections vary greatly in size. In some cases, even when an alveolus is of great size, the spherule may nearly fill it. In general, the size of the spherule is roughly proportional to the size of its alveolus. The spherules are more or less irregular in form. Often, especially in sections of animals which are a little shrunken by reagents or by the heat used in imbedding, one sees the spherules showing a shape that irresistably suggests that they have been coagulated and shrunken from a more fluid substance which previously filled the alveoles. The finely granular character of these irregular spherules is not inconsistant with such an interpretation.

The ectoplasmic spherules are often clearly seen in the living animals. They have usually a distinct yellow tinge. This is emphasized by acetic acid. In many acetic-carmine preparations their yellow color is sharply contrasted with the red of the spherules in the endoplasm (Fig. 31 *a*, Pl. XV).

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[1]) I make no attempt in any part of this paper to distinguish in terminology between the minutest alveoles and the larger spaces fonnd within the protoplasm, which may arise by the enlargement or fusion of minnte alveoles.

These spherules or drops of liquid in the ectosarc stain *intra vitam* with neutral red (darkly stained after half an hour), methylen blue (deep stain), toluidin blue (deep stain) (Fig. 20, Pl. XV). They do not stain *intra vitam* with Congo red, indigo-carmine, methyl violet, dahlia, Bismarck brown, gentian violet, thionin or eosin. With methylen blue and toluidin blue the anterior end of the body remains almost entirely unstained, showing either that the ectosarc spherules are wanting there, or are in a different condition. The study of sections shows that the ectosarc spherules are very small in that region. After *intra vitam* staining with toluidin blue (Fig. 20, Pl. XV) one finds a few bodies, larger than the ordinary ectosarc spherules, stained a much darker blue. These are evidently in a different condition, if they be not of a wholly different nature. It is probable that these larger, darkly staining bodies lie in the outer part of the endosarc. Compare the results, obtained by staining with iodine and with FISCHER's glycogen stain (page 218).

The statements in the last paragraph apply only to true *intra vitam* staining, the animals remaining alive and active. As the animals become inactive and die, the ectoplasmic spherules commence to stain with methyl violet, though later they again fade. If one sections animals which have been fixed in corrosive sublimate-acetic acid and stains the sections with the same dyes that were used for *intra vitam* staining, somewhat different results are obtained. Methylen blue stains the ectosarc spherules green, not blue as in life; methyl violet colors them violet like the protoplasm; dahlia stains them purple; gentian violet stains them pale violet; thionin stains them green; Bismarck brown colors them brown; though the last five reagents left these spherules unstained in the living animal. In the table in the appendix the color reactions of the different parts of the body to all the stains used are given. I would call attention to the fact that with safranin and light green the ectoplasmic spherules are all colored green, though with safranin when used alone they stain a good red.

The ectosarc spherules show little structure with most stains (Figs. 4, 7, 8, 9, 14, Pl. XIV). With dahlia some of the larger, more faintly stained ones show granules which seem to be peripheral (Fig. 7). Iron-haematoxylin when insufficiently extracted shows the spherules apparently homogeneous (Figs. 4, 9), but after longer decolorization one often finds them showing clearly the presence of peripheral granules and even apparently alveolar structure (Fig. 5). When found, these indications consist merely of faint dark lines

stretching across the interior of the spherule and connecting certain of the deeply stained granules which lie at the periphery of the spherule with smaller granules in the interior. I have never seen ectosarc spherules showing indication of division.

The ectosarc spherules do not stain at all with potassium iodide, so they cannot be composed of glycogen. They do not stain at all with a solution of iodine in aqueous potassium iodide. They do stain strongly with safranin in sections of animals fixed in absolute alcohol and treated, after sectioning, with tannin and potassium bichromate. Fischer (1905) regards this staining after such treatment as indicative of glycogen, but the entire absence of any reaction to iodine on the part of these spherules seems to indicate that they are not glycogen and probably are not of a substance closely related to glycogen.

It is difficult to form an idea of the function of the ectoplasmic spherules. The internal structure demonstrated by iron-haematoxylin, and less well by certain other stains, seems to argue against their being wholly secreted bodies. On the other hand, their position within the alveoli rather than upon the alveolar walls, would suggest that they are a product rather than a constituent part of the protoplasm. The fact that with certain dyes they readily stain *intra vitam* casts further doubt upon their interpretation as living constituents of the cell, though these dyes, neutral red, methylen blue, and toluidin blue, are well known to stain some kinds of living tissue in Metazoa. Léger & Duboscq (1904 *b*) think that the similar bodies in *O. saturnalis* are probably connected with nutrition and may be of a nature similar to lecithin. In the species I have studied, however, they are not soluble in warm alcohol and ether, so that they cannot be composed of lecithin. I know of nothing to indicate that they are excretory. They have no connection with the system of excretory vacuoles I have described (Metcalf 1907 *b* and *c*).

The ectosarc spherules are present in all species I have studied, though they are very small in some forms of *O. dimidiata*. In *O. caudata* they resemble closely those of *O. intestinalis*. In *O. ranarum* and *O. obtrigona* they are smaller, but otherwise similar. In *O. zelleri* they are still smaller, but are clearly recognised. In *O. dimidiata*, in all but the minute forms in the spring and the young forms in the tadpole, one almost fails to find them with certainty, for, they are little if any larger than the largest of the ordinary granules of the ectosarc. On the other hand, yellow ectosarc spherules of large size are abundant in this species in the

macrogametes and other small forms from the tadpole, except possibly the microgametes, and also in the smallest forms found in the spring in the rectum of the frog. I regret that my notes do not say as to the presence of the ectosarc sphernles in the microgametes of *O. dimidiata* and I do not remember with certainty, thongh I think they are present and of good size in this species as in the microgametes of *O. intestinalis* and *O. caudata.*

It is not easy to be certain who of the students of the *Opalinae* have seen the ectosarc spherules, for, excepting by Léger & Duboscq (1904 *b*) for *O. saturnalis*, they have not been figured or clearly described. Tönniges (1898) describes for *O. ranarum* certain greenish granules, disc-shaped, elongated, or of irregular form, and varying in size. He says that in most individuals they lie exclusively in the endoplasm and goes on to describe at considerable length their minute structnre. In the main his description applies surely to what I have called endosarc sphernles (Metcalf 1907 *a*), but the greenish color he ascribes to these is characteristic of the ectosarc spherules. Tönniges has not distinguished between the two kinds of spherules and it may be that the greenish color of the onter ones has been ascribed by him to them all. Conte & Vaney (1902) have evidently not distingnished the ectosarc spherules. Neresheimer (1906 and 1907) describes remarkable phenomena connected with certain disc-shaped spherules. I am not snre I understand correctly his description, but it seems to apply to the larger ectoplasmic spherules and not to the smaller sort of spherules which lie in the endoplasma. He says that these disc-shaped bodies change into spherical or ovoid spherules into which the reproductive chromidia migrate, each spherule with its chromidia constituting a new reprodnctive nncleus in which the spherule furnishes the achromatic portion and the chromidia the chromatic portion of the new nucleus. The conditions in *O. intestinalis* previons to and during the spring sexnal reproduction preclude any snch interpretation, useless *O. ranarum*, upon which Neresheimer worked, is fnndamentally different from the binucleated species. *Opalina ranarum* is a far less favorable species than the binucleate forms for the study of the ectosarc spherules, for in *O. ranarum* they are not only proportionally bnt actually much smaller.

Endosarc.

The endosarc of all the species of *Opalina* I have stndied shows usually a finely granular and fibrous appearance in which the real

foam-like alveolar nature can hardly be discerned. Occasionally one finds individuals whose endosarc clearly shows the alveoles, especially when stained *in toto* with DELAFIELD's haematoxylin (Figs. 15, Pl. XIV, 87, Pl. XX), or in section by EHRLICH's triacid mixture. MAYER's haemalum after FLEMMING's fluid shows the same structure but less clearly. The clearest pictures of the alveoles of the endosarc are found in very small individuals of *O. dimidiata* and *O. obtrigona*. The structure in *O. intestinalis* is the same, but is a little less clearly seen.

The endoplasma differs markedly from the ectoplasma in the very much smaller size of its alveoles, which are very minute. The nodal granules of the endosarc (Fig. 15, Pl. XIV) resemble in size the granules which lie at the nodes and along the films of the ectosarc foam. The endoplasmic alveoli are usually so minute that one is unable to see if any granules lie along their walls at other points than the nodes of the foam. When however one finds an animal in whose endoplasma some of the alveoles are enlarged, the walls of the alveoles are seen to bear frequent granules.

The endoplasma in *O. intestinalis* is more dense in the anterior end of the body, in front of and near the anterior nucleus. In all other species studied a similar greater density of the endoplasm in the anterior end of the body is observed, though it is less noticeable in the flattened species, especially in *O. ranarum*.

Endosarc spherules (Figs. 4, 5, 6, 10, 11, 12, 13, 16, Pl. XIV).

In the endosarc are many refractive bodies which I have called endosarc spherules (METCALF 1907 *a*). They have been observed by most of those who have studied *Opalina*. ZELLER describes little refractive bodies in the "parenchyma" of each of the five species of which he treats. These he says are slightly flattened and disc-shaped, and show a central dark spot which may be due to the presence of a central cavity or to a hollow in each face of the disc. He says their diameter is about 0·004 mm.

BARFURTH (1885) describes for *O. ranarum* certain masses of "glycogen" which stain brown with iodine, and near them many light yellow strongly refractive drops of another substance ("fat?"). The latter were probably the refractive spherules.

TÖNNIGES (1898) describes minutely the structure of the spherules as they appear in sections of *O. ranarum* stained with iron-haematoxylin. I can confirm his statements that they are disc-shaped,

elongated, or irregular in form and are of various sizes; that they lie usually [in this species] in a regular direction in the whole body, the flat side of the disc [when they are disc-shaped] being parallel to the surface of the body, so that in sections parallel to the flattened surfaces of the body one sees them almost all circular, while in other sections they appear almost all rod-shaped; that they appear homogeneons when strongly stained with [most] aniline dyes; bnt not so with [well extracted] iron-haematoxylin; that they show an alveolar (?) structnre; that one often finds them constricted in the middle like a dumb bell; that they are insoluble in alcohol, alcohol and ether, strong acetic acid, or weak mineral acids; that they are soluble in concentrated mineral acids. I can add that they are but slightly colored by osmic acid; that they are insolnble in cold water; that they are insoluble in tannic acid; that after boiling with hydrochloric acid or after digesting them with diastase, the solntion gives no sugar reaction with FEHLING's solution (not a very delicate test); that they do not stain at all with potassium iodide; and that for the most part they do not stain with iodine dissolved in a water solution of potassium iodide, though occasionally some of them in some part of the body stain a good brown with the iodine solution.[1])

It is of interest that, when treated with this iodine solution, most of the individuals of *O. ranarum* used for experiment did not stain at all; a few showed brown color in some large irregular masses which seemed to be on one side or the other of the boundary between ectosarc and endosarc, probably in the endosarc; many showed a diffuse brown stain in the endosarc in one or more regions of the body, this diffuse stain usually not affecting the spherules, though in a few instances the spherules in these stained areas were themselves tinged with brown. Heat does not change the stain; adding strong sulphuric acid darkens it but slightly and does not give a red or violet tone.

In *O. caudata* there is no color reaction to potassium iodide; the reaction to iodine dissolved in a water solution of potassium iodide is similar to that described in *O. ranarum*, except that I fonnd that the whole endosarc in all individuals stained strongly and a more reddish brown, the ectosare, like that of *O. ranarum* showing only a faint yellow tinge. Addition of strong sulphuric acid darkens

[1]) Glycogen is said to stain reddish brown with potassium iodide or with iodine, the color disappearing upon heating. If sulphuric acid be added to the stained glycogen the color is said to become redder or show a violet tone. (See especially BARFURTH 1885.)

the color of the endosarc stain but does not seem to make it more red or to give it a violet tone. The substance in the endosarc which stains seems for the most part to be in solution, though here and there dense irregular masses are seen which seem as if coagulated. The endosarc spherules also stain, but less strongly than the protoplasm.

Individuals of *Nyctotherus* and *Balantidium* on the same slides with the *O. ranarum* and *O. caudata* show no reaction to potassium iodide. With iodine dissolved in a water solution of potassium iodide they show dark brown bodies in the endoplasma, the endoplasma itself, like the ectoplasma, being merely tinged with yellow.

FISCHER (1905) has described a method of treatment which he says gives a distinctive stain for glycogen. The tissue containing the glycogen is fixed in absolute alcohol, sections are made by the paraffin method, these are brought through graded alcohols into a ten percent solution of tannin (to precipitate the glycogen) and are then placed in potassium bichromate to render this precipitate insoluble in water. Ofter washing the sections they are stained in safranin, only the glycogen bodies becoming red, the cytoplasm and nuclei being hindered from staining by the treatment with tannin. I tried this stain upon sections of *O. dimidiata.* The rectum of a *Rana esculenta* was opened and the contained Opalinas and Balantidiums were divided into two parts, one lot being fixed at once and stained according to FISCHER's directions; the other lot was kept two days in a solution of sodium chloride until the infusoria were mostly inactive, then they were fixed and stained by the same method. In the sections of the first lot of Opalinas, killed before starving, the ectosarc spherules were a bright red. In most individuals the endosarc was wholly unstained: in other individuals the endosarc was strongly stained, great irregular red masses, of what appeared like coagulated material, completely filling it; in still other individuals but little color and few masses of coagulum were seen in the endosarc. The endosarc spherules were usually unstained but in some animals with well stained endosarc and coagulum the endosarc spherules were also stained, but showed a fainter red than the protoplasm.

In the sections of *Opalinae* which were starved forty-eight hours before killing and staining, the ectosarc spherules were stained as strong a red as in the other sections. In almost all individuals the endosarc was wholly unstained; a few individuals, on the other hand, showed red masses of coagulum in the endosarc, the whole endosarc being stained. In all the Opalinas on this second lot of

slides the endosarc spherules were present in their usual abundance but were unstained.

In the *Balantidia* on both lots of slides there were abundant spherules in the endosarc which took the safranin well, each spherule showing a red peripheral layer and an unstained or faintly stained core.

The natural interpretation of all these microchemical tests and digestion experiments seems to be: 1) that the endosarc of *Opalina* contains a nutrient substance abundant in freshly taken *O. caudata* and in many individuals of *O. dimidiata* and in parts of the body of many individuals of *O. ranarum*; in starved individuals its presence is infrequent. This nutrient material seems to be usually in solution in the endoplasma, though even in living animals it may form some irregular semi-solid masses. In some cases the endosarc spherules as well as the endoplasma seem to be permeated by the nutrient fluid, but usually they are not so. 2) The nutrient substance is not true glycogen, but seems to be related to glycogen. The term paraglycogen which Bütschli has suggested seems to be appropriate. 3) The endosarc spherules are not oil for they do not stain with osmic acid or dissolve with alcohol or ether or xylol. 4) The endosarc spherules are not lecithin for they do not dissolve when warmed for four hours with a mixture of equal parts of absolute alcohol and ether. 5) The ectosarc spherules for the same reasons are neither oil nor lecithin. 6) Probably neither sort of spherules contains true glycogen; whether they contain any related substance is uncertain. I have failed to get a sugar reaction with Fehling's solution after diastatic digestion, but this test is not a very sensitive one. 7) The spherules of the endoplasma of *Nyctotherus* and *Balantidium*, seem to be composed of paraglycogen. It is, of course, natural to suppose that those of *Opalina* are of a somewhat similar nature, but they are not exactly similar chemically, as is shown by the difference in their reaction to iodine and to Fischer's glycogen stain.

The whole subject of the nutrient fluids and refractive bodies in the cytoplasm of the *Protozoa* needs more successful study than it has yet received. For valuable papers upon the subject see Certes (1880), Maupas (1885, 1886), Barfurth (1885), Bütschli (1885 *b*, 1880—1889 and 1906 [1])), Stolc (1900) and Bott (1907).

[1]) In some way I overlooked this valuable paper of Bütschli's upon the paramylon bodies of *Euglena*, and I have not yet had opportunity to study the spherules in *Opalina* in the light of Bütschli's work. There is much divergence

Contrary to Tönniges (1898) I do not find the endosarc spherules much, if any, more numerous near the periphery of the body even in *O. ranarum*. Tönniges says that these spherules very frequently divide. According to his description they become first dumbbell-shaped, then still more constricted, the connecting portion becoming a mere thread and then breaking, the two halves separating. He says that, before division, a spherule becomes smaller and more dense, loosing its visible alveolar structure, doubtless by exuding the liquid in the alveoles, and that in this condition they stain more strongly. In my preparations, the dumbbell-shaped spherules are not on the whole smaller than the others, nor do they show less internal organisation (Figs. 11 and 10, last two spherules, Pl. XIV).

Kunstler & Gineste (1905) describe the endosarc spherules of *O. dimidiata* as containing a central granule. They say the spherules divide by constriction, the central granules first dividing. This I cannot confirm.

I am unable to convince myself that the endosarc spherules divide. The dumbbell-shaped forms are not infrequent, but after long search I have not found a single spherule in which the connecting portion is very slender as if ready to part. By for the most constricted one I have seen is shown in Fig. 11. This point is an important one, affecting the question of the nature of these bodies, so I have studied it with care. At the time I wrote my preliminary paper (Metcalf 1907*a*) I assumed that the frequent dumbbell shape indicated division, but I now think that these bodies do not divide any more than do the ectosarc spherules. One never finds two of either sort of spherule in one alveole or any other indication of division in them.

The endosarc spherules are more numerous in the anterior part of the body, where the endosarc itself is denser (Fig. 1).

In *O. obtrigona* certain strands of minutely alveolar protoplasm stretch out from the endosarc and, passing between the large alveoles of the ectosarc, join the subcuticular layer (rather poorly shown in Fig. 6). Along these strands, and in the subcuticular layer near the outer ends of the strands, one finds endosarc spherules. In no other species have I seen the endosarc spherules outside the limits of the endosarc proper.

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even among the *Ciliophora* as to the character of their refractive spherules, and it is probable that the spherules in *Opalina* are more or less different from those in *Euglena*.

The endosarc spherules stain well *intra vitam* with neutral red, methyl violet, dahlia, and gentian violet. This fact makes it doubtful if they are living constituents of the cell.

The spherules of the endosarc are so similar, in all the species I have studied, that no distinctions of size, strncture or reaction to stains can be described. The questions of the origin, nature and functiou of these spherules will be further discussed after the description of the nucleus has been given.

One must agree with Tönniges (1898) that there is no indication that the endosarc spherules are either excretory or parasitic. To his interpretation of them as a diffuse macronucleus we will refer again.

Conte & Vaney (1902) believe that the spherules arise in the nnclens from chromatin and wander out into the cytoplasm through the nnclear membrane. They think that they are similar to zymogen grannles in gland cells and to yolk nuclei. To this we will return again.

Maier (1903) fails to confirm Tönniges' description of internal strncture in the endosarc spherules, saying that they are homogeneous. It must be that in the sections upon which this statement is based the haematoxylin (Haidenhain's) was insufficiently extracted (*cf.* Fig. 4). Sufficient extraction of the stain always shows the internal structure.

Léger & Duboscq (1904*b*) figure certain apparently similar bodies in what seems to be a microgamete of *O. saturnalis* (my Text Fig. XVII, page 338), and their Fig. 3, representing an optical longitudinal section of a full grown form of this species, shows in the endoplasma deeply staining bodies of the right size and form to represent endoplasmic spherules, yet these anthors say that the endoplasma is withont particular inclnsions, though showing here and there small spherical vacuoles with very sharp coutours. This appearance of vacuoles is exactly what is seen after staining with borax-carmine, Mayer's or Delafield's haematoxylin, or any of the unmerous dyes which do not color the endoplasmic spherules.

Kunstler & Gineste (1905) interpret the endosarc spherules as a "secretory apparatus".

Neresheimer (1907) did not see any alveolar structnre or any division stages in the endoplasmic spherules. He snggests that Conte & Vaney's description of the origin of the spherules from the nuclens may indicate that they saw the formation of reproductive chromidia, a process which Neresheimer describes at length. I feel

confident, however, that Conte & Vaney, who worked on *O. intestinalis*, refer to certain very evident chromatin spherules in the nucleus, which will soon be described.

Excretory organs

(Figs. 1, 17, Pl. XIV; 97, Pl. XXI; 248—250, Pl. XXVI).

A system of excretory vacuoles is present in the axis of the body. I have published a description of these organs (Metcalf 1907 *b* and *c*) for the full grown forms of *O. intestinalis*, *O. caudata* and *O. obtrigona* and for the small spring individuals and the macrogametes (not the microgametes) of *O. intestinalis*, *O. caudata* and *O. dimidiata*. Reference must be made to this very primitive excretory organ when we discuss the relationships of *Opalina*, so I include here a few figures showing its character, and summarize the chief points in the published description.

In *O. intestinalis* (Fig. 1) the excretory organ, when highly developed, consists of an axial series of more or less irregular fused vacuoles, opening to the exterior by a transient aperture at the posterior end of the body, and stretching forward usually as far as the posterior nucleus, or often nearly to the anterior end of the body. In its course, as it passes the posterior nucleus, it lies close against the nuclear membrane, usually bending spirally around it. It often has a similar relation to the anterior nucleus. Frequently the series of fused vacuoles branches behind the posterior nucleus, the branch running along another side of the posterior nucleus, which is thus almost enveloped by the excretory organ. I have recently found full grown *O. intestinalis* and *O. caudata* in which the excretory organ stretches as far forward as the anterior nucleus. Heretofore I had seen the elongated excretory organs only in small forms in the spring.

Generally the posterior end of the organ in *O. intestinalis* shows one or more enlargements of considerable size surrounded by unusually large granules in their walls (Fig. 97, Pl. XXI, also cf. Metcalf 1907 b). Usually one sees a mass of such larger granules in the cavity of the posterior chamber. These granules stain somewhat differently from the ordinary endoplasmic granules with most stains. They are from time to time extruded from the excretory aperture at the posterior end of the body and are cast away. One often sees individuals dragging after them a mass of these extruded granules (Figs. 248, Pl. XXVI, 147, 153, Pl. XXII).

In *O. caudata* the conditions are very nearly the same, but the posterior end of the organ is nsually branched, one or two shorter branches being seen in addition to the chief branch which runs forward along the axis of the body. The relation to the nuclei is like that in *O. intestinalis* and the excretory granules are similar.

In *O. dimidiata* (Fig. 17) the conditions resemble those in *O. intestinalis.* In this species there are many nuclei. One often sees that most, if not all, of these nuclei are surrounded by narrow perinuclear vacuoles. Many of the posterior nuclei are enveloped by the excretory orgau. Probably by no means all of the perinuclear vacuoles have any direct connection with the excretory organ, bnt their contained excreta probably reach the excretory vacnoles by dialysis through the intermediate alveoles of the endoplasm. In Fig. 17, Pl. XIV, which represents the posterior end of an unusually slender but nearly full grown *O. dimidiata*, the axial series of excretory vacuoles is very clearly seen to consist merely of enlarged and irregularly fused alveoles of the endoplasm. The most posterior nucleus, in mitosis, is shown entirely enveloped by the excretory vacuoles. One often sees individuals of *O. dimidiata*, especially small forms in the spring, dragging behind them masses of extruded excretory granules.

The three species already mentioned are circular in cross section and have the excretory vacuoles along the axis of the body. *O. obtrigona* and *O. ranarum* are very flat. It is possibly becanse of this flattening that their excretory organs are so much less developed. In *O. ranarum* I have found no trace of any excretory organ, though one often finds perinuclear vacnoles present. In *O. obtrigona* there is present only a slight rudiment of the posterior end of the excretory organ in the form of a small elipsoidal or semilunar vacnole at the extreme posterior tip of the body. This vacuole occasionally contracts and one sees the shrunken, depressed contour where the vacuole previously was. No excretory grannles have been found in this vacuole, nor have I seen the living animals dragging a mass of extruded granules after them, as is so frequent in the three spindle-shaped species above described.

Excretory organs have not been seen before in *Opalina*, the genns being always described as nnique among the *Ciliata* in having no excretory vacnoles.

The condition of the excretory canals in the new Ciliate *Pycnothrix monocystoides*, described by Schubotz (1908) is very interesting in comparison with the cylindrical Opalinae. This simple axial

system of irregular, branching canals is more developed than the excretory organ of Opalina in having 1) a definite limiting membrane, 2) a permanent external aperture, and 3) cilia lining the outer portion of its duct, and also in being evidently a permanent organ of the cell.

In my first paper on the excretory organs of *Opalina* (Metcalf, 1907*b*) I wrote: "Under pressure from a cover-glass, in gradually drying preparations, oil globnles are generally protruded from the body at different points on the periphery. The largest of these oil globules is generally found at the posterior end of the body" [in connection with the excretory pore] "and is usually the first to appear, in spite of the fact that the posterior end of the body is the most slender part and mnst be the last to feel the pressure." Further observation shows that I was mistaken in describing any special connection between the excretory pore and an especially large drop of this exuded liquid. Often a drop is fonnd here and it may be large, but observation of a much larger number of *Opalinae* under pressnre shows that it was an error to emphasize the size and early appearance of this drop. The exuded liquid is not oil, but is either the protoplasm itself, or is derived from the protoplasm. It cannot be derived from the ectosarc spherules, for similar exuded drops are found in *Ciliata* which have no ectosarc spherules (*cf.* also Kölsch 1902).

Nucleus, and mitosis.

Few if any known nuclei among the protozoa are clearer and better for study than those of *O. intestinalis* and *O. caudata;* the nuclei are large and the chromatin is small in amount and does not obscure the achromatic structures; the chromosomes are few in number, eight in *O. intestinalis* and six in *O. caudata;* all the structures usually found in typical nuclei, including a plasmosome nucleolus, are present and stain readily and distinctively; and, as already mentioned, all the structurec in the nucleus are sometimes very clearly seen in the living animal. I have therefore given chief attention to the nnclear phenomena, especially those of mitosis.

Opalina intestinalis has nsually two ovoid nuclei lying in the anterior half of the body, sometimes in the anterior third (Fig. 1 and Pl. XVII, Fig. 38). The ends of the nuclei which are tnrned towards each other are generally more or less pointed. Commonly these pointed ends are connected by a more or less elongated delicate strand consisting of the attenuated nnclear membrane, which was

constricted in the middle at the last division and drawn out to a thread (Pl. XVI, Figs. 34, 35, 37). This thread persists for a long time, disappearing, generally, as the two nnclei are entering upon the next division. Frequently the connecting thread is much bent or even coiled, being far longer than the shortest line between the two nuclei (Figs. 35 and 37), a condition which suggests that the thread elongates by its own growth.

The two nnclei divide at the same time, becoming first spindle-shaped, then dnmbbell-shaped, and finally separating into two daughter nuclei which are still for a long time united by the thread which indicates their common origin. While the division of the two nnclei is occurring, the body divides (Pl. XVII). This is usnally dnring the anaphases, but one often finds the body still bnt partially divided when the nuclei are entering on the telophases. One often sees a danghter cell with only a single nuclens, but this, if normal, is always in an anaphase or early telophase stage of division (Figs. 32, Pl. XVI; 43, Pl. XVII; 54, Pl. XVIII). It is during the early telophase that the nucleus constricts into two (Figs. 32, Pl. XVI; 60—65, Pl. XIX).

The nuclear membrane is very definite and clear, not thick, but very firm and strong. This is indicated by the fact that the connecting strand between the daughter nuclei persists for so long a time. It is seen even more clearly when the living animals are crushed by pressure upon the cover-glass, causing the nnclei to come ont into the surrounding salt solution. Such isolated nuclei, even when connected by very slender threads, one seldom succeeds in causing to break apart by the most violent currents one can produce by pressing npon the cover-glass. Often one of the two united nuclei will be held in place by its connection with the broken body, while the other nucleus projects into the clear liquid. One can then make it jerk about and tug violently npon the thread that holds it, yet withont breaking the thread. I kept one such pair of nuclei for three days, trying several times daily to break the thread by violent currents, bnt even the third day it held as firmly as ever. It is very evident that nnder this severe treatment the thread connecting the nuclei does not stretch. It seems not to be at all elastic.

Conte & Vaney describe the endosarc spherules as arising from the nucleus, from which they emerge through an opening in the membrane. We will return again to this point. It is well here merely to emphasize the remarkable toughness of the nuclear membrane, which could be penetrated only with the greatest difficulty, unless it were weakened (chemically?) at some point.

The nuclear membrane never disappears even during mitosis.

The nuclear membrane shows no structure. Under all conditions, whether living, or after treatment with acetic acid, silver nitrate, or fixing agents without staining, after all sorts of staining in total preparations or in sections, one finds it always appearing homogeneous and uninterrupted. There are no indications that the achromatic strnctures in the nucleus are in any way continuous through the nuclear membrane with the structures of the cytoplasm. Of course in each division the membrane is nltimately broken at the point of constriction, but this break occurs in the slender connecting thread at a distance from the cavities of the daughter nuclei and there are no wounds at the snrface of either nuclens.

The nucleus lies in the cytoplasm, as it were in a great alveolus, being snspended and held in place by the films of the cytoplasmic foam. Fig. 205, Pl. XXIV, gives a clear picture of this condition in the case of a male pronucleus in a zygote of *O. intestinalis.*

The resting nucleus (Pl. XX, Figs. 76 and 77).

The phrase "resting nucleus" of course does not imply that the nucleus is inactive physiologically, but only that it is not engaged in the movements which constitute or accompany mitosis. One can hardly speak with propriety of such a resting stage in the nuclei of *O. intestinalis*, for there seems to be no time throughout the year when changes in their visible structure are not constantly occurring. The divisions of the nuclei and of the body never cease, and every nucleus seen is either in actual division or is preparing for or recovering from division. There is no condition in which the nuclei seem to pause for any prolonged period. The stage which corresponds to the ordinary resting nucleus of metazoan cells is probably that in which the chromatic network is most branched and diffuse. I will begin the description with the stage just preceeding the formation of the mitotic spindle.

Achromatic foam.

The whole space within the nuclear membrane is seen to be filled with alveolar protoplasm, the alveoles in many places being fused to form vacuoles of different sizes (Fig. 77, Pl. XX). In many other nuclei the alveoles are not fused but are of fairly uniform size (Fig. 69, Pl. XIX). Granules of varying sizes and irregular

shape lie at the nodes of the foam. These are highly refractive in the living nucleus (Pl. XVI). Their reaction to stains shows them to be of achromatic, not chromatic, material. The granules not only differ in size in the same nucleus, their average size in different nuclei varies perceptably. They seem largest in nuclei in which the spindle is forming preparatory to mitosis (Fig. 47, Pl. XVIII). The lines uniting these granules (optical sections of the walls of the alveoli) generally show very clearly in well-stained nuclei both in preparations of total objects and in sections. The lines are not discernable in living nuclei, at least with the illumination I have used.

Nucleolus (Figs. 18, 19, 22. 25, 27, 29, Pl. XV; 55, 56, Pl. XVIII; Fig. 73, Pl. XIX).

The always spherical, or nearly spherical, nucleolus belongs to the achromatic group of nuclear structures. It is always present in fully formed nuclei and lies near the axis of the nucleus, never at the surface. It is held in an alveolus of the achromatic foam (Fig. 55, Pl. XVIII), completely filling this alveolus, so that the films of the foam are seen radiating from its surface. Where these strands touch the surface of the nucleolus they are seen to enlarge to form typical nodal granules, triangular in optical section, as are many of the other nodal granules.

The nucleolus stains strongly with plasma stains. One does not find it in total preparations stained with borax-carmine, but in many Delafield haematoxylin preparations it shows very distinctly and is sharply distinguished from the chromatin by its fainter color and browner tone. In other Delafield haematoxylin preparations, which are not so well decolorized, one often cannot distinguish the nucleolus from the chromatin. The most selective and distinctive stain for the nuclear structures is safranin followed by light green (*Lichtgrün*). The chromatic elements take the safranin strongly while the achromatic elements are green. With this stain the nucleolus is a clear bright green and is a very conspicuous object, for it is large (Figs. 22, 25, 27, 29, Pl. XV). Often with light green, and still better with Delafield's haematoxylin, one sees that the nucleolus is not homogeneous, from one to ten or more circular lighter areas being visible within it (Figs. 55, 56, Pl. XVIII; 73, Pl. XIX). Generally the more central light spot appears the larger. These vacuoles (?) are generally of different sizes in the same

nucleolus and their average size may be different in different nucleoli. As a rule the size of the vacuoles is in inverse proportion to their number.

Sections stained with methylen blue often show an interesting condition in many nucleoli (Figs. 18 and 19, Pl. XV). The nucleolus proper is stained a bluish green. This portion is spherical. In one or two regions on its periphery, it bears cap-like structures which are stained a clear blue darker than the pale blue of the nucleolus proper. This is no accidental condition, for it is present in almost all nuclei seen upon these slides which were made from two different lots of Opalinas. Vacuoles are not seen in the nucleoli which show these blue caps, though in other nucleoli upon the same slides vacuoles are found. The history of these peculiar nucleoli has not been followed, so nothing can be said as to the meaning of the conditions found.

The behaviour of the nucleolus in dividing nuclei is interesting. Zeller observed that in dividing nuclei the nucleolus did not divide but remained intact in one of the daughter nuclei, the nucleolus of the other daughter nucleus being a new structure. I can fully confirm this for *O. intestinalis* and *O. caudata*. The nucleolus is less easy to see in the smaller nuclei of the multinucleated species, and as my material of the multinucleated forms shows comparatively few nuclei in division I have not taken the considerable time required to study the nucleoli carefully in them.

To Zeller's interesting observation I would add the further facts: — first, that in *O. intestinalis* the old nucleolus remains in the posterior of the two daughter nuclei (Figs. 70—72, Pl. XIX), and second, that in *O. caudata* this relation is usually reversed, the old nucleolus remaining generally in the anterior daughter nucleus (Fig. 82, Pl. XX). In the many hundreds of nuclei of *O. intestinalis* examined I have found but a single exception to this rule (Fig. 74, Pl. XIX). In this young daughter cell whose nucleus is still in the dumbbell stage of division, the nucleolus was found in the anterior part of the nucleus near the constriction. The narrow tube connecting the daughter nuclei was not too small to allow the nucleolus to pass through it and reach its usual position in the posterior daughter nucleus, but that the nucleolus would have done so does not seem very probable.

In a large majority of cases, in dividing *O. caudata* the old nucleolus remains with the anterior daughter nucleus, yet one occassionally finds these relations reversed.

One of course must inquire as to the meaning of this puzzling difference between the two species. I can suggest no adequate explanation. The nuclei lie much further forward in *O. intestinalis* than in *O. caudata* (compare Fig. 38, Pl. XVII, with Fig. 81, Pl. XX). It suggests itself that the nucleolus in both species may remain in the nucleus which is nearer to the center of the body, or rather nearer to the protoplasmic rather than the geometric center of the body. The geometric and protoplasmic centres are not the same, for the protoplasm in the anterior part of the body is more dense than that further back. This suggestion fits the conditions in *O. intestinalis* and, for the most part, also, the conditions in *O. caudata*. If one takes into account the greater density of the anterior part of the body, it is true that in *O. caudata* in the cases in which the old nucleolus remains in the anterior daughter nucleus it is nearer the center of the protoplasm. I have looked through many preparations, comprising many hundreds of *O. caudata*, to see if in cases in which the old nucleolus remained in the posterior daughter nucleus the nuclei were unusually far forward, so that the protoplasmic center might in these cases be nearer to the posterior than to the anterior nucleus. In the majority of instances of this sort it was found that the nuclei were unusually far forward, almost as much so as in *O. intestinalis*, but, unfortunately for my suggestion, no less than six instances were found in which the old nucleolus remained in the posterior daughter nucleus, although this was placed not only not exceptionally far forward but even unusually far back. I believe, therefore, that there is probably no worth in the suggestion made.

The new nucleolus in the daughter nucleus of *O. intestinalis* arises always at the pointed end of the nucleus near the thread which connects it with its sister nucleus (Fig. 72, Pl. XIX). At first it is very small. It grows rather slowly, but by the time the nucleus is ready for its next division the nucleolus is again of full size.

I have little suggestion to make as to the nature or function of the nucleolus. I would merely emphasize 1) that it is wholly distinct from the chromatin elements and never at any time has any discernable genetic relation to them; 2) that a nucleolus once formed persists throughout the year and until the nucleus containing it is ready to throw off its vegetative chromidia and enter upon sexual reproduction (processes which will be described later). I have not found nucleoli in the nuclei of any forms between the

time of extrusion of the vegetative chromidia and copulation, though I have stained sections of all of these forms with safranin and light green, which gives such clear pictures of the nucleolus. In at least some zygotes which have grown a little since copulation, the nucleoli are seen. Its apparent absence from those nuclei which have recently cast off vegetative chromidia, and are probably but slightly active in nutrition, seems of much interest, though I would not venture to suggest what may be the real meaning of this relation. In the case of large dividing individuals in the summer, fall and winter, the new nucleolus appears in the anterior daughter nucleus at the time when this nucleus is forming the chromatin spherules, which seem to be essentially vegetative chromidia. These phenomena will soon be described. It seems probable that the nucleolus has some connection, not necessarily causal, with the nutritive activities of the nucleus. 3) In the third place I would emphasize that the new nucleolus, when it arises, seems to come from some substance in liquid form in the nucleus, and not from the immediate transformation of any previously visible structures. 4) The old nucleolus does not grow beyond a certain size, though it may persist for nearly a year. The new nucleolus, after each division, grows to the same size as the old and then stops its growth. This suggests that there is some balance between the nucleolus and the other structures of the nucleus (or cytoplasm?) which requires the presence in an ordinary fully-formed nucleus of a nucleolus of a given size. The diminution of the vegetative chromatin, preceeding and during the period of conjugation, seems to do away with the necessity of a nucleolus during that time, or to remove something which if present would have caused a nucleolus to form.

The various stages in the growth of the new nucleolus are of great assistance in determining the sequence of phenomena in the telophases of mitosis. Until this criterion was found it was almost impossible to be certain of the relative order in mitosis of several of the stages observed.

This description of the condition and behavior of the nucleolus in *O. intestinalis* is based upon series of preparations of animals from many different hosts. I have since studied a series of preparations of apparently normal Opalinas from an apparently normal *Bombinator*, in which the nucleolar relations are quite different. These Opalinas were killed at once upon opening the rectum of the host so that the divergent condition of the nucleolus is not due to degenerative

changes caused by living in cultures. In all of these animals, when binucleated, one nucleus contains a nucleolus and the other does not. Generally the nucleolus is in the posterior nucleus, but in 8 % of the binucleated forms the nucleolus is in the anterior nucleus. All of the forms found with the nucleolus in the anterior nucleus were anterior daughter cells formed by transverse division indicating probably that about 16 % of the divisions are transverse.

The conditions in these Opalinas show that the old nucleolus does not persist. It diminishes and disappears just before, or during, or some times just after, the spireme stage, which in these animals is less marked than usual. It reappears generally at about the time the spindle is forming for the next division, or before. Occasionally it does not appear until the spindle is formed. We find therefore some animals with no nucleolus in either nucleus (spireme stage), some (8 %) with a nucleolus in the anterior nucleus only, and the rest with a nucleolus in the posterior nucleus only.

The discrepancy between the different series of preparations as to the condition of the nucleoli necessitates more careful study of this subject. My notes upon the several sets of preparations do not say whether the hosts had been starved for a time or not. Until this relation has been carefully, observed one cannot be certain which of the nucleolar phenomena are normal and which abnormal. Possibly all are normal, varying with the conditions of nutrition. It is but a surmise that the conditions of nutrition explain the divergent conditions of the nucleoli, but it seems the most probable explanation *Opalina* must be very sensitive to surrounding conditions, and it is possible that in this genus we have an opportunity to study, with unusual hope of some success, the problem of the function of the plasmosome nucleolus.

Zeller saw and clearly described and figured nucleoli in all stages from the cysts to the full grown forms, in all five of the species he studied. He even figures a central dot in the nucleolus, which he calls a central vacuole. It is barely possible that in the cysts and in certain small forms he has mistaken certain chromatin masses for the true nucleoli, but there is no doubt that, at least in the case of full grown forms, he has described the true plasmosome nucleolus. This is easily demonstrated with acetic acid, which is the reagent he chiefly used.

Since Zeller, no student of the *Opalinae* has observed the true nucleolus. Pfitzner (1886), Tönniges (1899) and Léger & Duboscq (1904*b*) refer to certain chromatin masses as nucleoli, but make no

reference to the plasmosome nucleolus. Neresheimer (1907) says he has not seen a true nucleolus, not accepting the name nucleolus as applicable to the masses of chromatin described by Pfitzner, Tönniges, and Léger & Duboscq. Neresheimer is surely right in not applying the name nucleolus to the chromatin masses so characteristic of the nuclei of *Opalina*. They seem so entirely unrelated to the true plasmosome nucleoli that no one term should be applied to both. One must admit, however, that in some other animals it is not easy to distinguish clearly between plasmosome nucleoli and bodies related to chromatin, so that in general the word nucleolus must be allowed to have the broader meaning.

Chromatin.

The chromatic material of the nucleus in *O. intestinalis*, and all other species studied, lies near the surface of the nucleus just beneath the membrane (Fig. 59, Pl. XVIII).[1]) This seems to be true of all conditions of the nucleus except just before, during, and after encystment, when, in the multinucleated species, the chromatin contracts toward the centre of the nucleus. One sees in the resting nucleus that there are irregular masses of chromatin, of larger and smaller sizes, scattered here and there beneath the nuclear membrane (Figs. 23, 27, Pl. XV; 45—48, Pl. XVIII; 76, 77, Pl. XX). These chromatin masses are drawn out into numerous points each of which connects with a fiber of chromatin which runs over the surface of the nucleus beneath the membrane. These fibres branch and the branches anastomose with one another and with the branches of similar fibres from other chromatin masses (Figs. 27, Pl. XV; 46, Pl. XVIII). In other words, there is just beneath the nuclear membrane a network of chromatin fibrils, the chromatin masses described lying upon and being in connection with the network. One could say that the fibres seem like delicate reticulate pseudopodia from the chromatin masses.

The fibres of chromatin are sometimes quite even (Fig. 46, Pl. XVIII); again one finds them considerably enlarged at the nodes (Fig. 27, Pl. XV); in other nuclei one sees them as rows of different sized granules strung on a thread (*cf.* Fig. 50, Pl. XVIII, a nucleus in mitosis). These differences cannot be wholly due to differences in staining, but represent real divergent conditions of the chromatin threads during the so-called resting stage. The chromatic structures of the nucleus never seem

[1]) *Cf.* Tönniges (1899), Boveri (1900).

to be arranged in the form of an alveolated foam, but are in the form of masses and fibrils. It is difficult with most dyes to distinguish the chromatic fibrils from the achromatic, but double staining with safranin and light green gives a very clear demonstration of the distinctness of the two sorts of fibrils, the chromatin being red, the linin green. Care must, however, be taken not to extract the light green too much. The slides must be left in absolute alcohol but a moment, else the light green may be extracted from the nucleus and the whole endoplasma as well, remaining only in the ectoplasma.

The chromatin masses are not homogeneous, but contain many granules which after staining with safranin (Fig. 31, Pl. XV) iron-haematoxylin (*O. caudata*, Figs. 83, 84, 86, Pl. XX), or DELAFIELD's haematoxylin (Fig. 69, Pl. XIX), decolorize more slowly than the rest of the chromatin mass. The character of these granules can best be discussed in connection with the later stages of mitosis.

In the preliminary notice of this work (METCALF 1907*a*) I wrote "The chromatin net in the 'resting' nucleus consists of large and small chromatin masses and their branching anastomosing pseudopodia-like processes. In certain conditions of the nucleus no such processes are found". After further study, it seems that the last statement is mistaken and that more or less of a network of chromatin is always present, though in some conditions of the nucleus it may be very delicate and difficult to distinguish from the achromatic foam even with differential stains.

Prophases of mitosis.

One sees from the study of total preparations and of sections that, as the nucleus prepares for mitosis, the longitudinal fibres of the chromatin net become emphasized and the transverse fibrils become fainter (Figs. 45—52, 57, Pl. XVIII). One imagines that the latter are drawn in and that their substance is added to the longitudinal fibres. At this time the nodes that lie along the longitudinal fibres are especially emphasized (Fig. 50). While the chromatic spindle is thus forming, the longitudinal films of the achromatic foam thicken and the transverse films become fainter (left side of Fig. 47, Pl. XVIII), the whole nucleus at the same time becoming elongated.

The spindle is never regular and is hardly well enough formed to be comparable to the spindle in the mitosis of metazoan cells. Figs. 49 to 52, Plate XVIII, show it in its fullest development. The thicker fibres in the whole nucleus are seen to have an irregularly

longitudinal direction, yet they are always connected by transverse fibrils (Fig. 50). The general form of the group of fibres is spindle-shaped, it being thickest at the equator of the nucleus, where it bulges ont almost or qnite to the nuclear membrane. As the chief chromatic fibres converge toward the two poles of the nucleus they frequently, one can say usually, bend inward toward the axis of the nucleus, presenting a peculiar and very characteristic appearance of a spindle with acuminate ends.

Centrosomes.

There are no centrosomes visible either inside or outside the nucleus.[1]) The thick chromatic fibres extend to and are in contact with the nuclear membrane at the poles of the nucleus (Figs. 51, 55. Pl. XVIII). Usually these fibres are somewhat swollen at or near their ends, forming granules of qnite noticeable size (Figs. 51, 57, Pl. XVIII). There are no special aggregations of achromatic material. either granular or fibrous, at the poles of the nuclens. None of the structures found can be interpreted as a centrosome.

Equatorial plate stage.

These is no well defined equatorial plate stage in the mitosis. The chromatin masses make an irregular group scattered through the whole equatorial third of the nncleus (Figs. 45—48, Pl. XVIII). There is at this time no indication of any longitndinal splitting of the chromosome masses. If it occurs at all, it has occurred previous to this stage.

Anaphases.

This very irregular and imperfect eqnatorial plate stage soon passes into an early anaphase condition in which one sees the chromatin masses arranged in two transverse rows (Figs. 49—52, Pl. XVIII). These masses are still united one to another by the longitudinal fibrils and one often finds this connection so definite as to suggest that the masses so united in pairs are products of a transverse division of the chromatin masses of an earlier stage. Probably some have recently divided, but others divide at a considerably earlier stage, before the spindle is formed. Some of the

[1]) *Cf.* Pfitzner (1886), Tönniges (1899), Léger & Duboscq (1904*b*) and Nereshemer (1907).

masses of chromatin may be slower than the rest in coming to the center of the nucleus and in taking their place in this double transverse row, but in the end all do so.

Each chromatin mass is connected with the pole of the nucleus by one, or sometimes by two, of the thicker chromatin fibres (Fig. 50). The chromatin masses of the two groups are also connected with one another by thick fibres which cross the equator of the nucleus. Occasionally one sees a fibre start from a chromatin mass, cross the equator of the nucleus and pass on directly to the opposite pole without connecting on the way with a second chromatin mass (Fig. 50). This, however, is not very general. One may say that the chromatic spindle is composed of fibres which in general stretch from pole to pole of the nucleus and connect in their course with one or two chromatin masses. The fibres may branch and unite in a more or less irregular way.

The chromatin masses now begin to migrate toward the poles of the nucleus (Figs. 53—55, 58, Pl. XVIII). During these migration stages, one often sees that the chromatin fibres connecting the chromatin masses with the poles are thicker, while the fibres stretching across the equator are fainter (Fig. 54, Pl. XVIII; *O. caudata,* Fig. 81, Pl. XX). During this migration the chromosome masses assume more definite shape, becoming in general more elongated (Fig. 58). They lie side by side and because of their regular arrangement and compact form are best studied at this stage. They seem to be true chromosomes. They keep their regular parallel position during the whole migration, but, as thy approach the pole, first some and then others may divide into two (Fig. 54), and at about the same time they send out thin broad plates of chromatin which unite them together (*cf.* the upper end of the nucleus in Fig. 58). The stage, then, when the chromosomes can be counted and studied to best advantage, is but a brief one during the middle portion of the migration. As some chromosomes may be late in coming into the double transverse equatorial plate, and as others may divide precociously during the migration, one finds, in certain nuclei, conditions that are very confusing. I have, however, carefully counted the chromosomes in more than a hundred favorable nuclei in the middle anophase condition and find their number to be eight for *O. intestinalis* (Figs. 32, 33, 36, Pl. XVI; 58, Pl. XVIII; 65, Pl. XIX; 119, Pl. XXII, anterior nuclei of 201—203, Pl. XXIV). The apparent exceptions are due, I believe, wholly to precocious division of the chromosomes, or to their precocious fusion by means of the band-

shaped pseudopodia. In some dumbbell-shaped nuclei one sees, in one end, the chromosomes already thus fragmented or fused, while in the other end of the same nucleus the eight chromosomes are compact and distinct (Fig. 34, Pl. XVI; 63, Pl. XIX).

The chromosomes, even in their most compact condition, give off numerous threads which connect with the general chromatic network. Unless the staining is satisfactory the threads themselves are sometimes difficult to see, but one rarely fails to notice upon the surface of the chromosomes the pointed protrusions with which the threads connect.

The chromosomes differ in size and form and in the number of granules they contain. After much study I must confess that I am not sure whether these differences are constant. The granules are so small and, especially in this stage, so difficult to see, that the margin of error in the count in a single chromosome is greater than the difference betwen the numbers in different chromosomes. In the first attempts to count in different nuclei the granules in the chromosome which is the first to divide during the telophase, the numbers so nearly agreed as to give hope that evidence from this source would prove valuable, but further study has rendered the whole matter so doubtful that it is best to say nothing further of it. Similarly, after prolonged study of the form and size of the chromosomes, I feel that it would be unsafe to express an opinion as to the constancy of these characters. It is true that there is usually a remarkable degree of resemblance between the chromosomes of the two ends of the same nucleus in their size and form and in the time and manner of their division or fusion in the early telophase. It is also true that one finds nuclei in different animals whose chromosomes show equally remarkable resemblance in these regards. Yet the whole series of phenomena is so often confused by the early appearance of division or fusion in the telophase and the precocious appearance of the characteristic differences between the anterior and posterior nuclei, that is seems unsafe to conclude from the resemblance referred to that the chromosomes have constant and characteristic differences from one another. I incline to that belief, but cannot quite convince myself. It would be easy to give rather convincing drawings, if only the most favorable nuclei were selected, but the study of hundreds of nuclei shows the conditions to be too various for satisfactory solution of the question.

Telophases.

When the chromosomes have passed almost to the poles of the nucleus, they stop their migration and enter upon the changes of the telophase. These changes affect both the chromosomes themselves and the fibrillar portion of the chromatin, as well as the achromatic films. The chromatin fibres in the equatorial area are already, or soon become, more slender and more branched. The fibres nniting the chromosomes to the poles of the nucleus somewhat more tardily undergo the same change. The longitudinal films of the achromatic foam similarly become less emphasized until one sees no distinction between the longitudinal and transverse films. The nucleus has now wholly lost its longitudinally striated appearance.

Two sets of changes occur in the chromosomes, they constrict transversely, and they fuse. The transverse constriction of the chromosomes is very freqnently seen dnring the telophases (Figs. 53, 54, 58, Pl. XVIII). They do not all constrict transversely at this time for one never finds a nucleus in the condition which would thns resnlt. In many cases no transverse constriction occurs until later. In one of the chromosomes, the first to so constrict, the division is very unequal, the larger moiety lying toward the equator and the smaller toward the pole (Figs. 53,[1]) 54, Pl. XVIII). The two parts are generally clearly seen to be united by a distinct thread resembling one of the thick fibres of the chromatin spindle. Another of the chromosomes divides more nearly equally, the slightly smaller moiety being toward the pole of the nuclens. As already, said this transverse constriction of the chromosomes does not always occur before their fusion. One cannot say which is the proper and which the divergent time relation between these two sets of phenomena, transverse constriction and fusion.

The chromosomes nnite by sending out thin plates of chromatin which pass from one chromosome to the next (Figs. 58, Pl. XVIII; 60—63, Pl. XIX). At first perhaps bnt a single pair will nnite (Fig. 58), then others will become connected. There may thns arise a very irregular complete ring of chromatin just beneath the nuclear membrane[2]) (*cf. O. caudata*, Fig. 82, Pl. XX). This fusion may begin

[1]) This figure shows the only exception I have found to the rule that the smaller moiety of the chromosome first to constrict lies toward the pole. In the posterior end of the anterior nucleus the smaller moiety of the divided chromosome is nearer the equator.

[2]) Léger & Duboscq's Fig. 19 is interesting in this connection (*cf.* Text Fig. IV H, page 249).

during the early telophases, before the elongated nucleus has become dumbbell-shaped (Fig. 58), or may not have occurred by the time the daughter nuclei are quite distinct (Fig. 65, Pl. XIX).

Spireme.

Ultimatly a condition is reached in which the chromosomes are completely united to form a long ribbon coiled irregularly over the surface of the nucleus, beneath the nuclear membrane (Fig. 34, lower half, Pl. XVI; 67, Pl. XIX). Along the course of this ribbon, from very many points, threads run out to join the chromatin network. In the preliminary notice of this paper (Metcalf, 1907 *a*). I referred to the chromatin ribbon as a more or less compact mass. The more compact condition is found in animals which have been kept for a time in cultures, and is probably slightly abnormal.

Resting nucleus.

Later the ribbon breaks up into band-shaped portions of unequal size, varying in number from six to sixteen (Figs. 68, 70, Pl. XIX; 76—79, Pl. XX). Either at first, or at some time before the next division of the nucleus, the chromatin masses become sixteen in number (Figs. 35, 37, Pl. XVI), for we find sixteen of them, arranged in two rows of eight each during the next anaphase, as described (Fig. 36, Pl. XVI). Often, in nuclei in which the spindle is beginning to form, sixteen chromatin masses (chromosomes) can be counted (Fig. 35). In other similar nuclei fewer chromatin masses are found, the division of some of them evidently being retarded (Figs. 46—48, Pl. XVIII).

The constriction of the nucleus into two daughter nuclei connected by a thread occurs before the complete fusion of the chromosomes into a ribbon. By the time this ribbon has broken into its smaller portions the two nuclei are far separated in the cell, being connected only by a very slender thread consisting of the attenuated nuclear membrane.

After the separation of the chromatin ribbon into its several portions, the spindle for the next division begins to form as already described.

The nuclear membrane has remained intact during this whole mitotic cycle.

Division of the body.

The division of the body begins while the two parent nuclei are in a late anaphase of mitosis (Fig. 38, Pl. XVII), and the separation of the daughter cells, in normal vigorous animals, is complete during the latest anaphase (Fig. 43), or less often during the early telophase, when the daughter nucleus is dumbell-shaped (Fig. 42). In division one daughter cell receives the anterior nucleus of the parent, the other daughter cell receives the posterior nucleus, both nuclei soon completing their division and becoming double. The division of the body, after it begins, occupies, in the fall and winter, about one day. As this begins and ends generally during the anaphases, it is evident that the whole mitotic cycle must occupy many days. Less vigorous animals, weakened by being kept too long in unnatural conditions outside the host, may take two or three days for division, or may even fail to complete the division.

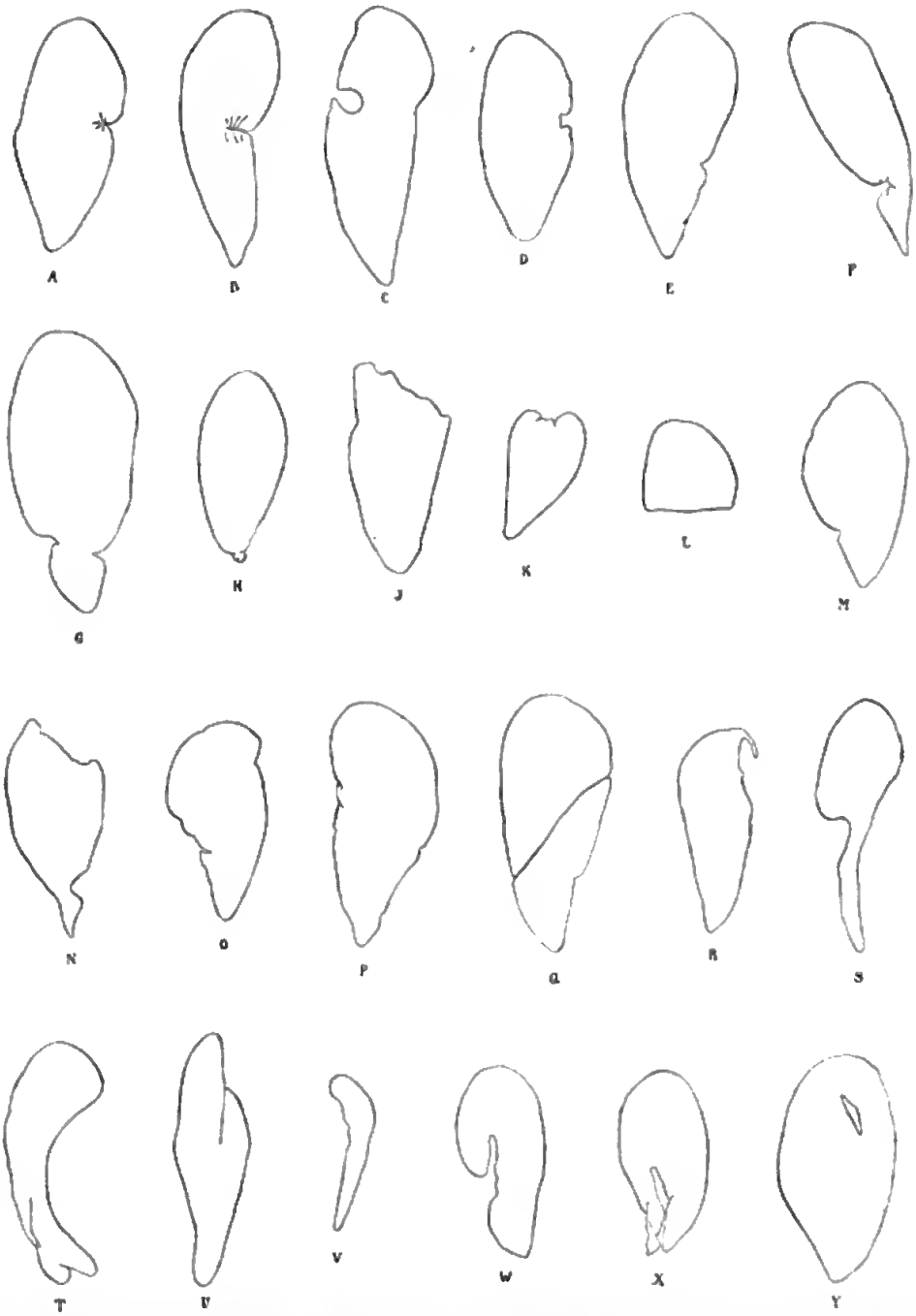
Occasionally one sees individuals fresh from division, one side of whose body is drawn out into irregular strands (Fig. 43, Pl. XVII). This appearance is explained when one observes the last stages of the division itself and finds the two daughter animals united by such strands that have been drawn out by the efforts of the animals to pull away from one another. ZELLER described these conditions.

Division of the body in *O. indestinalis* is usually longitudinal. In one series of preparation of individuals which were probably slightly abnormal, only one of the two nuclei in each individual having a nucleolus, I found that the conditions of the nucleolus gave a criterion enabling one to estimate the relative frequency of transverse division. In individuals resulting from transverse division, the posterior daughter cell, when its nucleus completed its division, showed the nucleolus in the posterior of its two nuclei; the anterior daughter cell, in a corresponding stage, showed the nucleolus in the anterior of its two nuclei. Only young anterior and posterior daughter cells can with certainty be distinguished by their form and general appearance. In these preparations of abnormal individuals the nucleolar relations were, without exception, as described, in the case of the young daughter cells, and doubtless held good for the older cells. In the case of longitudinal division of the body each daughter cell, when its nucleus divides, shows the nucleolus in the posterior nucleus. Eight per-cent of the individuals on these slides show the nucleolus in the anterior nucleus. We can therefore estimate that sixteen per-cent of the divisions were transverse. Probably normal

individuals would show a similar proportion of transverse divisions. Figs. 44, Pl. XVII, and 20, Pl. XV show individuals in transverse division. Zeller describes and figures transverse divisions for *O. intestinalis*, but does not speak definitely as to their relative frequency, though implying that they are numerous.

In *O. caudata* transverse division is of the same character and about as frequent as in *O. intestinalis*. The longitudinal divisions are exactly similar in the two species. In *O. dimidiata* longitudinal division resembles that of the binucleated species, except that in this multinucleated form there is no apparent connection between nuclear division and the division of the body. I have not observed transverse division in this species, but Zeller's observations show clearly that it occurs. I have once seen longitudinal division of the body in *O. zelleri*: it resembled that of *O. dimidiata*. In *O. obtrigona* and *O. ranarum* one finds longitudinal, transverse and irregular divisions, the latter in the spring when division is very rapid (Text Fig. III). In all species the longitudinal divisions follow the main axis of the body. As this is bent, [1]) the really longitudinal divisions, especially in the flattened species, appear to be oblique, as Zeller has described them. Cohn (1904) and Schouteden (1905) have shown that the so-called oblique division of *O. ranarum* is morphologically longitudinal. I have studied *O. ranarum* but little, but from observation of *O. obtrigona* I doubt if the longitudinal and transverse divisions are so definite in their sequence as Zeller describes them. Reference to the figures of irregular division in *O. obtrigona* (Text Fig. III) shows that in the spring one may find almost any sort of irregularily, even two or three entirely irregular

[1]) *Ciliata* and *Flagellata* have either the body form asymmetrical, or the organs of locomotion asymmetrically arranged, or both, so that the animals rotate on their main axes as they swim, producing spiral progression, *Opalina* is no exception to this rule. The spiral motion in *Opalina* is caused by two factors, first by the bend in the anterior end of the body, second by the spiral arrangement of the rows of cilia. The latter is not a result of the former, for, if one should straighten out the bend in the body of an *Opalina*, the rows of cilia would still be spiral. Dale (1901), Wallenoren (1903), quoted by Jennings (1906), emphasize also the direction of the beat of the individual cilia in *O. ranarum* in producing spiral progression, rows of cilia along the right side of the anterior end being said to beat forward and to the left, while the others beat backward (Text Fig. XVI, page 335). It seems to me these authors have failed to emphasize that the broad anterior end of the body in this species is bent "to the right" as is so evident in other more slender species, so that the morphological anterior end is not the actual anterior end. The cilia in all species seem to beat nearly if not quite in the morphologically posterior direction.



Text Fig. III. Outline drawings of *O. obtrigona* showing the irregular divisions in the spring, previous to the formation of the infection cysts. In each figure the anterior end is uppermost. Transverse division *A—H* and *M—P*. Oblique division *Q*. Longitudinal division beginning as a perforation of the central part of the body *Y*. Longitudinal division beginning at the posterior end of the body *X*. Three divisions occurring at the same time *O*, *Q*.

divisions occurring at once (*Q*). Sometimes the division furrow begins in the middle of the body, instead of at the edge, and spreads to the edge (*Y*). TÖNNIGES (1899) has described exactly parallel phenomena in the irregular divisions of *O. ranarum* in the spring.

Were the divisions of the body always as regular as ZELLER describes, one would be tempted to compare them with the regular divisions of an egg, in which each division plane has a definite and predictable direction. Vegetative division in most *Ciliata* is transverse; in the *Flagellata* it is longitudinal; in *Opalina* it is generally longitudinal, sometimes transverse, and, in the multinucleated flattened species, sometimes irregularly oblique.

Time of appearance of the new nucleolus.

We have already seen that the nucleolus in the parent nucleus does not divide, but remains in one of the daughter nuclei, the other daughter nucleus acquiring a new nucleolus. This new nucleolus appears near the pointed end of the nucleus, where it narrows to the thread which connects it with its sister nucleus (Fig. 72, Pl. XIX). The new nucleolus does not appear in the daughter nucleus until the chromatin ribbon is ready to break up, or has already broken up, into separate chromatin masses. It increases and is of full size by the time the new spindle for the next division begins to form.

Differences between the two nuclei.

Very often the two nuclei are not in exactly the same stage of mitosis. Frequently one half of the dividing nucleus will have its chromosomes all distinct, while the other half shows them beginning to unite by means of band-shaped pseudopodia (Fig. 58, Pl. XVIII; 34, Pl. XVI); or one nucleus may show a complete chromatin ribbon while the other has its chromatin ribbon already broken into a number of pieces (*cf.* Fig. 37, Pl. XVI, in which one nucleus shows fourteen (?) chromatin masses, while the chromatin ribbon of the other nucleus has not yet completely divided). In very many cases in nuclei which are forming and casting off the chromatin spherules, soon to be described, one sees these spherules larger in one nucleus than in the other, or already separated from the chromatin masses in one nucleus but not in the other. In abnormal nuclei of animals kept too long outside the host, there are often differences between the two nuclei (Fig. 95, Pl. XXI). Gener-

ally, but by no means always, it is the anterior nucleus, in *O. intestinalis*, which is in the more advanced condition of the two, if they be different, although in this nucleus the restoration of fully typical structure after mitosis is somewhat delayed because of the late appearance of the new nucleolus. One would naturally expect the chromatin of this nucleus to be in the less advanced condition of the two, if there is to be a difference.

Chromatin spherules.

In this description of mitosis no reference has been made to one of the most interesting series of phenomena, i. e. the diminution of the chromatin by the throwing off and dissolving of a large proportion of the material of the chromatin masses into which the chromatin ribbon constricts as described.

Mention has been made of the granules present in the chromosomes at all times. These are small, usually no larger than the achromatic granules of the nucleus. In addition to these, in *O. intestinalis*, many larger spherical granules appear during the late spireme stage, or as soon as the chromatin ribbon is broken into separate masses (Figs. 71—73, Pl. XIX; 21, 22, 28—31, Pl. XV). These spherules[1]) are formed at the surface of the chromatin masses and protrude beyond their contour. They are of different sizes, not only in the same nucleus, but upon the same chromatin mass. They are compact and seem homogeneous with all stains used. Before the spindle for the next mitosis forms, the chromatin spherules break away from the now divided chromatin ribbon and come to lie free in the nucleus (Fig. 72, Pl. XIX). At first they stain very strongly, but, by the time the spindle for the next mitosis is formed, they stain much more faintly (Fig. 75, Pl. XX, also Figs. 23, Pl. XV; 65, Pl. XIX; 79, Pl. XX). By the time the anaphase stage is reached they usually can no longer be recognised, though occasionally they can be faintly discerned even in the early telophases.[2])

The formation of chromatin spherules was not seen in the gametes or zygotes or in the gamete mother-cells. I did not

[1]) In the preliminary notice of this paper I called these structures sometimes spherules and sometimes spheres. It seems best to call them chromatin spherules and to reserve the term chromatin spheres for certain much larger bodies formed in and extruded from the nuclei in the spring before the sexual phenomena occur.

[2]) Fig. 94, Pl. XXI, shows a pair of abnormal nuclei in the anterior of which are granular bodies which may be dissolving chromatin spherules.

observe the chromatin spherules in the early part of my stndy of *Opalina* in the fall and early winter.[1]) They were abundant in the late winter and early spring. I have since fonnd them in material preserved in the fall. As I have not yet studied material from tadpoles preserved in the summer, I cannot say for how long a period their formation is interrupted, though it seems probable that they are absent from the nuclei which have cast off their vegetative chromatin (a process to be described later) and in which the ordinary proportions of vegetative and reproductive chromatin, characteristic of the vegetative phase of the life cycle, have not been restored by subseqnent growth.

Their fate is a little donbtful. They seem to go into solution. It is apparently these chromatin spherules which Conte & Vaney (1902) have described as passing through the nuclear membrane into the cell-body and there giving rise by division to the refractive sphernles in the cytoplasm. It seems as if they do occasionally pass undissolved through the ends of the nucleus at the place where the nnclear membrane broke in a former division. In several dozen instances I have found the end of a nucleus drawn out into an irregular protrusion and one (rarely more) body resembling a chromatin spherule lying in this protrnsion, apparently ready to pass out into the cell-body (Figs. 78—80, Pl. XX). In some instances the membrane bounding the protrusion is noticeably more delicate than that of the rest of the nucleus. The chromatin spherules in these protrusions generally stain but faintly, having already somewhat changed their character. On the other hand there were several times seen, in these protrusions from the nncleus, spherules which with iron-haematoxylin were deeply stained. Fig. 5, Pl. XIV, shows one such nucleus. The slide was well decolorized and the endoplasmic spherules are quite light colored, only their granules being dark. Even the chromosomes are lighter than usnal, but the chromatin spherule in the nuclear protrusion, and two other spherules at the base of the protrusion, are heavily stained. Apparently they stain almost as strongly as if newly formed, thongh this nucleus is in a late anaphase. Their persistence in this condition to so late a stage in very exceptional.

There are from twenty to one hundred or more, of the chromatin spherules in oue nucleus (*cf.* Pl. XV: Figs. 21 aud 22 show one nucleus, Figs. 28—31 another). If they all passed uudissolved throngh

[1]) Because my attention was not then directed to them.

the nuclear membrane, one would surely see more frequent evidence of their doing so. It seems certain that only a very small proportion, if any, pass as solid bodies out of the nucleus. In the great majority of nuclei apparently none do so.

It seems, however, not improbable that the chromatin spherules of the nucleus and the endoplasmic spherules may be somewhat related. Their staining reactions suggest this. Stained with DELAFIELD's haematoxylin the newly formed chromatin spherules are very dark blue; the older chromatin spherules stain less and less, and finally are not stained at all. The endosarc spherules are entirely unstained with this reagent. Similarly with safranin and light green the chromatin spherules, if newly formed, stain deep red; older chromatin spherules stain more faintly, and in nuclei in which the new spindle has appeared they are either very faint or have already disappeared. With the same dye the endoplasmic spherules are colored a very faint pink, resembling the almost dissolved chromatin spherules within the nucleus. As the chromatin spherules loose their staining capacity one sees that many are growing smaller; some, on the other hand, in the same nuclei often seem to be enlarged and more diffuse. Not infrequently one finds faintly staining irregular masses which look like dissolved chromatin spherules filling several alveoli of the nuclear foam, and I believe this is the proper interpretation (*cf.* Fig. 58, Pl. XVIII, in which near the centre of the nucleus are such faintly stained areas). Is seems well-nigh certain that the chromatin spherules dissolve, and it is probable that they pass in liquid form through the nuclear membrane into the cytoplasm. It is not improbable that, having reached the cytoplasm, this material reforms in the endoplasmic spherules. Very likely, however, material from the cytoplasm as well is used in the formation of the cytoplasmic spherules.

The origin of the endoplasmic spherules from the chromatin spherules is by no means assured. The granules that with proper staining are always seen in the endoplasmic spherules, and especially the lines occasionally seen within them, connecting their granules, suggest that they are formed elements of considerable complexity. If they really divided, as TÖNNIGES (1898) and KUNSTLER & GINESTE (1905) describe, their interpretation as living constituents of the cell would seem unavoidable. I do not, however, find evidence of their division, the constricted portions of the frequent dumbbell-shaped spherules never being very slender as if about to part.

If the two sorts of spherules be related as suggested, probably the material from one chromatin spherule is enough to form or aid

in the formation of many endoplasmic spherules; otherwise it would be difficult to explain the great number of the latter present in the body at all times. The whole body divides during each mitosis, so that the number of endoplasmic spherules is reduced to half. The chromatin spherules are formed during each mitotic cycle, but there are rarely, if ever, more than one hundred and twenty in one nucleus, while there are many hundreds of the endoplasmic spherules in an ordinary sized *Opalina intestinalis*. Kunstler & Gineste estimate eight thousand for an ordinary sized *O. dimidiata*, a much larger species than *O. intestinalis* and having many more endoplasmic spherules. If the endoplasmic spherules are in any way derived from the chromatin spherules, and if they do not increase by division, there seems no escape from the conclusion that the material of one chromatin spherule suffices for many endoplasmic spherules. The endoplasmic spherules are larger than the chromatin spherules. New chromatin spherules do not continue to form in the nucleus while the earlier formed ones are dissolving, for one does not find them showing all varieties of staining in the same nucleus. They stain all about alike. The period during which the chromatin spherules form is indeed a rather brief one, extending from the end of the spireme (chromatin ribbon) stage to the beginning of the formation of the spindle.

It is, of course, possible that the chromatin spherules are but by products of the metabolism of the chromosomes and that they have little significance. Yet their staining reactions, with all stains used, seem to indicate that they are composed of chromatin, and the gradations in their staining, as they dissolve, seem to connect them with the refractive spherules of the endosarc.

In *O. caudata* the condition of the chromatin spherules and endoplasmic spherules is like that in *O. intestinalis*. The multinucleated species, *O. dimidiata*, *O. zelleri*, *O. ranarum* and *O. obtrigona*, have endoplasmic spherules of the same character. Their nuclei are so small that it is not easy to study in them the formation of the chromatin spherules, and I have not attempted it.

Origin of the ectosarc spherules.

I have seen nothing to indicate any genetic connection between the spherules of the endosarc and those of the ectosarc, except that with iron-haematoxylin, when very thoroughly extracted, one sometimes finds, in the ectosarc, spherules showing an internally granular

and fibrous appearance exactly similar to that seen in the endosarc spherules. I am somewhat puzzled by this observation. Under all other conditions the two sorts of spherules seem very distinct. To most stains they react in an utterly different way (cf. the table in the appendix). It is difficult to belive that the bodies in the ectosarc, which with iron-haematoxylin show this structure, are really the ordinary ectosarc spherules. They are not endosarc spherules which have been misplaced by the microtome knife, and they cannot be endosarc spherules which have wandered unmodified into the ectosarc, for in sections stained with differential stains one never sees endosarc spherules in the ectosarc. These conditions sometimes seen in sections stained with iron-haematoxylin are not enough to indicate that the ectosarc spherules arise from the endosarc spherules. They are far more probably formed in situ.

Splitting of the chromosomes.

Pfitzner (1886) is the only student who has described splitting of the chromosomes in *Opalina*, and all more recent workers agree that he was mistaken in his description. The chromosomes do not form into a definite equatorial plate and then split, as he described for *O. ranarum*. I find no convincing evidence of splitting of the chromosomes at any stage of the mitosis, but in sections of *O. intestinalis* and *O. caudata*, stained with iron-haematoxylin, one often sees, in the early telophases, a condition that suggests that the chromosomes may possibly be splitting (Fig. 86, Pl. XX). The chromosomes, when seen in side view, have a lighter axis and darker edges. Close observation shows that the darker appearance of the edges is due to the presence there of deeply staining granules which are absent from the axis of the chromosome. It would seem very simple to find cross sections of chromosomes in this condition and to determine definitely if we do have here a true splitting involving a division of the granules, but, so far, I have failed to obtain convincing pictures.

Léger & Duboscq (1904*b*) describe for *O. saturnalis* the formation of an equatorial ring and its division which they interpret as equivalent to the ordinary splitting of the chromosomes (Text Fig. IV, *B*, *C*, *D*). To this we will return.

Nuclear conditions in other species compared with those in O. intestinalis.

The nuclear conditions in *O. caudata* are so similar to those in *O. intestinalis* that but one point needs mention, namely, that the

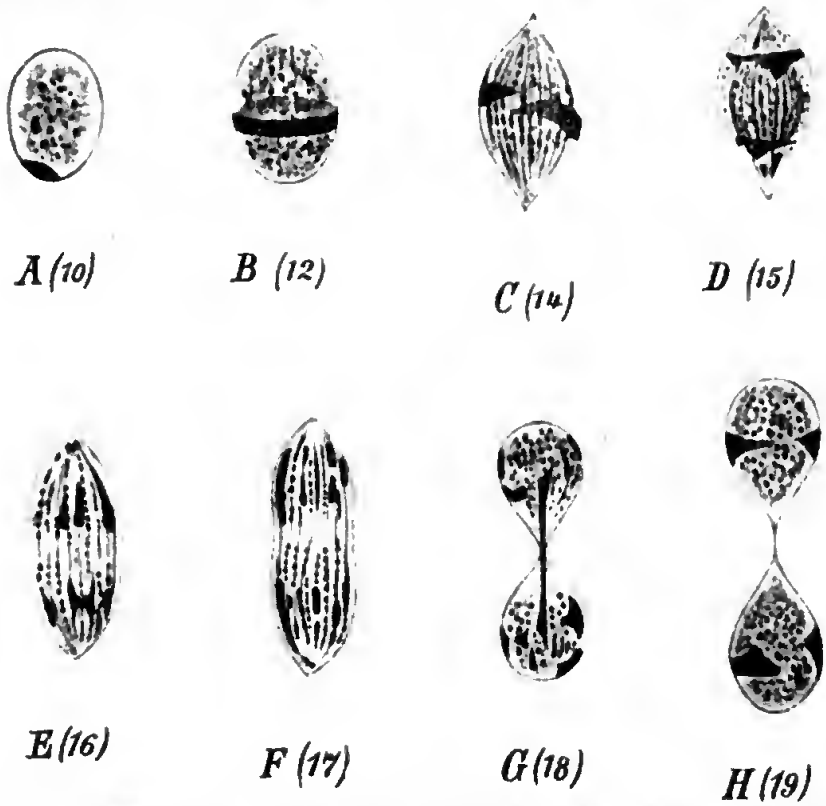
number of the chromosomes is six instead of eight (Fig. 81, 82, Pl. XX). In the multinucleated species the nuclei are much smaller and less favorable for study and I have given them much less attention. The nuclear membrane persists through the whole mitosis; there are no indications of centrosomes; the spindle is similar to that of *O. intestinalis*; the chromatin lies just beneath the nuclear membrane.

The number of the chromosomes in *O. ranarum* and *O. dimidiata*, as Neresheimer has said, seems to be twelve. The nucleus of *O. dimidiata* shown in Fig. 17, Pl. XIV, is unusually clear, bring surrounded by vacuoles of the excretory organ, and in this instance there seems little doubt that there are twelve rows of superficial granules, each row probably corresponding to a chromosome. The chromosomes in these multinucleated forms are more granular and less compact than in the binucleated species. The relations of the chromatin spherules are difficult to make out. The resting nuclei show a very characteristic appearance with a superficial chromatin network with enlarged nodes, and one to four disc-shaped chromatin masses closely applied to the nuclear membrane (Figs. 99—101. Pl. XXI; Text Fig. X, *a* and *b*). I have not yet attempted to follow the course of the mitosis, nor have I studied the nucleolus carefully. The chromosomes in multinucleated *Opalinae* seem to be more granular and less compact than those in *O. intestinalis* and *O. caudata*. They often appear merely as rows of granules. The chromosomes of *O. caudata* and *O. intestinalis* are always granular as described, but the granules instead of being in linear aggregates are scattered through a mass of less darkly staining chromatin, this mass with its granules composing the chromosome.

Léger & Duboscq (1904*b*) have described mitosis in *O. saturnalis* in a way that is somewhat difficult to reconcile with my description of the phenomena in *O. intestinalis* and *O. caudata*. Text Fig. IV shows eight of their figures: *A* is a resting nucleus; *B* shows the characteristic gathering of a part of the chromatin into an interrupted band around the equator of the nucleus; in *C* this band is shown dividing; in *D* the two parts are seen migrating toward the poles of the nucleus; *E* and *F* show how the daughter bands break into numerous parts which join the lines of granules ("chromosomes") and move with them to the poles; *G* and *H* show telophase stages in the reconstitution of the daughter nuclei.

Léger & Duboscq suggest that the division of the equatorial band of chromatin is equivalent to the ordinary splitting of the chromo-

somes in metazoan nuclei. It seems more probable that the equatorial chromatin ring in *O. saturnalis* is comparable to a chromatin nucleolus. It seems impossible that its division can be equivalent to the splitting of the chromosomes in *Metazoa*. I have not yet examined nuclei of *O. saturnalis* and cannot interpret the nuclear conditions in this species in comparison with those described for *O. intestinalis* and *O. caudata*. It may be that the equatorial band

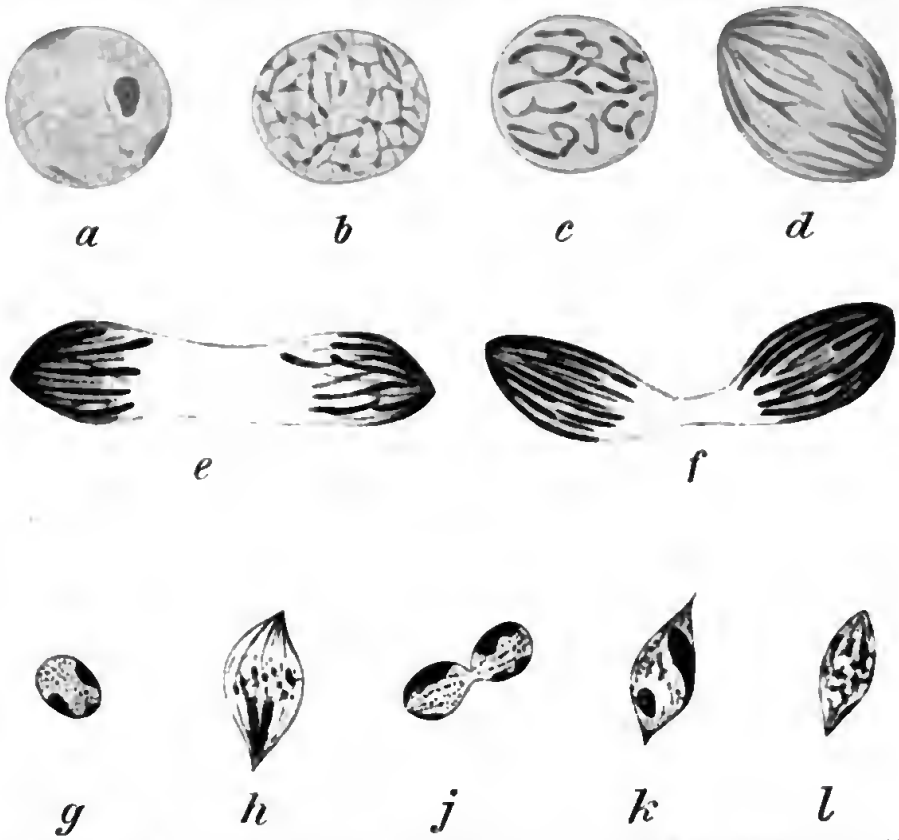


Text Fig. IV. Mitosis in *O. saturnalis*. After Léger & Duboscq. The numbers in parentheses are their figure numbers. × 1500 diameters.

of chromatin in *O. saturnalis* is homologous with the irregular ring of chromatin in daughter nuclei of *O. intestinalis* and *O. caudata* formed by the fusion of the chromosomes, as described, preceeding the formation of the chromatin ribbon (spireme). In multinucleated species and in *O. macronucleata* (Bezzenberger 1904) much of the chromatin often gathers in one or more large masses beneath the nuclear membrane (Figs. 99—101, Pl. XXI, and Text Fig. V, *a*). It is possible that these superficial chromatin masses correspond to the equatorial band in *O. saturnalis*.

Bezzenberger has described for the binucleated *O. macronucleata*

a type of mitosis that resembles that of the multinucleated species much more than that of *O. intestinalis* and *O. caudata* (Text Fig. V, *a—f*). The resting nucleus is like that of the multinuclear species; the chromosomes are numerous and are linear. In his Text Fig. XV (my Text Fig. V), *a* shows superficial chromatin masses like those in



Text Fig. V. BEZZENBERGER's figures of mitosis. *a—f* in *O. macronucleata*; *g—l* in *O. lanceolata*: *a* resting nucleus; *b—f* stages in division: *a—f* × 2000 diameters; *g—l* × 350 diameters.

a resting nucleus of *O. ranarum*; *b* shows the chromatin net without such larger masses; *c* is a spireme stage with the chromatin thread apparently ready to fragment to form the many chromosomes; *d*, *e* and *f* show anaphases. BEZZENBERGER gives also five figures of nuclei of *O. lanceolata*, whose mitosis seems to resemble somewhat that of *O. saturnalis* (Text Fig. V, *g—l*).

Enlarged individuals of *Opalina caudata* and of other species.

In both species of *Bombinator* one finds frequently, especially in the spring, certain very thick individuals of *O. caudata* (Fig. 88.

Pl. XX). These are generally associated with other normal forms, but rarely may be the only sort present in the rectum. I have never seen these very large individnals in division. That they are not a distinct species bnt are really *Opalinae caudatae* is proven by nnmerous transitional stages between the two forms. The chromatin in their nuclei is often, through not always, aggregated into larger masses than is the case in normal nuclei of ordinary forms, and one suspects that the animals are not entirely normal, yet they are as active as other forms and are freqnently fonnd in large numbers in freshly taken material.

It is chiefly the finding of these broad individuals of *O. caudata* that makes one a little donbtful as to the status of *O. zelleri* as a trne species rather than as a condition of *O. dimidiata.* I have never seen similar enlarged forms of *O. intestinalis* or *O. obtrigona.* In one lot of *O. ranarum* from the rectum of an apparently normal *Rana temporaria,* there were, among a large number of ordinary forms, a few (abont ten) individuals which were very much thicker than usual, being almost cylindrical. Their length was twice their width and their width half again as great as their thickness. I have not sectioned these thick individnals, but stained total preparations show nothing nnnsual in their appearance except the unnsual thickness of the endosarc, the nuclei being a little less closely set in the endosarc than in individuals of ordinary thickness.

Léger & Duboscq (1904 *b*) describe certain individnals of *O. saturnalis* as very broad and thick in comparison with their length. In these individuals the increased thickness is due to the greatly increased thickness of the ectosarc, in which the alveoles and spherules are of remarkable size. In *O. caudata* and *O. zelleri* and in the few thick individnals of *O. ranarum* seen, the increased thickness of the body is dne to the nnusual development of the endosarc. Léger & Duboscq suspected that the broad individuals of *O. saturnalis* might be prodncts of transverse division, bnt it is difficult to see what suggests this interpretation.

It is well known (Zeller 1877, Neresheimer 1907) that in the spring, when division becomes very rapid and most of the *Opalinae* become very small, some individnals in all species remain almost of full size, apparently not dividing any more rapidly than dnring the rest of the year. These large individuals do not encyst, but remain in the host and secure a continnance of its infection. It is possible that the great enlargement of some individnals is related in some way to the retardation of division. It is possible that

O. zelleri may be but a similar enlarged form of *O. dimidiata*. I have found *O. zelleri* only twice and then in the spring, and the thick forms of *O. caudata* are rather rare, except in the spring at a time when many individuals have already become small through repeated division. Both ZELLER and I have found *O. zelleri* and *O. dimidiata* together in the same individual host. NERESHEIMER describes these two species as from *Rana esculenta*, but does not say if both occur in the same individual host. In no other instance, except one very doubtful one in *Bombinator*, have I ever found two species of *Opalina* in one rectum. BEZZENBERGER describes *O. lata* and *O. longa* as occurring in *Rana limnocharis*, but does not say if the two species are found together parasitic in the same individual. It seems to be very unusual to find two species of *Opalina* together in the same host. The presence of individuals of *O. dimidiata* with those of *O. zelleri* in the same host casts some doubt upon the status of *O. zelleri* as an independent species. Until, however, we have more evidence of its connection with *O. dimidiata*, we must, as NERESHEIMER has done, treat it as independent. ZELLER, the discoverer of this form, expressed doubt as to its connection with *O. dimidiata*.

The chromatin spherules which are formed and dissolved, or extruded from the nuclei, during the course of each mitosis, seem to be especially connected with nutrition and growth. It is not impossible that careful study of the chromatin spherules in these large individuals of *O. caudata* and *O. ranarum* and in *O. zelleri*, might throw some light on their origin, but as yet I have found nothing of special import in this direction. I have not enough preparations of nuclei of any of these thick forms, in the right stage of mitosis, to allow me to study the point with sufficient care.

General considerations in connection with the structure of Opalina and of the phenomena of mitosis.

Ectosarc and endosarc.

The ectosarc and endosarc of *Opalina* are quite sharply distinct, both the protoplasmic granules and films and the refractive spherules of the two regions staining very differently with many stains. It is difficult to suggest to what this may be due. Is the primary

difference in the spherules or in the protoplasm itself? If the refractive spherules are products of the cytoplasm then, of course the primary difference between the ectosarc and endosarc must lie in the protoplasm itself. It seems, however not unlikely that the endosarc spherules are derived in part at least from the chromatin of the nucleus. On the other hand, the spherules of the ectosarc probably are formed by the ectoplasma itself. In *O. obtrigona* a few of the smaller spherules in the ectosarc are merely endosarc spherules that have wandered toward the periphery (Fig. 6, Pl. XIV). In other species studied, the endosarc spherules seldom, if ever, leave the endosarc. Even in *O. obtrigona* the migrated endosarc spherules lie in endosarc-like tissue which has protruded in strands between the alveoles of the ectosarc. In any case one can ignore these displaced endosarc spherules in inquiring as to the differences between endosarc and ectosarc.

That the peculiar staining reactions of the ectosarc are probably not due to the presence of the ectosarc spherules is shown by the fact that in the anterior end of the body, where only a few very small ectosarc spherules are present (Fig. 1), the ectosarc stain is just as divergent from that of the endosarc as it is in the rest of the body. That the difference in staining is not due to the absence of endosarc spherules from the ectosarc is shown by the fact that in *O. obtrigona* the ectosarc shows the same peculiar staining reactions as in other species, although in this species the endosarc spherules migrate into the ectosarc between the large alveoles, even reaching the sub-pellicular layer.

Apparently we can safely emphasize two points, first that there is very decided structural difference between the two regions, and second that there is an equally marked chemical and physiological difference, as indicated in the staining reactions and by the divergent character of the refractive spherules of the respective regions.[1])

One might suspect that the ectosarc spherules are excretory and that one of the chief functions of the ectoplasm itself is excretion, were there not present in the body, in several species, such a well developed system of excretory vacuoles. As these vacuoles lie in the endosarc and have no discernable connection with the ectosarc, we seem debarred from attributing any special excretory function to the ectosarc. We must rest for the present with the mere statement

[1]) Schubotz (1908) finds, in *Pycnothrix monocystoides*, that when stained by van Gieson's method the ectosarc is yellow, the endosarc red.

of the fact of a chemical and physiological difference between the ectosarc and endosarc, leaving unexplained the nature of this difference.

Excretory organs.

The special connection of the excretory organs with the nuclei is worth emphasis, though just what its physiological meaning may be is unexplained.

It is also of interest that the granules massed in the posterior end of the system of excretory vacuoles, which are from time to time extruded from the body, seem to be derived from granules of the cytoplasm, as indicated by their size and their exact resemblance to the granules of the cytoplasm bounding the posterior vacuole of the excretory system. In the processes of excretion certain of the cytoplasmic granules seem to be thrown away bodily.

The very primitive character of the excretory organs in *Opalina* has been emphasized in a previsious paper (Metcalf 1907 *c*).

Anterior end of the body.

The divergent character of the anterior end of the body also deserves special note. One sees that in this region the granules of the endosarc are more numerous, and the endosarc spherules much more abundant (Fig. 1), while in the ectosarc the very large alveoli and the large ectosarc spherules are wanting. As division of the body is constantly going on, growth must be constant, and one naturally thinks that the denser character of the anterior end may be related to special activity in this growth, yet this is not easy to prove. There are no definite points in the body which can be taken as landmarks in estimating the relative growth of different regions. The nuclei move within the plasma and so cannot be used as a fixed point for reference in studying the relative growth of different regions. That they so move is shown by the fact that one daughter cell, in each division, receives the posterior nucleus from the parent and that in a short time this comes to lie as near, or almost as near, to the anterior end of the body as does the nucleus in the other daughter cell (Pl. XVII).

Absence of centrosomes.

The absence of centrosomes in the mitosis is of interest. Centrosomes are well known among the *Protozoa* (e. g. in the *Sporozoa*). Among *Flagellata* and *Foraminifera* and in certain *Ciliata*, structures

which seem clearly to be related to true centrosomes are found both inside and outside the nuclei. The absence of centrosomes in *Opalina* is apparently not a primitive character.

In so for as the function of the centrosome is a mechanical one, furnishing a point of resistence in the movements attending mitosis, it is not needed in the mitosis of *Opalina*, for the ends of the spindle are attached to the nuclear membrane and this membrane can furnish the necessary resistence points, if any such be really needed. At each constriction of a nucleus in division, both the chromatic and the achromatic fibrils at the equator of the nucleus are pinched and held by the constricted nuclear membrane, and apparently the attachment of at least the chromatic fibrils to each end of the nuclear membrane persists even during the resting stage.

The spindle.

The mitotic spindle in *Opalina* is interesting in its simplicity, being formed merely by the enlarging of those fibrils and films which run lengthwise in the oval nucleus and by the concomitant diminishing in size of the transverse fibrils and films, the latter, apparently, for the most part, being drawn in like pseudopodia. There is nothing that can be interpreted as an outgrowth of fibres from any formative center, as seems to occur in connection with the centrosomes in many mitoses.[1])

The mitotic spindle in *Opalina* is also interesting in the fact that it is formed from both chromatic and achromatic material. In the resting nucleus the achromatic foam fills the whole nucleus, a network of chromatin fibrils being also present over the surface of the nucleus just beneath the nuclear membrane. The appearance of longitudinal striation in the dividing nucleus is due to the emphasizing of the longitudinal strands of the chromatin net and the longitudinal films of the achromatic foam. The spindle, therefore, is composed of a central achromatic portion and a superficial chromatic portion. To what extent the two are connected in either the resting or dividing nuclei it is difficult to say.[2])

There seems to be little true resemblance between the condition in *Opalina*, with an outer spindle composed of chromatin and an inner spindle of achromatic substance, and the condition in many

[1]) Very similar conditions have been described by Boveri (1887 *b*, p. 21) in the formation of the spindle in the maturation divisions of *Ascaris megalocephala*.

[2]) Wilson (1895, 1:00) believes that the linin which gives rise to the mitotic spindle in sea-urchin eggs arises from the chromatin.

metazoan mitoses in which one distinguishes a central and an outer portion in the achromatic spindle. It seems somewhat doubtful if the mitotic spindle in *Opalina* is really comparable at all to the mitotic spindle of a metazoan cell, though the presence of perfect spindles of nearly, if not exactly, the metazoan type in *Sporozoa*, and the occurrence in other *Protozoa* of spindles of intermediate character, seem to justify our regarding the structure in *Opalina* as a true, through very lowly developed, spindle. Its achromatic portion is evidently more nearly related to the inner than to the outer spindle of *Metazoa*, being as Boveri (1900) has shown, a "netrum".

The mechanism of mitosis.

The machanism of mitosis in *Opalina* seems as difficult, in some regards, to nnderstand as it is in other forms. One sees nothing in the cytoplasm which seems to be acting upon the nucleus. So far as one can judge, the nucleus is automatic in its movements, for even the separation of the danghter nnclei cannot be due to the pull of the cytoplasm as the body elongates, since the thread connecting the daughter nuclei is often long and coiled, indicating that it has itself been in rapid growth. The whole nucleus wanders forward in that daughter cell which receives the posterior nucleus of the parent, and some sort of contraction in the cytoplasm seems necessary to explain this migration, but the changes of form in the nucleus itself seem due to its own activity.

In the changes of shape and in the movements accompanying mitosis are certain portions of the nucleus active and others passive? Does the nuclear membrane elongate and become spindle-shaped because it is pnshed upon by the fibres of the spindle forming within, or is the nuclear membrane the active agent, itself elongating by growth at the same time that it serves to supply points of resistence to the pull of the spindle fibers? Do the chromosomes migrate of their own accord along the chromatin fibres of the spindle; or do the latter contract and pull the chromosomes towards the poles of the nucleus; or do those portions of the spindle-fibres between the chromosomes elongate and push the chromosomes apart? Are both the chromatic fibres and achromatic films in the spindle active, or is one set of structures active and the other passive? A little evidence upon some of these points can be found.

The change in form of the nucleus from oval to elongated eliptical or spindle-shaped seems to be due to growth of the nuclear

membrane. The spindle usually does not fill the whole nucleus, the nuclear membrane apparently growing more rapidly than the rest of the nuclear structures. During the whole mitotic cycle the nuclei are in constant growth, as is indicated by their increase in size. That the nuclear membrane shares in this active growth is shown not only by the fact just mentioned that the spindle does not fill the nucleus, but also by the fact that in the late telophases the thread connecting the two daughter nuclei grows even to unnecessary length and becomes coiled.

The usual peculiar form of the spindle, with acuminate ends, is instructive. It does not seem as if the chromatin fibres uniting the chromosomes to the poles of the nucleus can be contracting, for they are much, and quite irregularly, bent. They are not taut, as if pulling upon the chromosomes. Yet it is barely possible that the minute transverse fibrils connecting the longitudinal fibres of the spindle are drawing these together with sufficient force to bend them into the irregular bows which are seen. Naturally, at the narrowed ends of the nucleus, these transverse fibrils are more numerous in a given area than they are near the equator, where the nucleus has nearly its original diameter. The first impressions one receives from such a nucleus as that shown in Figs. 49—52, Pl. XVIII, may be that the spindle is elongating and pushing the nuclear membrane in front of it; yet the whole character of the chromatin fibres of the spindle is such as to suggest that they are pliable and not so stiff as any such hypothesis of the force of their elongation would imply. The fibres are usually quite irregular and curved, and it seems impossible to think of their pushing with any appreciable force. Such irregular fibres may exert some pull, but that they can effectively push is unbelievable.[1])

The migration of the chromosomes is accompanied by a perceptable thickening of the chromatin fibres connecting them to the poles of the nucleus, suggesting that the chromosomes are pulled toward the poles by the shortening of these fibres. As the chromosomes approach the pole they become less branched, less irregular in form and larger. On their way to the pole they seem to absorb most of the substance of the fibres of the chromatin spindle, drawing in the transverse strands of the chromatin net and taking up the substance of the longitudinal fibres and adding it to their mass. Thus, during the late anaphase, a very large proportion of all the chromatin in

[1]) Prandtl (1905, 1906) believes that the equatorial portions of the spindle-fibres in *Didinium* elongate and push the chromosomes apart.

the nucleus is in the chromosomes. Soon the chromosomes begin again to send out processes and the chromatin network of the resting nucleus is formed. The whole migration of the chromosomes and the reformation of the chromatin network suggests comparison with the movements of a reticulate foraminiferan. All the chromatin seems to be active in this movement.

No explanation of the migration of the daughter chromosomes as dependant upon some repulsion between them, connected with their splitting, can apply here, for no such splitting occurs in the equatorial plate stage or immediately preceeding it. The splitting, if it occurs at all, is found in the telophases of mitosis, after, not before, the migration of the daughter chromosomes.

A chromosome cannot crawl upon nothing any more than can an animal. There must be some resistant substance upon which the moving chromosomes can advance. The resistant substratum in this case seems to be the alveolar achromatic material which fills the center of the nucleus, and whose alveoles with their delicate walls and contained liquid seem to furnish the necessary resistence for the movements of the chromosomes and their pseudopodia (chromatin fibrils). The attachment of the spindle fibres to the nuclear membrane also, of course, aids in these movements.

What causes the chromosomes to arrange themselves at first in the equatorial third of the nucleus and later to crawl to the poles of the nucleus, and what causes the changes in the form of the nucleus, are questions whose answer the conditions in *Opalina* do not help us to approach.

Such a mitotic division as we see in *Opalina*, in which all parts of the nucleus seem to be active, membrane, chromatin and achromatin all sharing by active growth and movement, seems not only less specialized than the mitosis of higher forms, but also less removed, at least mechanically, from amitotic division, in which also probably all parts of the nucleus share by active movements. The presence of centrosomes in a cell allows the nuclear membrane and the chromatin and, in the most highly developed mitoses, the achromatic foam of the nucleus also, to be less active.

The polarity of the nuclei and the planes of division of the nuclei and of the body.

The nuclei of the binucleated *Opalinae* are always somewhat elongated, their two poles being always clearly recognisable. This

polarity is seen not only in the shape of the nucleus but also in the fact that the chromatin network is attached to each pole of the nucleus. This attachment is very clearly seen during mitosis and apparently persists through the "resting period". The orientation of the nuclei is constant and unchanging, their long axis being about parallel with, usually coincident with, the long axis of the body. The nuclei never rotate, except possibly upon their long axes, which would be without significance in this connection. This constancy of orientation may be due in part to the fact that the two nuclei are generally connected by a thread. The longitudinal axis of a daughter nucleus always remains in the same position as that of the parent nucleus. This enables us to clearly see the interesting fact that the nuclei of the binucleated species of *Opalina* always divide in the same direction and that they do so whether the accompanying division of the body is longitudinal or transverse.

In *Metazoa* and plant cells and in most *Protozoa* in which the relations are clearly discerned, the plane of division of the cell-body is parallel to the plane of division of the nucleus, both being perpendicular to the long axis of the mitotic spindle. The binucleated *Opalinae* show the same relation in the case of the unusual transverse divisions, but in the more frequent longitudinal divisions the plane of division of the body is parallel with the long axis of the nuclear spindle. A similar discrepancy between the directions of the division of the body and of the nucleus is seen in the Trypanosomes, but there the nuclear relations are not so clear, there being, especially, no certain indication that the orientation of the parent and daughter nuclei remains constant generation after generation. The constancy in the direction of the division of the nuclei and the variability in the direction of the division of the body in *Opalina* show that there is a lack of coordination between the direction of division of the nucleus and of the body. This lack of coordination is much more marked in the multinucleated species.

The conditions are interesting to consider from the point of view of phylogeny. If the anterior end of *Opalina* is homologous with the anterior end of a Flagellate, and doubtless it is so, we see that the two agree in dividing longitudinally. In most *Flagellata* we have only longitudinal divisions.[1]) Probably in *Opalina* the longitudinal division is primitive and the unusual transverse division secondary. The latter is probably comparable to the transverse division char-

[1]) *Oxyris marina* is said to divide transversely.

acteristic of the higher *Ciliata*. *Opalina* shows, therefore, a condition intermediate between that of *Flagellata* and that of the higher *Ciliata*, since, while retaining the more primitive type of division, it shows occasional divisions of the secondary type. The flattened multinucleated species of *Opalina* show more frequent transverse divisions than do the probably more primitive cylindrical binucleated species.

The phylogenetic significance of these phenomena is further considered on page 274 in connection with the discussion of the relationshipes of *Opalina* and the origin of distinct micro- and macronuclei in higher *Ciliata*.

Time relations in the division of the body and of the nucleus.

The two sets of daughter chromosomes in each nucleus of the binucleated *Opalinae* remain for one cell-generation in the same daughter cell, though soon separating into different nuclei; that is division of the body lags one step behind the division of the nuclei. At the second division of the body the daughter chromosomes of the preceeding division become distributed to separate cells. Probably originally the binucleated condition was brought about by the delay of one division of the body, the temporary binucleated condition thus secured persisting until the body itself finally divides.[1] The multinucleated condition of other species seems due to the further suppression of other divisions of the body, nuclear division and division of the body in them being still more loosely related.

Splitting of the chromosomes.

In the mitosis of *Opalina* we do not find any longitudinal splitting of the chromosomes in connection with the imperfect equatorial plate stage. The chromosomes are then already present in nearly or fully the double number and, while one or two chromatin masses may constrict transversely during the equatorial plate stage, the majority merely rearrange themselves in two transverse rows preparatory to migration to the poles. Careful observation of the chromosomes in both ends of many nuclei during the anaphases shows that generally each chromosome in one end of the nucleus corre-

[1]) I find that Boveri (1900, p. 189) has similarly interpreted the binucleated condition of *Opalina* as due to delay in the division of the cell-body.

sponds more or less closely in size and form and in number of contained granules to one of the chromosomes at the other end. These corresponding chromosomes are opposite to one another. This all suggests very strongly that the daughter chromosomes in a dividing nucleus of *Opalina* are paired, just as truly as they are in a metazoan nucleus.

We have already seen in the early telophase a condition which suggests that the chromosomes may be splitting longitudinally. The fate of the two halves that may be so formed is well-nigh impossible to follow, for the chromosomes almost at once unite to form a continuous ribbon. I have never found sixteen chromosomes at each end or even at one end, of a nucleus in the telophase, nor have I seen evidence that the chromatin ribbon is double. This, very interesting stage in the mitosis, needs further study, though I have little hope of obtaining conclusive results. At present we can only say that splitting of the chromosomes does not occur at the equatorial plate stage, that it may occur in the telophases, and that in the anaphases the daughter chromosomes seem to be paired as in metazoan mitoses.

Gonder believes that splitting of the chromosomes may often occur during the anaphases or telophases of maturation mitoses, but I know of no description of other mitoses in which the splitting of the chromosomes occurs after their distribution to the daughter nuclei instead of in the equatorial plate or in the prophases.

If there be true splitting of the chromosomes in the telophase, one cannot be certain whether each granule in a chromosome divides (Fig. 86, Pl. XX). Some of the granules at the edges of the chromosome, seem spherical, others eliptical, others elongated rod-shaped, it is possible that adjacent granules often lie in contact, or even fuse, and so give an appearance of an elongated rod. In comparing the two rows of granules at opposite edges of the apparently split chromosome, one sees a general resemblance but often the fusion or the contact of the granules in one line does not correspond exactly to that in the other line, and the two rows are not alike. Very likely, of course, even if the granules were all perfectly distinct, the two rows would not be found to be alike.

Of course, if, in general in mitosis, chromosomes retain their individuality during the spireme stage, is makes no real difference whether splitting of the chromosomes occurs before or during or after the spireme stage, so that mitosis in *Opalina* is not fundamentally different from that in most *Metazoa*, if splitting of the

chromosomes really occnrs during the telophases. If, on the other hand, the chromosomes and their contained granules do not divide into eqnivalent parts in each division of the nncleus, it seems that nuclear division in *Opalina* mnst be mnch simpler than mitotic division in higher forms.

I at first inclined toward the first hypothesis in interpreting the mitosis in *Opalina* and tried to imagine how these daugther chromosomes, formed in the telophase, could pass into the spireme and reappear later in the next double equatorial plate. Three salient facts are seen: 1) the fusion of the chromosomes in the late telophase is not a union end to end, bnt is instead an irregular lateral union, more or less broad plates of chromatin passing from the side of one chromosome to the side of the next, nntil they all become united to form the chromatin ribbon, 2) the chromosomes when they again become distinct, previous to the next mitosis, arise by transverse constriction of the chromatin ribbon and 3) the chromosomes remain permanentle, attached at each end to the poles of the nucleus by means of fibres some at least of which do not split. The transverse constriction of some of the chromosomes in the early telophases is a transient phenomenon, all the unconstricted chromosomes and the parts of the constricted chromosomes soon completely fusing in the spireme.

If the eight chromosomes split longitndinally in the telophase and then unite laterally to form the chromatin ribbon of the spireme stage, this ribbon might consist of the sixteen daughter chromosomes lying side by side in a single row. When now the spireme constricts transversely to form the sixteen chromosomes of the next mitosis, these might be the original sixteen daughter chromosomes from the longitudinal division in the last telophase. On the basis of such a schema, the relations of the grannles in the chromosomes would not be very difficult to bring into harmony with the usual conception of the chromosomes as consisting of a linear aggregate of chromioles which retain their individnality and which, in division, give one half of their substance to each daughter chromosome. But the chromosomes which in the telophase show appearance of splitting do not, as already noted, show their grannles arranged in pairs, so that we have, even on the basis of this schema, no satisfactory indication that equivalent daughter chromioles are distributed in the daughter chromosomes to each daughter nucleus: and furthermore, the attachment of each end of each parent chromosome to the corresponding pole of the nucleus by means of a per-

sistent fibre which, at least in some instances, does not itself split seems to introduce insuperable difficulty into the schema.

Calkins & Cull (1907) describe splitting of the chromosomes in the maturation divisions of *Paramaecium*, with a clearness which leaves no doubt that this type of mitosis occurs in at least some of its divisions, and so is known among the *Protozoa*.

Professor Boveri directed my attention to conditions in *Ascaris megalocephala* which seem similar to those in the telophases of mitosis in *Opalina* and which make it seem likely that the appearance of splitting in the chromosomes of *Opalina* during the telophases is not significant. In *Ascaris* the chromosomes are seen to be granular during the telophases, and the granules lie more or less distinctly in two rows at the edges of each chromosome, leaving the axes of the chromosomes lighter, as in *Opalina*. Van Beneden (1883, p. 343; 1887, Plate V, Fig. 8) has referred to these conditions in *Ascaris* as showing a second longitudinal division of the chromosomes in the telophases, one having already occurred in the equatorial plate stage of mitosis. Heidenhain, at the Anatomical Congress in Würzburg, 1907, described an appearance of splitting in the chromosomes in the telophases of mitosis in cells of the skin of salamanders. Boveri, during the discussion of Heidenhain's paper, suggested that, inasmuch as this appearence, when seen, is found in all parts of all the chromosomes, whatever their position may be, it cannot indicate a splitting of the chromosomes, but probably shows that the axes of the chromosomes in the cells of the salamander skin stain at this stage less deeply than the periphery. Chromosomes with unstained or faintly stained axes have since been interpreted by Haidenhain (1907) as having an axial linin fibril. The lighter axis of the chromosomes of *Opalina* during the telophases seems not to be due to the presence of an axial fibril of linin, but to the absence of granules at the axis and their presence at the edges of the chromosomes.

The appearance of granular edges and a lighter axis in the chromosomes of *Ascaris* is said by Boveri to be transient, disappearing when the chromosomes branch to form the network of the resting nucleus, and not showing in the chromosomes when these reappear from the resting nucleus preparatory to the next mitosis. There is no reason to believe that the splitting of the chromosomes in the new mitosis is a reappearance of a double condition in the previous telophase. The appearances of doubling of the chromosomes in the telophases seem quite similar in *Ascaris* and *Opalina*, and the condition in the cells of the salamander skin is somewhat

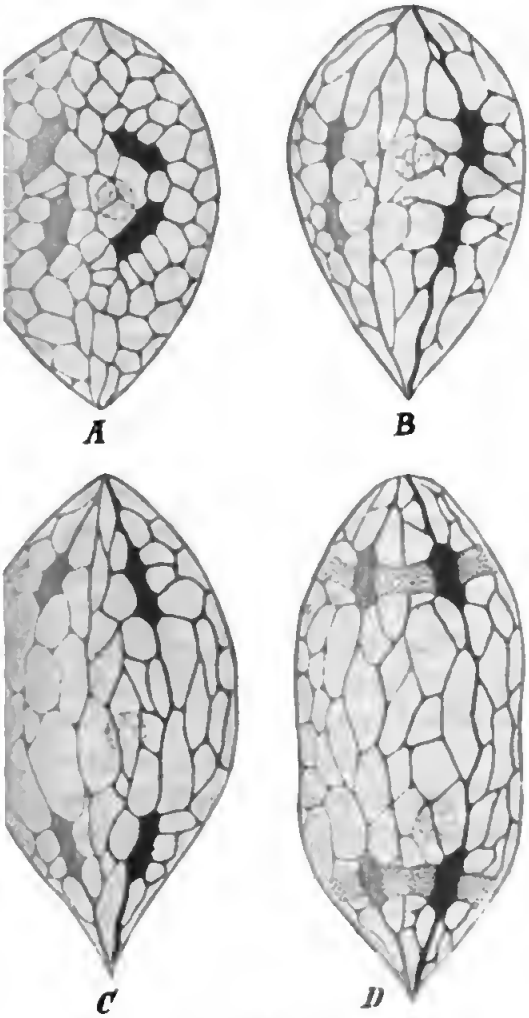
comparable. In *Ascaris* and the salamander the condition in the telophase seems to be unrelated to the true splitting of the chromosomes, which occurs during the next mitotic cycle, a resting stage intervening. This comparison with *Ascaris* and the salamander adds more doubt to the already very doubtful interpretation of the appearance in the telophases of *Opalina* as indicating a splitting of the chromosomes.

CALKINS & CULL (1907) found that splitting of the chromosomes occurs in the two maturiation divisions of *Paramaecium*, but not in the third division by which the conjugating nuclei are formed. The ordinary vegetative divisions of *Paramaecium* have not been so studied as to show whether splitting of the chromosomes occurs in them or not. Neither HAMBURGER (1904) nor CALKINS & CULL show its presence in the nuclear divisions immediately following conjugation. These conditions suggest the possibility that in *Opalina* the maturation divisions may differ from the vegetative divisions and that splitting of the chromosomes may be found in the maturation divisions. I have used for the most part, with the minute individuals of *Opalina* in the spring, methods which do not show the finest details. My study of carefully stained sections has not yet shown in detail the phenomena of matnration. Another spring's work will propably be necessary to determine this interesting point.

An alternative explanation of the mitosis.

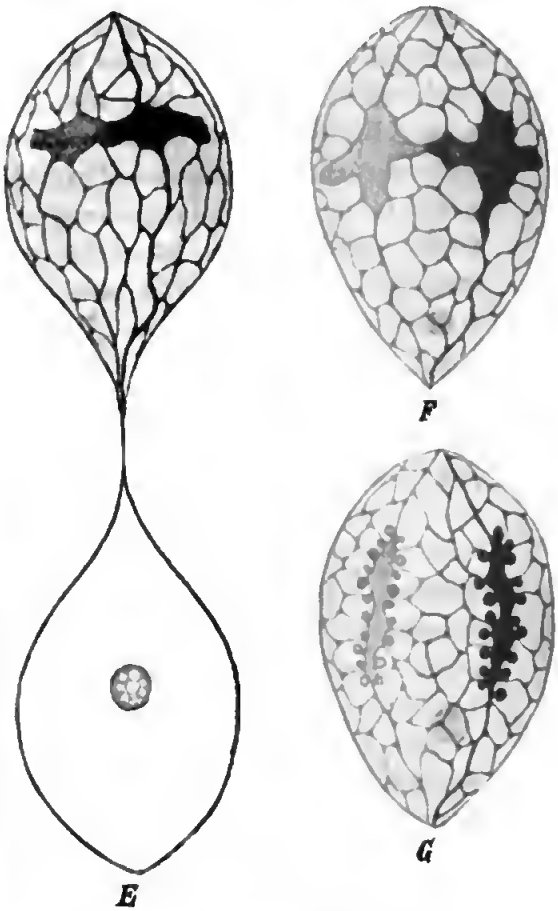
The alternative interpretation of the nuclear division in *Opalina* as a very primitive mitosis in which there is no longitudinal splitting of the chromosomes needs fnrther development. We have seen that the chromatin masses (properly called chromosomes only in certain conditions) are always branched, their branches being connected to form a network just beneath the nuclear membrane; and we have also seen that some of the fibres of the network are attached to the nuclear membrane at each of the two poles of the nucleus. This attachment of the fibres is very clearly seen when the spindle is well developed. The manner in which the nucleus constricts in each mitosis until the membrane at the equator of the nucleus pinches and holds the fibres of the spindle, explains the fact that these fibres are attached to the membrane only at the two poles. One night conceive each longitudinal chromatin fibre of the mitotic spindle in *Opalina*, with all its branches and with the two chromatin masses upon it, as forming one unit, the units being in con-

nection by means of their united branches (Text Fig. VI, B). When the division of the nucleus is completed, one would find in each daughter nucleus eight daughter units each consisting of a single mass of chromatin with many branches which unite with branches



Text Fig. VI.

agrams of mitosis in *O. intestinalis*, only 'o chromatin nnits, instead of eight, being own: *A* = an anterior nncleus in the irre- ılar "eqnatorial plate" stage, four danghter romosumes being present; *B* = an early aphase; *C* = a later anaphase; *D* = an rly telophase, the chromosomes being con- cted by their broad lateral ontgrowths.



Text Fig. VII.

Diagrams of mitosis in *O. intestinalis*, only two chromatin units being shown. *E* = a late telophase (spireme). The chromosomes are united into a ribbon which, if all the chromosomes were shown, would extend over nearly the whole nncleus. The nndivided nncleolus is shown in the posterior danghter nucleus. *F* = an anterior daughter nuclens in an early prophase; the chromatin ribbon is breaking np again into chromosomes; the new nncleolus is forming near the more pointed end of the nnclens. *G* = an anterior nuclens in a late prophase; chromatin spherules have been formed.

of the neighboring chromatin masses to form the nuclear net which is always recognisable at all stages of mitosis, however faint the transverse fibrils may become. The chromatin masses of the neighboring units send out also broad plates of chromatin (Text Fig. VI, D) which soon completely unite them into a chromatin ribbon (Text Fig. VI, E). Preparatory to the next mitosis the chromatin masses of neighboring units again separate (Text Fig. VII, F and G) and each constricts into two thus producing sixteen chromatin masses, whose branches are all interconnected, each unit of course having two chromatin masses (Text Fig. VI, A). The eight units now draw in most of the substance of their lateral branches and reassume their position side by side in eight more distinct parallel lines stretching from pole to pole of the nucleus, each line having upon to two chromatin masses (Text Fig. VI, B). The cycle then repeats itself.

Upon this interpretation it would be seen that the division is not a highly developed mitosis, but that, still, by means of the persistence of the longitudinal chromatin fibres in all stages, even in the resting nucleus, and through their retention of their connection with the two poles of the ovoid nuclear membrane, the chromatin masses, after they divide, are able to send one of their daughter masses to each pole of the nucleus, securing thus a result somewhat similar to that obtained in the fully developed mitoses of higher animals. There is no means in this division to secure the distribution of one half of each granule of each chromosome to the daughter nucleus, but each daughter nucleus does receive about half of the mass of each chromosome of the parent nucleus. In this case, we see that the emphasis is upon the chromosomes and not upon the chromioles.

The evolution of mitosis.

Is this type of mitosis in *Opalina* aberrant or does it correspond to a stage in the phylogeny of the more highly developed mitoses of *Metazoa*? The nuclei of many, probably of all, *Plasmodroma* are centronuclei (Boveri 1900) each containing, in addition to the chromatin elements and indifferent plastin, a differentiated karyosom which functions as a more or less perfect centrosome. Compare *Euglena* (Keuten 1895), *Trypanosoma* (Schaudinn 1904, v. Prowazek 1905, Salvin Moore & Breinl 1907), *Amoeba* (Schaudinn 1894, Hartmann & v. Prowazek 1907). It is somewhat doubtful whether the simplest nuclei have differentiated karyosomes, but at any rate,

we may probably assume the former existence of such simple nuclei, even though they may not exist to-day.

One naturally conceives a series of stages in which both the plastin and the chromatin constituents of the nucleus are becoming more highly developed. On the one hand the phylogenetic development of the chromatic structures probably showed at one time a stage with the chromatin in the form of diffuse granules not grouped into chromosomes, and this may have been succeeded by a stage such as we now seem to have in Trypanosomes, in which we have diffuse granules irregularly arranged during the vegetative mitosis (Salvin Moore & Breinl), but true chromosomes during some of the divisions preceeding conjugation (Schaudinn, v. Prowazek). A further evolution gives definite chromosomes persisting throughout the whole life of the cell. Ultimately the chromosomes show morphological differences corresponding to their differences in function. On the other hand we conceive the plastin elements of the nucleus as giving rise to an intranuclear centrosome[1]), which soon becomes developed to the point of containing a centriole. The final development, showing a spindle and astral rays, is best seen when the centrosome becomes extra nuclear. The original centronucleus has thus evolved into an elaborate double set of structures, one consisting of the chromatin elements associated with some indifferent plastin, and the other being the kinetic plastin in the form of the centrosome, whose structure in some phases of mitosis becomes elaborately developed.

The conditions in *Opalina* seem to throw light upon this phylogeny, though its own nuclear structure seems aberrant and not to correspond to any stage in the phylogeny of the mitosis of higher forms. It seems to have substituted another and simpler mechanical device for the centrosome; has developed its mitosis to a certain point and has stopped there, unable to go further because of the absence of developed centrosomes. Its method of mitosis is simple and is efficient to a degree, but is incapable of producing the remarkably perfect results reached by those cells which kept and further evolved their centrosomes.

The indication that in the mitosis of *Opalina* the emphasis seems to be placed upon the chromosomes and not upon the chromioles is

[1]) The karyosom of *Plasmodroma* seems to consist of both plastin and chromatin and to be therefore more than a centrosome. It contains the centrosome. These relations are shown with especial clearness in an as yet unpublished paper by Hartmann upon *Amoeba tetragena*. See also Hartmann & v. Prowazek 1907.

not without significance. May it be that the longitudinal splitting of the chromosomes has been overemphasized, and is not so fundamental as is often thought, having been evolved from a less definite simpler method of division in which the chromosomes but not their granules divide into equal halves? Probably the vegetative divisions of the micronuclei of *Paramaecium* are of this type. Has this simpler type of division itself been evolved from lowly mitoses, like those in many *Plasmodroma*, in which there is no method of securing so equal a distribution of the mass of each chromosome to the daughter nuclei, no distinct and constant chromosomes, indeed, appearing to be present? In such divisions as these in the *Plasmodroma* the masses of chromatin in the two daughter nuclei may be about equal. Possibly amitotic division stands as still more primitive. It is difficult to distinguish the two in some cases. Has the individuality of the chromosomes and perhaps of the chromioles been developed *pari passu* with the elaboration of the process of division? Are the conditions in *Opalina* intermediate between ordinary amitotic division and highly developed mitoses, *Opalina* having a method of preserving the distinctness of the several chromosomes generation after generation, but not having a perfect method for securing such an exact equality of the daughter chromosomes as results from the longitudinal splitting in highly developed mitoses, in which one half of each chromiole goes to each daughter nucleus? The latter is secured only in mitoses in which the chromosomes split longitudinally and this, it seems, is not the case in *Opalina*, in which the persistent attachment of the two ends of each chromosome (chromatin unit) to the two poles of the nucleus is the means of securing the separation of the daughter chromosomes and the independence of the several sister chromosomes. The conditions in the binucleated *Opalinae* seem to favor such a general interpretation as that here developed, but mitosis among the *Protozoa* must be better understood before one can accept as sufficient the evidence in favor of such a phylogeny of mitosis.

Opalina seems to have the chromosomes not only distinct but somewhat different from one another, as is indicated by differences in size and form. Of course differences between the chromosomes, once established, could readily persist if the schema of division suggested for *Opalina* is correct, and it seems to be so, provided one assumption is correct, namely, the assumption that when the chromatin ribbon constricts to form the chromosomes for the new mitosis, constrictions occur at points where the eight chromosomes

of the previous telophase nnited to form the ribbon. Fundamentally the same assnmption is involved in the belief in the individuality of the chromosomes in any animal. In the binucleated *Opalinae* the chromatin is less diffuse in the "resting nuclei" than it is in most animals, so that it is easier to conceive of the chromatin masses which appear before the new mitosis as being merely the old chromosomes of the previous telophase which have again become distinct. Each chromosome itself soon constricts into two, this being apparently the true division of the chromosomes. Division of some of the chromosomes may occnr at the time when the chromatin ribbon is constricting to reform the chromosomes, so that the number of masses coming out of the chromatin ribbon may be more than eight, but this, of conrse, does not affect the interpretation of the phenomena.

Nuclear condition and cytoplasmic movements.

Attention has been called to the fact that the two nuclei in the binncleated *Opalinae* are often in slightly different condition. This divergence is never great. In the multinucleated species the conditions of the numerous nnclei are very different. No disturbance of the movements of the cell arises from this diversity in condition of the different nuclei, which tends to confirm the general belief that the chromatin of the nuclens is not directly concerned in the control of protoplasmic movements. Meves (1899) showed that secretion in the cells of the kidney in salamander larvae is interrupted during mitosis, the chemical activities of the cytoplasm being influenced by the condition of the chromatin in the nncleus.

Analogies of the chromatin spherules.

Some light may be thrown npon the problem of the nature of the chromatin spherules and cytoplasmic sphernles by comparison with the conditions in other ciliate *Infusoria*. The macronucleus of most *Ciliata* consists of grannles aggregated into a more or less compact group. This gronp breaks np nnder certain conditions, the granules scattering through the whole endoplasm and soon disappearing by solution. When a new macronucleus is formed, it arises from one (or more?) of the micronnclei. The macronncleus of the *Ciliata* is generally regarded as especially connected with nutrition.

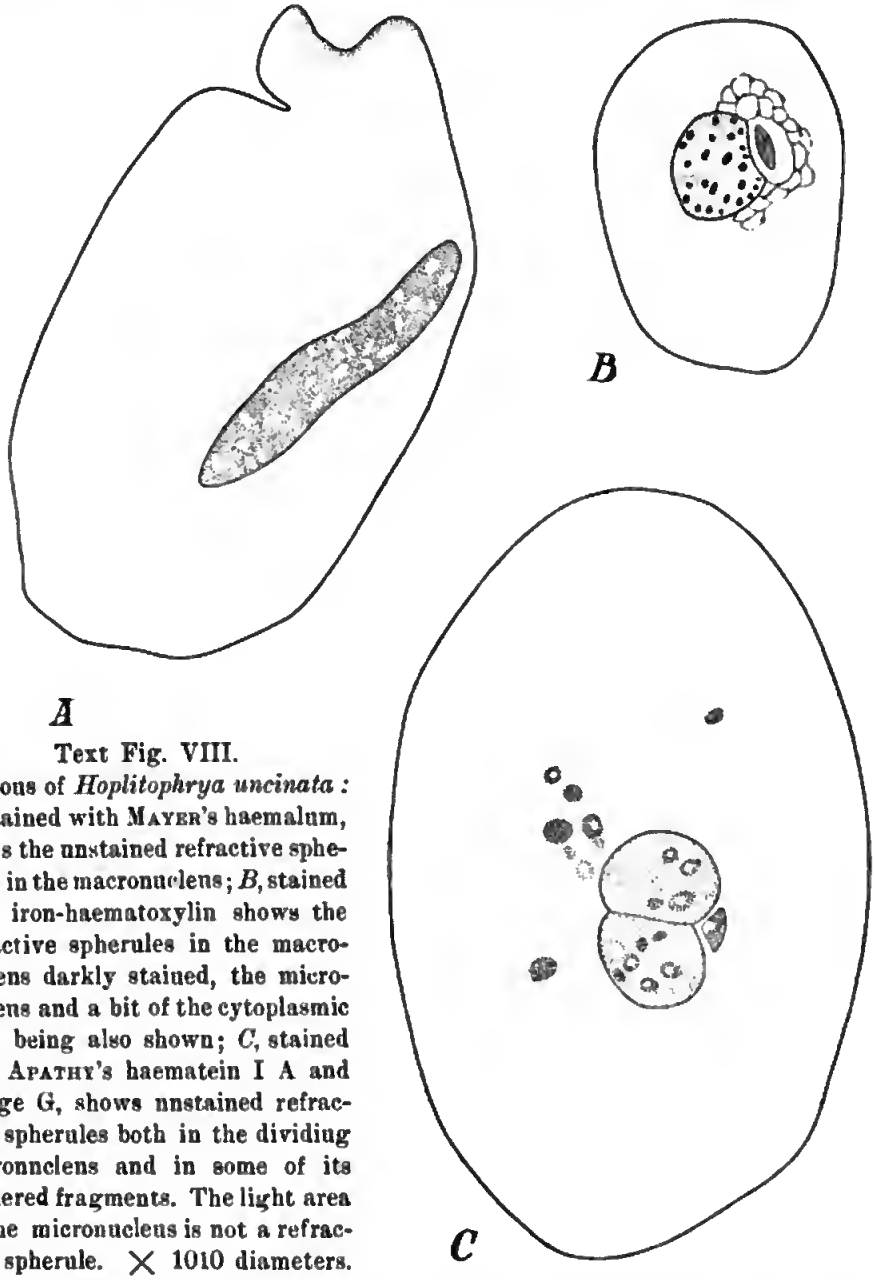
The chromatin spherules in *Opalina* are derived from the chromatin of the chromosomes and by their origin from chromatin and their solution and disappearance remind one of the macronuclear

granules of other *Ciliata*. But one notes a decided difference between the two. In *Opalina* the chromatin spherules are formed and dissolved in the course of every cell division, while in most of those *Ciliata* which have been most carefully studied the macronucleus dissolves and is reformed only during or after conjugation, or under conditions of nutrition which often induce conjugation. In *Hoplitophrya uncinata*, a probable near relative of *Opalina*, the macronucleus is very often found fragmented into granules or groups of granules which are scattered through the cell (Text Fig. VIII, C). One cannot believe that this fragmentation is usually connected with conjugation. I have not followed the fate of the scattered granules in *Hoplitophrya uncinata*, nor have I as yet had time to study carefully the abundant refractive spherules in the endoplasm of this species. They seem to be formed in the macronucleus and also in the scattered fragments of the macronucleus when this breaks to pieces (Text Fig. VIII). They do not react to *intra vitam* stains exactly as do the endoplasmic spherules of *Opalina*, yet they are probably of a generally similar nature. The apparent connection of refractive spherules and macronucleus in *Hoplitophrya uncinata*, and the freqnent fragmentation of the macronucleus, make this species a pecnliarly favorable one for the study of the refractive spherules and their relation to the macronucleus. I hope soon to give the matter further study. The scattered macronuclei of *Loxodes rostrum* (JOSEPH 1907) are perhaps comparable to the scattered groups of macronuclear granules seen in *Hoplitophrya*.

The relation of the refractive spherules in the endosarc to the chromatin spherules in *Opalina* is not fully established. The resemblance between the two in their staining reactions is not a demonstration of their relation, but it does suggest that the substance of the chromatin spherules may find its way into the endoplasmic spherules. This, is rendered still more probable by the fact mentioned that in *Hoplitophrya* apparently similar refractive spherules arise in the macronucleus and in the scattered groups of macronuclear granules when the macronucleus fragments.

Refractive spherules somewhat comparable to those in the endosarc of *Opalina* are not rare among the *Ciliata*, *Nyctotherus* and several species of *Balantidium*, present in the same hosts with *Opalina*, have many such refractive spherules in their endoplasma, which, however, seem always to stain darkly with iodine. They are not composed of true glycogen but are apparently paraglycogen of a somewhat different nature from that in the spherules of *Opalina*.

It seems probable that some of the refractive bodies found in *Flagellata* and *Foraminifera* are of the same general nature. There seems to be something the same doubt as to the origin of the



Text Fig. VIII.
Sections of *Hoplitophrya uncinata*: *A*, stained with MAYER's haemalum, shows the unstained refractive spherules in the macronucleus; *B*, stained with iron-haematoxylin shows the refractive spherules in the macronucleus darkly stained, the micronucleus and a bit of the cytoplasmic foam being also shown; *C*, stained with APATHY's haematein I A and orange G, shows unstained refractive spherules both in the dividing macronucleus and in some of its scattered fragments. The light area in the micronucleus is not a refractive spherule. × 1010 diameters.

refractive bodies in *Pelomyxa* that there is as to their origin in *Opalina*. GREEF (1874) and GOLDSCHMIDT (1905) describe them as arising from the nucleus, GOULD (1893) says that they divide by

constriction. Stolc (1900) and Bott (1907), on the other hand, are certain that they arise in the cytoplasm and that they do not increase by division. Stolc describes them as consisting of two parts, an outer envelope and an inner substance, the latter glycogen, the former a carbohydrate. Bott confirms Stolc, agreeing that the refractive bodies are probably reserve nutritive material. The morphological structure of the endosarc spherules in *Opalina*, with their outer layer of granules and more lightly staining central-portion, resembles the structure of the refractive bodies of *Pelomyxa* and their interpretation as reserve nutrient material seems altogether probable, though they are not true glycogen. As already shown, the chromatin spherules of *Opalina* and the macronuclear granules of other *Ciliata* may be comparable. If, then, the endoplasmic spherules of *Opalina* are derived from the chromatin spherules, our series of comparisons would include the macronuclear granules of most *Ciliata*, the chromatin spherules in the nucleus of *Opalina*, the endoplasmic spherules of *Opalina*, and the refractive bodies in *Flagellata* and *Foraminifera*. There is a general resemblance also between the refractive spherules of *Protozoa* and the pyrinoids of plant cells. Both seem to be a reserve food supply and both are handed down from parent to child when the cells divide. We know nothing, however, to indicate any special connection between the pyrinoids and the substances in the nuclei of plant cells.

Phylogeny of the nuclei of Ciliata.

The question as to what in *Opalina* is the full homolog of the macronucleus in higher *Ciliata* can best be approached through a discussion of the evolution of the condition with two functionally diverse nuclei. The macronucleus of higher *Ciliata* arises by the metamorphosis of a nucleus which has itself arisen by division from the micronucleus.[1]) It is therefore phylogenetically a complete nucleus and not a mere mass of granules extruded from a nucleus and gathered into a group. The macronucleus seems to be specialized in connection with the nutrition of the cell. It is able to divide, as does the micronucleus, in the vegetative divisions of the cell, but it takes no part in the special phenomena, interpreted as maturation, which preceede conjugation. The micronucleus, apparently

[1]) Neresheimer (1908) does not describe the origin of the macronucleus in *Ichthyophthirius* (species?). The remarkable phenomena which he does describe, if correctly described, make it improbable that the macronucleus in this species arises by metamorphosis from a micronucleus.

holds in abeyance the functions connected with the nutrition of the cell, but the potentiality of these functions must be present, since daughter nuclei from the micronuclei are able to transform into macronuclei. Probably each type of nucleus is a complete nucleus, the nucleus especially connected with conjugation being slightly specialized by diminution of some of its functions and probably of the chromatic material upon which these functions rest. The macronucleus is specialized by the great development of its nutritive activities and a corresponding great increase in the amount of chromatin especially associated with these functions. The specialization and hypertrophy of the macronucleus seems to have gone so far that it is difficult to secure conjugation of the macronuclei, and so, partly as a consequence, the macronuclei degenerate. The germinal (unspecialized) chromatin is so overbalanced by the nutritive (specialized) chromatin in the macronuclei that it is unable to assert itself and bring about conjugation of these nuclei, and, without occasional readjustment such as is secured through conjugation, ultimate degeneration seems unavoidable.

Before copulation or conjugation there seems to be quite generally, among the *Protozoa* at least, a process of reestablishing the proper balance of the nutritive and other chromatin in the nucleus. It is apparently the nutritive chromatin which especially increases in amount during growth and ordinary vegetative divisions and the excess of this nutritive chromatin is gotten rid of before conjugation by the formation of chromidia, either the excess of vegetative chromatin leaving the nucleus, or the excess of this specialized chromatin being left in the nucleus, the ordinary chromatin going out into the cytoplasm and there reforming into a new nucleus or new nuclei, or, as in *Chromidina* (Gonder 1905), all the chromatin passing into the cytoplasm where after a time part degenerates and the rest forms the generative nuclei. We doubtless do not know the full significance of these phenomena, but this much seems probable, that there is division of labor between different parts of the chromatin and consequent hypertrophy of some parts during their periods of special activity. The specialization and hypertrophy of chromatin in connection with nutrition has gone so far in the macronucleus of *Ciliata* that it is simpler to secure a new macronucleus than to reestablish in the old macronucleus such a balance of the respective parts as will allow it to share in conjugation.

What was the phylogenetic origin of the condition with two nuclei, one of which is highly developed for nutrition while the other

remains minute and hardly shares in the activities of growth. The divergence mnst have occnred in a binucleated (or mnltinncleated) condition. We have in *Opalina* such a binncleated (or multinncleated) form. In what way could its condition with similar nnclei be changed into a condition with dissimilar nnclei?

First let ns note again the fact that the nuclei of the binucleated *Opalinae* are often slightly dissimilar in regard to mitosis, one being often in a slightly more advanced condition than the other. There is a similar divergence in regard to the formation of the probably nutritive chromatin spherules, one nncleus showing these in a more advanced stage of formation. The exact balance of the two nuclei seems already somewhat disturbed in *Opalina*.

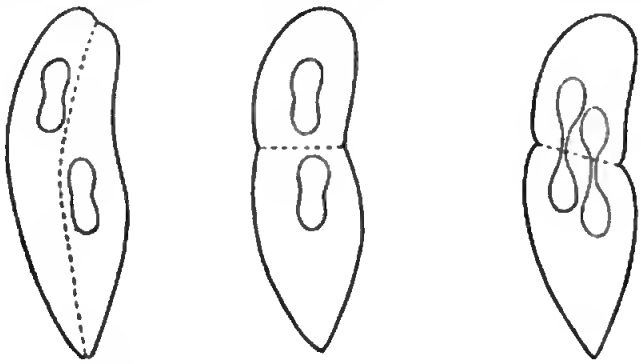
May we conceive this divergence as going further, the nntritive chromatin becoming hypertrophied in one nucleus and not in the other, the second nucleus ultimately giving up almost all its connection with nutrition and becoming, much smaller, giving us ultimately the condition seen in higher *Ciliata* with very divergent micro- and macronuclei?

One thing seems to stand in the way of such an interpretation so far as *Opalina* is concerned: in the division of *Opalina* one whole nncleus, and not two half nuclei, is given to each daughter cell. The condition in *Opalina* is not a true binncleated condition. We have merely a delayed division of the body, which causes two danghter nuclei to lie for a long time in one cell, indeed even until they have entered upon the next mitosis. Division of the cell when it does occur is not associated with the mitosis in the nuclei which is taking place at the same time, but is really the delayed celldivion that belonged with the last nuclear mitosis. Division of the cell-body lags one step behind the division of the nuclei. To get a proper understanding of the real meaning of this division we must bring together that division of the body and that division of the nucleus which really belong together.

In attempting to do this we see at once that the direction of division of either nucleus or body must be changed. At present the long axes of nuclei and body coincide and remain constantly in this relation. The nucleus divides transversely and the body generally longitudinally.[1]) Can we find a plausible scheme which will get around this difficulty?

[1]) Longitudinal division of the body is characteristic of *Flagellata* and is doubtless, primitive for *Opalina*. Many *Flagellata* show nuclei which when dividing elongate at right angles to the plane of division of the body and then divide

The present condition in *Opalina*, with an apparent but not a true binucleated state, could be changed into a true binucleated state comparable to that of *Paramaecium*, if cell-division should change from longitudinal to transverse and at the same time should *bisect each of the two nuclei.* Instead of the condition shown in Text Fig. IX, *A*, as now, we would have that illustrated in Text Fig. IX, *C*, each cell recieving two daughter nuclei instead of one whole nucleus. From this condition, that of *Paramaecium* could be reached by functional and accompanying structural divergence of the nuclei, as suggested above. The ordinary infrequent transverse divisions of the binucleated *Opalinae*, do not help us in this schema, for they do not bisect the nuclei (Text Fig. IX, B). The false binucle-



Text Fig. IX. Illustrating the development of the truly binucleated condition of the higher *Ciliata* from a pseudobinucleated form like *Opalina*. *A* and *B* are drawings of actual conditions found in *O. caudata*; *C* shows a hypothetical transverse division which bisects the two nuclei.

ated condition of *Opalina* can be changed to a real binucleated condition only by the complete suppression, not the mere delay, of one division of the body. Were this to occur, then a transverse division of the body, such as we occasionally find, would bissect the two nuclei, being not a delaged division belonging to the last mitosis, but the division which properly belongs with the present mitosis of the two nuclei (Text Fig. IX, C). We can conceive the same result as following still longer delay in the postponed division of the

across the equator, their plane of division coinciding with that of the body. In Trypanosomes as in *Opalina* the division of the body and that of the nucleus are not synchronous and the planes of division of nucleus and body do not coincide. One cannot say which is the more primitive condition, that which does or that which does not show coordination between the division of the nucleus and the division of the cytoplasm.

body, it not occuring until the daughter nuclei are separated to a considerable distance, so that the division of the body (transverse in this case) conld easily pass between the daughter nuclei, producing thus in each daughter cell a truly binucleated condition.

It seems to me quite probable that snch has been the history of the evolution of the binncleated condition in higher *Ciliata*: first delay in division of the body, establishing a temporary binucleated condition; then complete snppression of this delayed division of the cell-body, establishing a true binucleated condition, each nucleus, as apparently now in *Paramaecium*, belonging to a potentially, but not actnally, independent individual.

The remarkable phenomena which Neresheimer (1908) describes for *Ichthyophthirius* probably cannot be brought into harmony with the interpretation of the nnclear conditions in the *Ciliata* here suggested. There are gaps in Neresheimer's work, and an absence of detail in both figures and description, which make it desirable that this genns be further studied.

Compound nature of the Ciliata.

The trnly binucleated forms, as well as the falsely binncleated *Opalinae* are really potentially double individuals; and similarly the mnltinucleated *Opalinae*, arising by further temporary suppression of divisions of the body, are highly compound forms composed of many potential individuals. These individuals all become ultimately distinct before or in connection with copulation, even in the mnltinucleated *Opalinae*, the temporarily suppressed divisions of the body finally appearing rapidly in the spring and producing nnicellular gametes.[1])

The phenomena in the spring which preceede and accompany copulation.

Phenomena in *Opalina intestinalis*.

As the breeding season of the host approaches most of the *Opalinae* in the rectum increase the rapidity of their division, be-

[1]) The fact that the macrogametes are often still binucleated at the time of conjngation does not indicate that they are really binncleated forms, but merely that conjugation may occur before the complete separation of the definitive gametes, as if fertilization in a Metazoan should occur before completion of the maturation of the egg. Compare pages 285 and 290.

coming very minute, a few individuals retain nearly their full size and do not encyst, but remain in the rectum of the frog, apparently continuing the infection of the host. The minute individuals encyst, pass into the water with the foeces of the host, and are eaten by tadpoles, in whose alimentary canals the little *Opalinae* work their way out of the cysts and divide, forming micro- and macrogametes which copulate.[1])

Decrease in the number of chromosomes.

In the later mitoses before encystment one finds but four (Fig. 120, Pl. XXII) instead of eight (Fig. 119, Pl. XXII) chromosomes. This change in the number of the chromosomes takes place in animals from four to eight times as large as the individuals which enter the cysts. It occurs before the vegetative chromatin is thrown out of the nucleus, the latter process, under normal conditions, taking place just before encystment and in the cysts or in the rectum of the tadpole. The decrease in the number of the chromosomes might be due either to their union in pairs (synapsis of Montgomery) or to an actual "reduction division" at this stage. The chromatin ribbon breaks into eight instead of sixteen parts, and these do not seem to be unusually large. Possibly counting the granules in these chromosomes would show whether they are double or not. I have not yet done this, such work being necessarily slow, and my material needing restaining before any such counts can be made. It will probably be necessary to wait until another spring in order to have sufficient favorable material for studying this interesting point. The reduced number of chromosomes persists until copulation occurs (*cf.* Fig. 148—152, Pl. XXII; 168, 173, Pl. XXIII; 183, 185, 187—191, Pl. XXIV).

The last division before encystment.

One sees very clearly that in the last (?) division by which uninucleated animals ready for encystment are formed no mitosis of the ordinary type occurs (Figs. 121, 122, Pl. XXII). The nuclei seem not to be in division at all, but rather are occupied in getting rid of a part of their chromatin, a process which will soon be described.

[1]) It seems necessary to accept the german use of the word copulation to denote fusion of two gametes to form one zygote, and of the word conjugation to denote the mutual fertilization of two gametes each by the other, as in higher *Ciliata*. The natural English use of these words would be the exact reverse.

The uninucleated condition is reached by suppressing one nuclear division while the body divides. There is at no time any such degeneration of nuclei as NERESHEIMER has described for *O. ranarum* and *O. dimidiata*, nor is there any formation of new nuclei from chromidia in the cytoplasm. The old nuclei discharge a portion of their chromatin and themselves persist as the reproductive nuclei.

Extrusion of vegetative chromatin.

In living nuclei, at this stage, which are getting rid of a part of their chromatin in this peculiar manner, one observes two large balls or discs which by staining are clearly shown to be composed of chromatin (Figs. 121—139, Pl. XXII; 236, Pl. XXV). Occasionally instead of two such chromatin spheres one finds three (Figs. 132, 134, Pl. XXII), one or two of these being smaller. In other cases but one sphere is found, but in these cases another may have been present and have been extruded. The rest of the contents of the nucleus lies in the form of granules, generally in an hour-glass-shaped group, transversely, between the two chromatin spheres when two are present (Fig. 121). In the nuclei of the cysts one finds sometimes one (Figs. 131, 135, 138, 139, Pl. XXII; 236, Pl. XXV), sometimes two (Figs. 136, 137, Pl. XXII), sometimes three (Figs. 132, 134, Pl. XXII) such chromatin spheres. In the animals hatched from the cysts one finds usually but one such compact sphere of chromatin, or often none, the granules remaining in the nucleus being often also gathered into a spherical group, which however in both the living animals and in acetic carmine preparations can be distinguished from the denser sphere. When stained with DELAFIELD'S haematoxylin the difference between the two is very clearly seen (Figs. 136, 137, Pl. XXII). By the time the gametes are ready for copulation, the dense chromatin spheres have entirely disappeared from their nuclei (Figs. 148—152, Pl. XXII; 168, 173, Pl. XXIII, also Pl. XXIV).

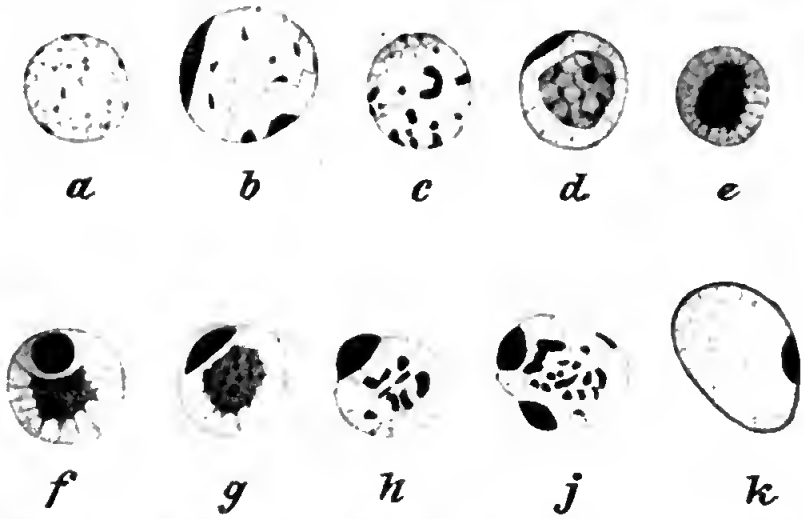
These compact spheres of chromatin are extruded from the nucleus into the cytoplasm and there degenerate. I have studied but little the minute animals in the rectum of the frog, before their encystment, and have but twice found in them the extrusion of the first chromatin sphere (Fig. 124, Pl. XXII; 279 [*O. dimidiata*], Pl. XXVII); I think, though, this usually occurs at this stage, as NERESHEIMER has said. In the cysts (Fig. 253, Pl. XXVI, *O. caudata*), and in young forms hatched from the cysts in the rectum of the tadpole (Fig. 143, Pl. XXII, and *O. dimidiata*, Pl. XXVIII, Figs. 306,

308, 309), I have often seen one or two compact chromatin spheres already extruded and lying in the cytoplasm, or in the process of being extruded (Fig. 302, Pl. XXVIII, *O. dimidiata*). In animals which, without being encysted, have been ingested by the tadpoles and have passed unencysted through the whole alimentary canal to the rectum, one often finds the extrusion of great masses of chromatin from the nuclei. These masses, sometimes before, but generally just after their extrusion, become reticulated, with lighter areas in the meshes of the heavily stained chromatin net (Figs. 237—247, Pl. XXVI). In some cases the nuclei from which the chromidia have been extruded show very distinct chromosomes (Fig. 238, 239, 241). In other cases irregular chromatin masses are left in the nuclei (Fig. 240). In still other instances the chromatin left in the nucleus is very finely granular (Fig. 245). In Fig. 241, in the posterior end of the body, is shown a hollow sphere of net-like chromatin surrounding an unstained central sphere. In many degenerating nuclei of *O. obtrigona* exactly similar structures were found (Figs. 104—109, 111—115, Pl. XXI). In a few instances, when the staining was exactly right, I have seen that the chromatin spheres in the cysts were composed of a net-like, darkly staining envelope surrounding a central sphere (Fig. 133, Pl. XXII: the central sphere was present but is not indicated in the drawing which shows the reticulated surface of the sphere). I have not yet attempted to test microchemically the nature of these central spheres. The unencysted ingested animals just described showed very clearly that the chromatin spheres fragment and scatter through the cell, there disappearing. It is not quite certain that always two chromatin spheres are extruded, but that there are usually two appears certain from the phenomena observed. It seems altogether probable that we have in these phenomena a throwing away of nutritive chromatin similar to that described by Hertwig, Schaudinn and others for numerous *Plasmodroma*.

I have described the formation of chromatin spherules during the course of each mitosis throughout the year (except perhaps just before and after copulation) and have suggested that these chromatin spherules are nutritive — comparable to the granules of the macronucleus of higher *Ciliata*. Their formation and extrusion is positively useful, being probably connected with nutrition and perhaps with the formation of the refractive spherules of the endosarc. The formation and extrusion of the large chromatin spheres before copulation is apparently negatively useful, leaving the nuclei in the right condition for copulation. It seems likely that the chromatin

spheres are composed of nutritive chromatin essentially similar to that seen in the numerous small chromatin spherules throughout the year.

LÖWENTHAL (1904) describes the presence and manner of formation of one or more large dense chromatin spheres in the nuclei of encysting *O. ranarum* (Text Fig. X). He did not observe their extrusion into the cytoplasm. He interprets them as homologous with micronuclei, whereas they are probably more comparable with



Text Fig. X. LOEWENTHAL's figures of the formation of a "micronuclens-like body" in the nuclei of *O. ranarum*: *a*, a nucleus with chromatin net and nodal thickenings; *b*, a nucleus with the chromatin gathered mostly into large masses at the periphery; *c*. shows the chromatin masses again fragmented; *d* and *e*, show the gathering of the chromatin at the center of the nuclens; *f*—*h*, show the separation of a compact darkly-staining chromatin sphere from the central mass, and its wandering to the periphery and suggest its possible extrusion from the nuclens: the material remaining in the central mass fragments and scatters through the nuclens; *j*, shows two chromatin masses at the periphery of the nuclens; *k*, a nucleus figured but not described by LOEWENTHAL. × 2250 diameters.

macronuclei, being probably composed of nutritive chromatin. I have not studied the process of formation of these chromatin spheres with sufficient care to justify me in commenting upon LÖWENTHAL's description of the manner of their formation. I would only suggest that it must be difficult to be certain of the sequence, of the isolated phenomena observed.

NERESHEIMER (1906 and 1907) gives an account of these chromatin spheres essentially similar to that I have given above. He emphasises the comparison of the two spheres with the two polarbodies of *Metazoa* (*cf.* p. 302).

It is interesting to note that at least some of the individuals which pass unencysted through the alimentary canal of the tadpole to the rectum [1]) form and extrude chromatin spheres but not quite in the normal manner. I have not observed that they encyst if living in the natural species of tadpole. I have, however, had such individuals of *O. intestinalis* and *O. caudata* encyst in the recta of large tadpoles of *Rana esculenta* four days after the infection was secured, and have successfully infected tadpoles of *Bufo vulgaris* with these cysts from the *Rana esculenta* tadpoles. In two series of sections of the rectum of an tadpole of *Bombinator pachypus* infected six days with *O. intestinalis*, I find numerous large individuals with eight chromosomes, and numerous smaller forms with four chromosomes, extruding their chromatin. Perhaps in the rectum of the frog extrusion of the chromatin may occur either before or after the reduction in the number of the chromosomes, though usually, if not always, under normal conditions the extrusion occurs after the number of chromosomes has been reduced.

Encystment.

The number of nuclei in the cysts varies with the species and within the same species. In one series of preparations of *O. ranarum* out of 146 cysts tabulated 1 had no nucleus (abnormal), 25 had 1 nucleus of the ordinary size, 70 had 2 nuclei, 32 had 3 nuclei, 16 had 4 nuclei, 1 had 5 nuclei and 1 had 6 nuclei. According to Neresheimer (1907) three to five nuclei are most frequent. Six to twelve or more nuclei in the cysts are described by Zeller, Tönniges (1899), Loewenthal and Neresheimer (1906 and 1907), though these larger numbers are infrequent. In *O. obtrigona* (Zeller) and *O. dimidiata* (Zeller, Neresheimer, Metcalf 1907 *a*) conditions are the same (Figs. 285—288, Pl XXVII). In the binucleated species *O. saturnalis* (Leger & Duboscq), *O. intestinalis* (Zeller, Metcalf) and *O. caudata* (Zeller, Metcalf) the cysts have generally one nucleus (*O. intestinalis*, Figs. 130—136, 140—143, Pl. XXII; 236, Pl. XXV; *O. caudata*, Figs. 137—139, Pl. XXII; 252, 253, Pl. XXVI) though I have often found two nuclei in the cysts of the latter two species (Figs. 254, 255, Pl. XXVI). Occasionally one finds the binucleated encysted animal in the process of division (Fig. 255), though I do not think the division can often be completed until the animal emerges from

[1]) Compare the chapter on infection experiments, page 314.

the cyst, the free action of the cilia apparently being of great assistance in all divisions at all times of year, helping to separate the daughter cells. I have, however, found, two cysts of *O. caudata* each containing two individuals either entirely distinct or so nearly so that the connection between them could not be observed (Fig. 256, Pl. XXVI). Zeller and Loewenthal describe cysts of *O. ranarum* containing individuals in division, and Doflein quotes Przesmecki as having seen the animals in the cysts divide into several offspring. I have seen nothing of this multiple division in the cysts.

The cysts do not need to lie in water in order to produce successful infection.

Tadpoles eat, often eagerly, the foeces of frogs and toads, so that it is easy to infect them. After feeding cysts to the tadpoles the cysts will be found throughout the whole alimentary canal including the rectum. The little *Opalinae* leave the cysts usually in the rectum of the tadpole but occasionally they are found in the small intestine. Wherever hatched the little *Opalinae* collect at the upper end of the rectum of the tadpole, just as the larger forms do in the rectum of the frog or toad. They mostly keep together, lying between the foecal mass and the rectal wall, a few individuals only being found scattered through the foecal mass.

I have studied the spring reproduction in *O. intestinalis*, *O. caudata* and *O. dimidiata*, and will describe the phenomena for all three species beginning with *O. intestinalis*.

The minute forms of *O. intestinalis* ready for encystment do not have the body form characteristic of larger individuals, but look more like *Amoeba limax* (Figs. 121—129, Pl. XXII). They are ciliated, but the cilia are unusually delicate, being distroyed by acetic carmine, while the cilia of larger individuals in the same preparations are only considerably injured. The narrow posterior end of the body often shows a peculiar minutely lobulated appearance similar to what one often sees at the posterior end of an actively moving *Amoeba proteus* (Figs. 122, 123, Pl. XXII). Ectosarc and endosarc are distinct and each contains the usual sphernles. The last division before encystment is almost always longitudinal (Figs. 121, 122, Pl. XXII) but possibly may sometimes be transverse (Fig. 123, Pl. XXII). I have not had the good fortune to see the whole process of encystment in any species. Zeller describes the process for *O. ranarum* as follows (p. 359). — "*Die Tierchen schwimmen nur noch eine Zeitlang mit großer Lebhaftigkeit umher, dann aber werden sie zusehends langsamer in ihren Bewegungen, ziehen sich kugelförmig*

zusammen und scheiden, indem sie sich dabei schneller oder langsamer drehen. eine farblose, glashelle Cyste um sich ab."

The cysts vary considerably in size. They are mostly spherical or nearly so, some are oval (Fig. 130, Pl. XXII), and a few somewhat irregular in form. One frequently sees encysted animals many of whose ectosarc spherules are at the extreme outer edge of the ectosarc (Fig. 134, Pl. XXII), and in a few instances, I have seen cysts in which, between the cyst wall and the ectosarc, there were numerous refractive globules which seemed to be extruded ectosarc spherules (Fig. 135, Pl. XXII). It is easy to see in some cases a mass of granular debris left behind, in the cysts which the animals are leaving (Fig. 140, Pl. XXII). Other cysts are left entirely empty when the animal hatches, no such debris being found (Figs. 142, 143, Pl. XXII). It seems as if some individuals, during encystment, got rid of an excess, through generally not of all, of the ectosarc spherules.

Cysts of *O. ranarum* stained with Moore & Breinl's lithium-iron-haematoxylin often show the presence of endosarc spherules. On the other hand many cysts contain no endosarc spherules. In minute individuals ready for encystment and in slightly larger forms, similar diverse conditions are seen. In the binucleated species and in *O. dimidiata* most if not all of the animals hatching from the cysts contain endosarc spherules. The presence or absence of the spherules in *O. ranarum* is probably dependent on nutrition.

It happens that none of my sketches of *O. intestinalis* show binucleated cysts, though I have seen many. Fig. 124, Pl. XXII, shows a binucleated individual in the process of encystment.

Before hatching from the cysts the little *Opalinae* become very active, the speed of their revolutions increasing until one becomes fairly dizzy as he watches them. Cysts in the duodenum of the tadpole may contain these very active animals, indicating that hatching may take place soon after ingestion. Much more frequently hatching occurs in the posterior part of the intestine or in the rectum. The cyst wall weakens at some point and the little *Opalina* squezes through, sometimes slowly, sometimes rapidly, and swims off with a very rapid motion quite different from the motion of other individuals. The newly hatched individuals have well developed cilia, longer in proportion to the size of the body than are the cilia of larger forms Figs. 140—144, Pl. XXII). The cysts begin to hatch within three hours of the time of their ingestion, probably even earlier.

Leger & Duboscq (1904*a*) describe a second type of "endogenous cysts" for *O. ranarum*. In an ordinary multinucleated individual, a bit of protoplasm containing one to four nuclei is said to isolate itself from the rest of the protoplasm and acquire a cyst wall. The cyst so formed is extruded from the body of the parent. This description, which is very brief and unillustrated, needs confirmation before it can be accepted. No other student of *Opalina* has seen anything of this sort.

The formation of the gametes.

Longitudinal division is observed almost as soon as one finds the animals hatching from the cysts, (Figs. 146, 147, 153, Pl. XXII). I have found no way of determining how many divisions take place before the gametes are formed. The minute animals do not live long enough outside the body of the tadpole to allow one to directly follow the phenomena from the time of hatching from the cyst to conjugation. Size is not a safe criterion, for the cysts and the animals that hatch from them are of various sizes; so also are both the micro- and macrogametes. Time relations have failed to determine the point, for one cannot be certain how long a time is required for one division, and the time when one observes copulation is different in different infections.

In animals removed from the tadpole and placed in salt solution with a bit of the rectal wall and some of the rectal contents, divisions just begun before removal from the host require generally from two to twelve hours to complete even under the most favorable conditions; in many other cases division is not completed at all, even pairs which upon removal from the host were almost distinct remaining unseparated after more than twelve hours. It is not improbable that division under natural conditions in the rectum may be more rapid than in even the most favorable artificial cultures. In one series of infections both micro- and macrogametes were abundant after forty-two hours and frequent instances of copulation were observed. Generally copulating pairs are abundant fifty to eighty hours after ingestion of the cysts. In material five and a half days after feeding the cysts I have found many zygotes but not copulating pairs. The average time required between ingestion of the cysts and copulation is therefore uncertain, as is also the number of divisions that intervene, if indeed the number be constant. In the multinucleated *Opalinae* the number of nuclei in the

cysts varies from one to twelve, so probably the number of divisions intervening between hatching from the cyst and the formation of definitive gametes is not constant. We have already seen that the condition of the nuclei in different cysts is different, indicating that the time required after hatching to produce the ripe gametes may be different in different cases.

As a result of the divisions following emergence from the cysts, two sorts of gametes are formed 1) macrogametes which do not markedly differ from the individuals of the asexual generation[1]) and 2) microgametes of very minute size and peculiar form. The macrogametes differ from the asexual forms in being smaller and in having relatinly longer cilia (Figs. 144, 148, Pl. XXII; 164, Pl. XXIII). They have one (Figs. 164—168, Pl. XXIII; 183, 185, Pl. XXIV; 210, Pl. XXV) or two (Figs. 170—180, Pl. XXIII) nuclei. Probably the typical fully mature gamete would be uninucleate, but copulation may occur before the final division which produces the uninucleate condition. The nuclear phenomena following copulation will soon be described. The macrogametes have large excretory organs, often very clearly seen in these small bodied forms (*cf.* Metcalf 1908*b*). In the Opalinas, at all times of year, the excreta, which are for a time dragged behind the body, are sticky. They are no less so in the macrogametes (Figs. 147, 153, Pl. XXII), but this stickiness of their excreta is not a phenomenon comparable in any way to the stickiness of *Paramaecium* when ready for conjugation (Calkins 1906) and has no relation to copulation.

The microgametes are much smaller (Fig. 163, Pl. XXIII). Their cilia are few in number and are long and weak. They are absent from the posterior end of the body, which is drawn out into a long and slender tail which is bent, generally at right angles, near its base, at a point usually about midway in the whole length of the body. This bend in the tail probably aids in securing spiral movement in swimming. Near the tip of the tail is usually a small swelling. Perhaps this is always present in functional microgametes. The tail appears homogeneous and transparent. It seems to be composed of ectosarc alone. It is very sticky. One imagines that the little swelling near the tip is a special accumulation of the sticky material, but the animals are so small that it is not easy to find conclusive evidence of this point. The stickiness of the microgametes is

[1] *Opalina* has no true alternation of generations, so that this term whil convenient to use, is not strictly accurate.

clearly an adaptation to copulation and is probably comparable to the stickiness of the isogametes in *Paramaecium*.

The microgametes usually swim tail foremost, though sometimes one finds them swimming in the opposite way. Doubtless the habit of swimming with the sticky end of the body foremost is an adaptation to copulation, helping the gametes to become attached. I think that the microgametes which an found swimming with the tail behind lack the ball of sticky material near the tip of the tail. In every case in which this point was noticed it was found to be so, but the observations are too few to make one certain that mature and immature microgametes can always be distinguished by their mode of swimming. The microgametes contain but one nucleus. This is usually difficult to see in the living animal, though sometimes it shows clearly. No excretory organs or extruded excretory granules are seen in the microgametes, but the extruded granules are sometimes found in the mother-cells from which the microgametes arise and in individuals of the preceeding generation. Endosarc spherules are present in the microgametes (Fig. 161, 163, Pl. XXIII). Ectosarc spherules are found in the microgametes of *O. caudata* (Fig. 259, Pl. XXVI) and *O. dimidiata*, and are doubtless present in the microgametes of *O. intestinalis* also, though I find nothing in my notes upon this point.

The gametes arise by longitudinal division in every case which I have observed (Figs. 146, 147, 143, XXII, 159, Pl. XXIII). Apparently transverse division does not occur between the time of hatching from the cyst and copulation, though it might be about as frequent as in the asexual generation and still very likely not be observed. There is nothing of special note in the divisions which result in the formation of the macrogametes. Division begins almost always at the anterior end (Fig. 146, 153, Pl. XXII) rarely at the posterior end (Figs. 207, 209, Pl. XXIV) sometimes at both ends at the same time (Fig. 147, Pl. XXII). The strand of tissue, which ultimately is all that is left connecting the two daughters, may lie at any level in the posterior two-thirds of the bodies; usually it is quite near to the posterior end.

In the formation of the microgametes the divisions for at least two generations do show some divergence from the ordinary divisions. The daughter cells are more slender than usual and they seem to have unusual difficulty in pulling apart. The division begins apparently always at the anterior end of the body and moves backwards until the daughter cells are connected only by the extreme

posterior tips of the bodies (Fig. 159, Pl. XXIII). The daughter cells, by attempting to swim in opposite directions, draw the posterior ends of their bodies out into slender points before they finally separate (*cf. O. dimidiata*, Figs. 307—309, Pl. XXVIII). The very pointed animals which thus arise (Figs. 154, 155, 160, Pl. XXIII) can by this feature be distinguished from the macrogametes and the cells from which the latter arise. This division required in some observed instances from two to fonr hours, reckoning from the first appearance of bifurcation of the anterior end of the body until the complete separation.

A second division of the same type follows. It may begin in one of the danghter cells before the last division is complete (*cf. O. dimidiata*, Figs. 308, Pl. XXVIII). I have never seen both daughter cells so dividing again before their separation. One cannot say how many divisions of this sort occur before the definitive microgametes resnlt. In the final division which forms the microgametes a very long and very slender thread is drawn out between the two danghter cells. It seems as if the animals become more sticky at their posterior ends and so have more difficulty in separating. One can watch the elongation of this thread until it becomes finer than one of the cilia of the body. It may reach a length more than twice as great as that of the body proper of one of the animals. Generally, on this thread, the point of original contact of the two bodies is indicated by a few granules resembling excretory granules with sometimes a little debris. One can thus determine that the elongation of the two bodies is frequently unequal, even as much as two-thirds of the thread coming from one daughter-cell. It seems probable that the tail of the microgamete is derived from this thread. In several cases in which I saw one danghter-cell dividing before it had completely separated from its fellow, the dividing cell had by far the longer tail. In each instance the undivided cell was somewhat pointed posteriorly, but had practically no tail (*cf. O. dimidiata*, Fig. 308, Pl. XXVIII). It is possible that the gametes are formed by differential division, the two sorts diverging at least one generation before the formation of the definitive gametes. This is, however, merely a suggestion with very insufficient evidence in its favor.

There are two sorts of tailed forms 1) larger ones with short straight tails (Figs. 154, 155, Pl. XXIII, *cf. O. dimidiata*, Pl. XXVIII, Figs. 307, 308) and 2) smaller forms with much longer tails usually bent at a right angle (Fig. 163, Pl. XXII). Only the latter have

the ball of sticky material near the tip of the tail. These are the true microgametes. I have seen individuals of the first type in a late stage of division and have followed the division to its completion seeing two forms with very long tails arise from one short-tailed form (Figs. 159, 160, Pl. XXIII). I have never seen the transformation of such danghter-cells with long straight tails into typical microgametes with bent tails bearing a ball of sticky matter near their tips. This transformation does not occur immediately after the division. It seems to be the tailed microgamete mother-cells which NERESHEIMER has described as the gametes of *O. dimidiata.* I do not find either these or the true gametes of either type "*ganz platt*", as he describes them. All are generally circular in cross section, thongh they may be broadly oval. The microgametes vary in size; so do the short-tailed forms from which they arise. The largest of the true microgametes are nearly or quite as large as the smallest of the short-tailed forms.

In my preliminary notice (METCALF 1907) I wrote "The tailed gametes are of two sizes, one about twice as large as the other, the smaller being found from the larger by longitudinal division". This was probably an error. There are larger and smaller microgametes, the largest being fully twice as large as the smallest, but it is doubtful if the latter arise from the former, both probably arising by division of the short-tailed forms, as described. In one case, I have seen, in *O. dimidiata*, a microgamete mother-cell, in process of division, attached by its unusually long straight tail to the center of the body of a uninucleated macrogamete no larger than the microgamete mother-cell (Fig. 313, Pl. XXVIII). The attachment was a firm one, lasting over three quarters of an hour while the animals were actively swimming. During this time no change occurred. The individuals were then lost. This seems to have been probably an abnormal attempt to copulate on the part of a microgamete mother-cell. I donbt if it wonld have been successful or if it indicates that fully formed and functional microgametes are accustomed to divide.

Both sorts of gametes are often numerous in the rectum of the tadpole. The forms resembling macrogametes are far the more numerous, probably in part becanse one cannot distinguish the definitive macrogametes from forms destined to divide further. The largest number of microgametes seen in one rectum was seventy eight; in the same rectum the number of pairs was forty-two; and the number of the larger, tailed forms (parent-cells requiring probably

bnt one more division to form definitive microgametes) was twenty-two. Similar proportions, hnt with smaller numbers, were freqnent.

Copulation.

In copnlation a microgamete fuses completely with a macrogamete (Figs. 210—217, Pl. XXV, also 164—182, Pl. XXIII). The first contact is purely accidental, there being no evidence of any attraction of the gametes for one another. NERESHEIMER describes the short-straight-tailed forms in *O. dimidiata*, which he interpreted as gametes, as circling aronnd each other, as if under the influence of some mutnal attraction. May this not have been merely the usual spiral movement seen in all *Ciliata* and *Flagellata*, appearing circular in this case hecause confined hy the slide and cover-glass almost to one plane? The microgametes cling to anything which they tonch with their sticky tails, thongh they seem never to cling to one another. Indeed I helieve I have never happened to see two of them in contact even for a moment.

The animals in the rectum gather chiefly in a single group (sections of the recta show this clearly). They have the same habit of gathering in groups in the slide cultures, collecting usually ahont some bit of foecal matter. In such a group there are often thirty to one hundred forms which look like macrogametes and perhaps a dozen microgametes. The latter are constantly striking the macrogametes, clinging to their cilia and again hreaking away, either because of the active swimming movements of the macrogametes, or hecause of violent contact with other individuals. Even trne copnlating pairs may be torn apart by other individuals swimming between them. In two such instances copulation between the intrnder and the microgamete immediately followed. Frequently two (*O. caudata*, Fig. 271, Pl. XXVII) and once three microgametes were seen attached to the cilia of one macrogamete. Microgametes were seen attached to individuals in an advanced stage of division to form two macrogametes (*O. caudata*, Figs. 269, 270, Pl. XXVII). They seem readily to attach to any of the individuals of the macrogamete type.

The loose attachment of the microgamete to the cilia of the macrogamete changes to the closer union and ultimate fusion of copulation (Figs. 210—217, Pl. XXV, various, stages on Pl. XXIII, *cf. O. caudata*, Pl. XXVII). The tail of the male fuses at its tip with the hody of the female: then the tail of the male becomes

shorter and thicker and, after from half an hour to an hour, the two bodies are almost completely fused. Frequently one can distinguish for quite a time upon the zygote a slight protuberance bearing a few weak cilia of the microgamete type, thus marking very clearly the point of fusion, which may be in any region of the body (Fig. 182, Pl. XXIII, *cf. O. caudata*, Figs. 265, 268, Pl. XXVII). I have seen all possible stages in the copulation, have three times followed the whole process from the first contact (observed) to the complete fusion, and have many times followed the process through the earlier or later or middle period and have then killed and stained the animals in order to observe more closely the nuclear phenomena.

The macrogametes may have either one or two nuclei (Pl. XXIII). The microgamete has always but one. When the macrogamete is uninucleated the nucleus may be in the resting stage (Fig. 164, Pl. XXIII) or in mitosis (Figs. 167, 169, 170, 182, Pl. XXIII); similarly the two nuclei in the binucleated females may be either resting (Figs. 171—173, 178, Pl. XXIII) or in mitosis (Fig. 180, Pl. XXIII). I have never seen the nucleus of the microgamete in mitosis before the complete fusion of the two bodies.

When the macrogamete has but one nucleus this unites obliquely end to end with the nucleus from the microgamete (Figs. 187—196, Pl. XXIV). The two nuclei apply themselves closely, and ultimately the double membrane between them breaks down (Figs. 191—193), sometimes first in the middle sometimes first at one edge.

If the macrogamete has two nuclei, conditions become more complicated. If the two female nuclei are in the resting condition, the male nucleus may fuse with either one. In one instance of copulation of this type I have watched the entrance of the male nucleus and have seen it fuse with the posterior nucleus of the macrogamete, the resultant fusion nucleus being considerable larger than the other nucleus in the same zygote (Figs. 174—177, Pl. XXIII). I had a single acetic-carmine preparation which suggested that the male nucleus had entered and had passed by the posterior nucleus of the binucleated macrogamete and was just uniting with its anterior nucleus (Fig. 186, Pl. XXIV). Sometimes one sees the male nucleus lying between the two female nuclei, generally nearer to the anterior one (Figs. 197—200, Pl. XXIV). In one lot of fine material from an eighty-eight hour infection I found that over fifty of the binucleated forms had one nucleus (almost always the anterior one) much the larger. In some of these larger nuclei it was easy to see

eight chromatin masses which were donbtless eight chromosomes (Figs. 201—203, Pl. XXIV), showing that the nuclei were syncaria resulting from copulation.

Copulation may occur while the single nucleus of the macrogamete is in division. In this case the male nuclens waits until the division is complete and then fuses with one of them, the anterior one in the best case I have observed (Fig. 200, Pl. XXIV).

Both nuclei of the macrogamete may be in division when copulation takes place. One preparation showing this condition was very clear (Fig. 204, Pl. XXIV). The male nuclens, by its longitndinal striation and the position of its chromosomes in two polar gronps, showed that it also was dividing. A second preparation shows a zygote in division with each of the femali nnclei already divided, giving fonr daugther nuclei and the male nucleus, in an early stage of mitosis, lying beside them (Fig. 208, Pl. XXIV). Under perfectly normal conditions division of the cell-body of this animal shonld have occnrred before the complete division of the nuclei. Another preparation of a daugther cell [1]) just from division and whose nnclens was in a little later telophase than the female nuclei in the next to the last case, showed a male nuclens lying against the constricted portion of the dumbbell-shaped female nucleus (Fig. 206, Pl. XXIV). This male nucleus was in a very early anaphase stage of division. It mnst have entered before the division of the macrogamete and have passed to one of the daughter cells (*cf.* Fig. 204, Pl. XXIV).

I have seen two instances of another condition which may possibly stand as the next term in this series of copulation conditions. These individuals seemed each to show four nuclei. In one instance the four nnclei were in an oblique row (Fig. 207, Pl. XXIV). As the posterior end of the body showed a slight division-furrow, it is somewhat donbtful if this was a zygote. It may possibly have been a dividing binuclear macrogamete in which the dumbbell-shaped nuclei overlapped, somewhat more than in Fig. 204, Pl. XXIV, producing in edge view the appearance of fonr nuclei. The preparation, stained with acetic-carmine was not entirely clear. The other instance was of a living animal, a dividing macrogamete, with a considerable division furrow at its posterior end (Fig. 209, Pl. XXIV). It seemed to show four unusually small nuclei side by side in pairs, but the pictnre was not very clear. Both of these

[1]) That it was a daughter cell fresh from division was indicated by the irregular contour of one side, the side by which it had been attached to its sister cell.

cases are so doubtful that they can hardly be taken into account in endeavoring to understand copulation. They may also have been abnormal, division of the body being delayed beyond the proper time, giving nuclear relations not found in perfectly normal cells. In all other cases described the phenomena were clear.

It is uncertain what would have been the further history of each type of zygote described. When the male nucleus unites with one of the nuclei of a binucleated macrogamete, it seems probable that the next division would separate the syncarion from the smaller nucleus, one going to each of the daughter cells, but I have not observed the division of these forms. If the binucleated zygotes do divide in this way, one sees no reason why the daughter cell which receives the nucleus with four chromosomes might not itself fuse with another microgamete to form a uninucleated zygote, just as a polar body may be fertilized, but I have made no observations bearing upon the point. If it does not do so, it would seem to indicate that changed cytoplasmic conditions due to the entrance of the microgamete stand as a bar to further copulation. Professor BOVERI tells me he has never succeeded in fertilizing a nonnucleated fragment of an already fertilized sea-urchin egg, though it is easy to fertilize fragments of unfertilized eggs. Sufficient study of old infections in *Opalina* would probably determine this interesting point. The comparison of these binucleated macrogametes with an egg, which has not yet completed its maturation seems a correct comparison, for doubtless the typical fully-formed macrogamete in *Opalina* would be uninucleated. Entrance of the male cell in *Opalina* seems to occur either after or during the maturation divisions, as in *Metazoa*.

The later history is much more doubtful in those cases in which the male nucleus divides before fusing with any of the female nuclei (*cf.* Fig. 204 and Fig. 206, Pl. XXIV). Fig. 206 seems to interpret Fig. 204 to the extent of showing that the dividing male nucleus all goes to one of the daughter cells. Apparently there it must so behave as to give four of its eight daughter chromosomes to each of the daughters of the female nucleus, but just how this is effected we cannot say. It might be either by fusing while the female nucleus is still incompletely divided, or by waiting until both nuclei are completely divided and then the four daughter nuclei conjugating in pairs. I hope to find in the sections of infected recta of the tadpoles answers to some of the questions still unsettled, but the preparation of the sections is difficult because of the dirt in the

rectum and their study with an immersion lens necessarily takes much time. I am, therefore, not delaying the publication of this paper until all of these sections have been made and studied. If from this study further results of interest are obtained, they will be communicated later.

In the material from the older infections one finds very many peculiar nuclei of huge size, much more pointed than usual and with peculiar spindles and chromatin (Figs. 222—227, Pl. XXV). It seems probable that these are copulated nuclei (syncaria). This is strongly suggested by such a condition as we see in Fig. 188, Pl. XXIV, in which we see the very pointed distal ends of the nuclei developed even before the two nuclei have fused at their apposed surfaces. I have not yet found such a spindle form in each nucleus in binucleated individuals, or in one nucleus while the other nucleus is in division according to the regular "asexual generation" type. I have however often found uninucleated gametes in a late stage of nuclear division in which the forming daughter nuclei were both of the peculiar type just described (Figs. 226, 227, Pl. XXV). It is doubtful whether the spindle form of these nuclei indicates division. The syncaria in many species of Protozoa are spindle shaped when not in division.[1]) It is probable that such of my material as is carefully preserved does not include old enough infectious to determine fully the phenomena following the copulation of the nuclei.

Cohn (1904) has described fundamentally different phenomena in the conjugation of *O. intestinalis*. It is evident that the animals seen apparently in conjugation could not have been *Opalinae*. The character of the nuclei, as well as the manner of the conjugation shows this. The brief unillustrated description of conjugation in *O. ranarum*, given by Léger & Duboscq (1904 *a*) is also fundamentally different and is probably erroneous. They say that two *Opalinae* "resembling those of the ordinary cysts" come together by their anterior ends, lie for a long time rubbing against each other and turning, and then from a cyst which contains the two, each animal occupying half of the cyst. These phenomena are so divergent from those observed by other students that the description can hardly be accepted without confirmation. It is possible that Léger & Duboscq mistook the encystment of a dividing individual for conjugation, though with such experienced observers this seems improbable.

[1]) Somewhat similar spindle-shaped nuclei of peculiar appearance are found in degenerating *O. obtrigona*.

Neresheimer (1906, 1907) saw both microgametes and macrogametes and saw them in copulation (Compare his Fig. B, on p. 26. One of his drawings from this figure is reproduced here in Text Fig. XI), but he failed to recognise this as copulation, interpreting it as abnormal budding. The forms which he describes as isogametes seem from his figures to have been microgamete mother-cells. He once saw two of these come together by their anterior ends and gradually fuse, closing together like the blades of a pair of shears. The nuclear phenomena in this case were not followed. This he took as showing the presence of isogamous copulation. Doubtless it was abnormal. In one instance I have found in an acetic carmine preparation two short-tailed forms of *O. caudata*, apparently microgamete mother-cells, attached, the tail of one being united to the side of the other (Fig. 276, Pl. XXVII). This instance, with the single instance which Neresheimer describes, seems to indicate that very rarely microgamete mother-cells of the same size may unite. I have in one instance in *O. intestinalis* seen a microgamete attached by the whole anterior half of the body to a macrogamete, the tail of the microgamete, being free (Fig. 181, Pl. XXIII). Neither the previous nor the subsequent stages of this copulation were seen. It was of course abnormal in the sense of being a departure from the regular method of copulation, but it may not have been pathologic. It is similar to the instance that Neresheimer describes in that the male was not attached by its tail as in every other of the several hundred instances of copulation I have seen.

Text Fig. XI. One of Neresheimer's figures of "abnormal division in the formation of the gametes in *O. dimidiata*". This was probably the attempted copulation of three microgametes with one macrogametes.

Chromatin spherules in the gametes and zygotes.

In the mature nuclei, after the formation and extrusion of the chromatin spheres, I have not observed the formation of chromatin spherules, nor have I seen their formation in the syncaria soon after copulation. I cannot say positively that there is an interval when they are not formed, but this seems to be the case.

Nucleoli in the gametes and zygotes.

I have failed to find nucleoli in the nuclei of the gametes and young zygotes. In older zygotes they are present.

Can the heterogamous copulation described be abnormal?

When I first saw heterogamous copulation in *Opalina*, it seemed possible that it might be abnormal, for several reasons: — 1) because it was observed in material that had been about three hours in a slide culture; 2) because the cysts from which the infection was secured had not lain in water before being fed to the tadpoles; 3) because there were present in the culture individuals of different sizes which had passed unencysted through the alimentary canal of the tadpole to its rectum. All doubt however was later removed. One of my best series of infections was secured from cysts which lay 36 hours in water until all the unencysted *Opalinae* with them were killed. When opening, under the microscope, the recta of the tadpoles infected from these cysts, I very often immediately found typical heterogamous copulating pairs in different stages of copulation. Indeed nearly half of the drawings of gametes and zygotes of *O. intestinalis* on the accompanying plates were made from this series of infections. Other good infections were made in the same manner, giving similar results. Six instances of heterogamous copulation have since been found in a series of sections of the rectum of a tadpole infected sixty hours with *O. intestinalis* (Figs. 183—185, a, Pl. XXIV).

Encystment following copulation?

Neresheimer has described encysted individuals which he regards as zygotes. He believes that encystment normally follows copulation. The zygote cysts, according to his description, are of the same size as the infection cysts, but are distinguishable by their usually spindle-shaped nuclei, by the fact that the animals more nearly fill the cysts, and by the faint concentric striation of the contained body. Neresheimer saw individuals containing two spindle-shaped nuclei become quiescent, change to oval form and throw off part of their cilia, and he interprets these changes as the early phenomena of encystment. Engelmann had already described for *O. ranarum* cysts with large nuclei in the rectum of the tadpole. Zeller described, for the multinucleated *Opalinae*, multinucleated cysts in the rectum of the frog and uninucleated cysts with large nuclei from the rectum of the tadpole. Influenced by the observations of these three students, I fully expected to find encysted zygotes in the recta of the tadpoles, but I have not seen them for any of the three species studied.

It seems to me that the encystment, which I have often seen in dying zygotes and in dying individuals of other sorts, is abnormal. In material from infected tadpoles, which has been kept too long in slide cultures, one sees very many individuals of all sorts rapidly change the form of the body, becoming first oval, then spheroidal. Following this change of form, they throw off most or all of their cilia with many of their basal granules, and extrude part of their protoplasm in the manner so characteristic of *Opalina* when under pressure (Figs. 219—221, Pl. XXV). The pellicula remains as a very delicate cyst. Within this the body becomes very transparent and the nuclei and their contained chromatin become very clearly visible. Observe especially the granular chromosomes in figures 219 and 220 which show such pseudoencysted individuals drawn from life, or rather death (*cf. O. caudata*, Fig. 275, Pl. XXVII). Figs. 210—218, Pl. XXV, show successive conditions in copulation and pseudoencystment in one pair of gametes from material of O. *intestinalis* forty-two hours after infection. In this case, after complete fusion of the two bodies, the male nucleus broke down, entirely disappearing in the cytoplasm of the zygote. The female nucleus remained long intact, showing considerable changes in the character and arrangement of its contents. Fig. 220 shows the condition in another pseudocyst of *O. intestinalis*, formed after the union of a binucleate macrogamete and a microgamete. The four granular chromosomes of each female nucleus were very clear. The male nucleus had but a single chromatin mass in one side of which an elongated body, probably a group of contiguous granules, was clearly seen. The excretory vacuole, with its contained excretory granules in Brownian movement, was also clearly seen. It lies uppermost in the figure. Fig. 219, is from a macrogamete of *O. intestinalis* which was found in copulation and was followed through a similar series of changes. In this case the nucleus of the macrogamete was in mitosis. Before copulation was complete the animals separated again and each formed a pseudocyst in the typical manner. There seems no doubt that these instances of encystment and all others observed were all abnormal and pathologic. I have never found encysted zygotes in material from old infections and I believe encystment after copulation does not normally occur.

I have occasionally seen infection cysts of *O. dimidiata*, with oval nuclei or with one of their nuclei oval while the rest appeared spherical (Fig. 286, Pl. XXVII). I doubt if the oval form of the nuclei can be taken as evidence that cysts are copulation cysts

(*cf.* Figs. 136, 138, 139, Pl. XXII). Many of the infection cysts are almost completely filled by the little animals within them (Fig. 130, Pl. XXII). A faint appearance of striation, which is usually spiral but may be concentric, is often found in the infection cysts, being due to the rows of cilia and their basal granules, the direction of the course of the lines being determined by the position of the animal within the cyst. These three features, which NERESHEIMER gives as distinctive criteria between the copulation cysts and infection cysts, seem insufficient to establish the presence of copulation cysts. NERESHEIMER's description may possibly apply in part to the phenomena of abnormal encystment.

On the other hand, the description by ENGELMANN and by ZELLER, for multinucleated *Opalinae*, of uninucleated cysts with large nuclei, in the recta of the tadpoles, seems to indicate the presence of a second type of cysts, which might well be copulation cysts. If, however, there really are such copulation, cysts, I do not understand why I have not found them. The ease with which cross infections are secured in aquaria in which adults and tadpoles of different species of frogs are kept (see the chapter on infection experiments, p. 314) suggests that possibly some cysts of *O. intestinalis* or *O. caudata* might have been present in ENGELMANN's and ZELLER's tadpoles, the infection cysts in these species having usually a single nucleus. I have had collected tadpoles of *Rana temporaria*, *R. esculenta* and *Bufo vulgaris* become infected with both *O. intestinalis* and *O. candata* by leaving them an hour in a jar with adult *Bombinator pachypus*, while on the way from the field to the laboratory. Some of these tadpoles were already infected with Opalinas of the natural species, so that, upon opening them, three species of *Opalina* were found living together. As the cysts of *Opalina* live in water for quite a time without injury, of course such cross infection could be secured by placing tadpoles in aquaria in which the *Bombinator* adults had been placed, perhaps only for a short time, many, days before. Encystment after copulation seems to me doubtful. The matter needs further study.

LÉGER & DUBOSCQ (1904 *a*) describe copulation cysts of an utterly different type in *O. ranarum*. These cysts are said to enclose two distinct gametes which lie side by side, each occupying one hemisphere of the cyst. I can suggest no satisfactory explanation of the phenomena, which were evidently not true copulation.

Phenomena in other species.

Opalina caudata.

In *O. caudata* all the phenomena are like those described in *O. intestinalis*, except that the reduced number of chromosomes in the gametes is three instead of four (Figs. 249, 250, Pl. XXVI), as one would expect from the fact that in the nuclei of full-grown individuals there are six chromosomes. Compare Fig. 248, Pl. XXVI, in which each nucleus is in an early stage of mitosis showing twelve chromosomes ready to arrange themselves in double rows preparatory to migration to the poles. Compare also Figs. 81 and 82, Pl. XX, which represent anaphases and a telophase in mitosis, the number of chromosomes being clearly six.

The cysts have either one or two nuclei (Figs. 252—255, Pl. XXVI), and these show the characteristic chromatin spheres which are thrown off in the process of getting rid of the vegetative chromatin (Figs. 252—254). The gametes and the manner of their formation and the external and nuclear phenomena of copulation are similar to what has been described. (See Figs. 257, Pl. XXVI, to 276, Pl. XXVII. Observe especially the number of the chromosomes in Figs. 273—276.) I have not, however, followed the nuclear phenomena in the zygotes to quite so late a stage as in *O. intestinalis*. Fig. 276, Pl. XXVII, shows the only instance I have seen of the apparent copulation of equal gametes. The drawing is from an acetic-carmine preparation and I do not know what would have been the later behaviour of the animals.

Large chromatin spheres, ready for extrusion from the nuclei, are shown in Fig. 251, Pl. XXVI, in a rather small individual from the rectum of an adult *Bombinator pachypus*.

Opalina dimidiata.

In *O. dimidiata*, which is a multinucleated species, the earlier phenomena are somewhat different. The nuclei are not so easy to study, for they are small and the number of chromosomes is greater, apparently twelve in the full-grown forms. The chromatin is generally arranged in numerous granules just beneath the nuclear membrane, giving a very characteristic appearance (Figs. 277—280, Pl. XXVII). I find no evidence of degeneration of nuclei, or of formation of new nuclei from reproductive chromidia, as Neresheimer

describes. This species shows phenomena exactly similar to those described for *O. intestinalis* and *O. caudata* in the extrusion of the vegetative chromatin, the original nuclei persisting as reproductive nuclei (Fig. 285, Pl. XXVII; 299—306, Pl. XXVIII).

It is evident that the number of the chromatin masses in the nuclei of the minute *Opalinae dimidiatae* in the recta of the tadpoles is much less than in the larger animals in the frog's rectum. The number of chromosomes seems to be either five or six (Figs. 307—309, Pl. XXVIII, indicating ten or twelve chromosomes in the nuclei of the full grown forms. I neglected to study this point carefully in the nuclei of the living animals and in the acetic-carmine preparations last spring, and have not yet worked through my preserved material of the gametes of this species, so that the statement of the reduced number of chromosomes is based on sketches made without that thought especially in mind. There is, however, no doubt that the number of chromosomes is reduced. The only doubt is as to the exact number present.

The cysts have from one to seven or more (Neresheimer twelve) nuclei (Figs. 285—288, Pl. XXVII), and the animals which hatch from the cyst have as many (Figs. 289—292, Pl. XXVII; 299—303, Pl. XXVIII). On this account, as well as because the nuclei are less clear, this species is not so favorable for study. Macro- and microgametes arise as in the binucleated forms. The microgametes are simular to those described for other species, their tails sometimes being of very great length (Fig. 310, Pl. XXVIII). The true long-tailed microgametes arise by longitudinal division from short-tailed forms, as in other species (Fig. 308, Pl. XXVIII). The macrogametes, like the full grown individuals in the asexual generation, are often rather pointed posteriorly. On this account they frequently somewhat resemble the short-tailed mother-cells of the microgametes, but the latter can be distinguished by their fewer, longer and weaker cilia.

Copulation occours between uninulecated microgametes and macrogametes which have one or two (or possibly more?) nuclei (Fig. 312, Pl. XXVIII). The compound nuclei resulting from fusion are unusually large, and they have a characteristic elongated spindle form which may indicate division; it is less pointed, however than that seen in the division of syncaria in the zygotes of the binucleated species. In this species the nuclei are more independent of one another than in the binucleated species, not dividing simultaneously but usually one dividing while the other remains quiescent (Figs. 315, 316,

318—321, Pl. XXVIII). This fact renders the species unfavorable for the study of the nuclear phenomena following copulation.

Neresheimer's Text Fig. B. (page 26) is very interesting. That which he interprets as budding was probably copulation. The second figure seems to indicate the copulation of three microgametes with one macrogamete (my Text Fig. XI, Page 294). I have often seen two males attached to one female, but in every case the attachment was a loose one and not true copulation (*cf. O. caudata*, Fig. 271, Pl. XXVII). In the instance figured by Neresheimer the union seems to be an intimate one indicating true conjugation. It is to be regretted that Neresheimer does not figure the nuclei. If the macrogamete had several nuclei there seems no reason why several male nuclei might not enter at the same time and fuse with them.

Opalina ranarum.

I have not seen the gametes of *O. ranarum*. I have found the multinucleated cysts as described by other students (Fig. 326, Pl. XXVIII). The zygotes show large spindle-shaped nuclei, resembling those of *O. dimidiata*, *O. intestinalis* and *O. caudata* (Fig. 327, Pl. XXVIII).

Further general considerations.

Vegetative chromidia.

The extrusion into the cytoplasm of a portion of the chromatin, preceeding sexual reproduction, seems in all probability to be a throwing off of vegetative chromatin [1]) comparable to that described by R. Hertwig, Schaudinn and others in so many *Plasmodroma*. The disappearance of the macronucleus in conjugating *Ciliata* is propably a similar phenomonen. The solution, and osmotic expulsion from the nucleus, of the chromatin spherules during each mitosis in the asexual generation is probably also a somewhat related phenomenon, the chromatin spherules being probably nutritive, and their extrusion into the cytoplasm probably having to do with nutrition,

[1]) It seems to me altogether probable that there is no fundamental distinction between generative and vegetative chromatin, the latter being derived from the former. Generative chromatin is probably complete perfect slightly specialized chromatin, vegetative chromatin being secondary modified chromatin. If the modification is too great the vegetative chromatin is unable to share in the processes of conjugation and so degenerates. (Compare Hertwig 1907.)

In the latter case the "purpose" of the extrusion is positive, to aid in nutrition: in the former it is more negative, to free the nuclei from that which must in some way be a hinderance to reproduction.

Before comparisons can confidently be made between *Metazoa* and *Protozoa* as to the phenomena preceeding fertilization, the former must be carefully studied in the light of recent work upon the freeing of the generative nnclei in *Protozoa* from nutritive chromatin before sexnal union, and the *Protozoa* mnst be studied more successfully with reference to reduction division. Boveri (1887 *a*, 1892, 1899) has shown the extrusion from the nncleus and subsequent degeneration of a definite part of each chromosome in those blastomeres of *Ascaris* which are to develop into soma cells, but we have no satisfactory understanding of the formation of vegetative chromidia in the germ cells of *Metazoa*. Yolk nuclei are well known. They seem to be somewhat comparable to vegetative chromidia, but their formation seems generally to have a positive value comparable to the formation of zymogen granules in metazoan gland cells, and of the chromatin spherules in *Opalina*, all bring connected with the manufacture of nntritive substances, in the cytoplasm. On the other hand, the degenerating residual chromatin in the germinal vesicles of many *Metazoa* seems more strictly comparable to vegetative chromidia. There is in *Protozoa* a somewhat similar formation of vegetative chromidia which degenerate before sexual union (chromatin spheres of *Opalina*, vegetative chromidia in many *Plasmodroma*), the result of the process being to free the generative nuclei from the nutritive chromatin which seems in some way to be an obstruction to the sexual process.

Reduction.

In the *Metazoa* the ripening divisions in ovogenesis and spermatogenesis are accompanied by a redncton of the number of the chromosomes. Phenomena of this sort have been described in maturing Protozoan cells; by Schaudinn (1904) and v. Prowazek (1905) for Trypanosomes and by Prandtl (1905 and 1906) for *Didinium*. The phenomena in *Paramaecium* are not quite clear, but Calkins & Cull (1907) interpret them as involving reduction. The accuracy of the observations of Schaudinn and v. Prowazek on Trypanosomes has recently been questioned by Salvin-Moore & Breinl (1907), though it is difficult to see upon what grounds, since they did not stndy the species or stages in which the phenomena were described as occnring. Dr. M. Hartmann tells me that Schaudinn's further work,

soon to be published by v. Prowazek, leaves no doubt of the existence of reduction in Trypanosomes. Several as yet unpublished papers by Hartmann and his students show reduction division in *Amoeba* and *Flagellata*. In *Opalina* we find clearly a reduced number of chromosomes in the gametes, and it is easy to see that the usual full number is restored by copulation. I have not found how the reduction is effected, but that it occurs is beyond doubt.

It is interesting to note that the diminished number of chromosomes appears long before copulation, in animals from four to eight times too large for encystment. Four chromosomes are found in the nuclei for at least four generations before copulation, and probably for a still longer period. It is impossible to say how many times the animals divide after leaving the cysts before they are ready for copulation. It is possible that for a time the appearance of fewer chromosomes is due to their bivalence.[1]) If the smaller number is due from the first to true reduction division, and apparently this is true, we have the interesting fact that the condition with a reduced number of chromosomes persists for several generations. In *Metazoa* fertilization immediately follows the maturation divisions.

Neresheimer (1907) has compared the extrusion of the two chromatin spheres in *Opalina* to the formation of the two polar bodies in *Metazoa*, a comparison which seems wholly unjustified.[2]) The chromatin spheres neither of them consist of whole chromosomes, but, like the chromatin spherules, are formed from chromatin given off from all the chromosomes. In some nuclei in which the chromatin spheres are present one sees eight chromosomes remaining in the nucleus, in other cases four chromosomes are found, showing that the formation of these spheres may occur before the number, of chromosomes is diminished and that it is a process distinct from that by which the diminished number of chromosomes is brought about. As a further objection to Neresheimer's interpretation of the chromatin spheres, which he seems to have based only upon their number and

[1]) In the preliminary notice of this work (Metcalf 1907*a*) I wrote "These chromosomes [of reduced number], as seen in the living animals, show about half as many granules as do the chromosomes of the full grown individuals". Further study of carefully stained material in all stages of the spring phenomena is necessary before further treatment of this interesting point will be profitable.

[2]) Neresheimer had already described a series of phenomena which would necessitate the nuclei at this stage being interpreted as purely generative nuclei formed from generative chromidia, which prevented his interpreting the chromatin spheres as vegetative chromidia.

their extrnsion from the nuclei, is the fact that we often find three of these spheres, and that very likely but one is formed in some cases. When only one is seen, it may be, of course, that another was present bnt has already heen extruded.

It may be well to note, hefore leaving this suhject, that, in *Opalina*, each nnclear division is associated which a division of the cell. The phenomena in *Paramaecium* seem less primitive, two cell-divisions heing suppressed during maturation, and as a consequence three nnclei in each gamete degenerating. In *Opalina* all the cells produced by the divisions preceeding copulation are functional gametes as in the spermatogenesis of *Metazoa*. Douhtless the conditions in the matnration of the eggs of *Metazoa* and in *Paramaecium* are secondary.

Relationships of Opalina.

There seems no ground for douhtiug the close relationship of the several species of *Opalina*. Possihly the binucleated species should be placed in one genus and the multinucleated species in another genns. The character of the nuclei, as well as their number, is somewhat different in the two groups. It seems, however, better to retain the genns as at present constituted and to recognise the hinucleated species as a rather distinct subgenus.

The connection of *Opalina* with the *Ciliata* has recently been qnestioned hy Neresheimer (1906 and 1907) who concludes, on the basis of the method of reproduction hy gametes, that *Opalina* is more nearly related to the *Plasmodroma* than to the *Ciliophora*. I find myself unahle to agree with this suggestion. The cilia with their hasal granules, and the manner of the arrangement of the cilia in spiral rows, so exactly agree with what we see in *Ciliata*, and these organs are so highly developed, that their independent origin in two distantly related groups should not he assnmed without convincing evidence. The resemblance between the excretory organs in *Opalina* and *Hoplitophrya* suggests relationship, and the excretory organ of *Pycnothrix monocystoides* (Schubotz 1908) shows still closer resemblance. The presence of a macronucleus containing refractive spherules in *Hoplitophrya* makes this form a good transition between *Opalina* and other *Ciliata*. The freqnent fragmentation of the macronucleus in *Hoplitophrya*, without connection with conjugation, is again a character somewhat intermediate between higher *Ciliata* and *Opalina* in which chromatin spherules are formed in the nucleus during each mitosis and are dissolved and extruded into the cytoplasm.

On the other hand the restriction of sexnal phenomena in *Opalina* to one period of the year is qnite different from what we find in most *Ciliata*. This is probably associated in some way with its parasitic habit.

The gametes are trne ciliates with characteristic basal granules upon their cilia. They are not formed during encystment, or in great numbers simultaneously from one individnal, as is so usual among *Plasmodroma*. They result from ordinary, though very rapid, division. They differ in no very decided manner from the individuals of the asexual generation, though the microgametes do show special adaptation in the naked sticky tail. The posterior end of *O. saturnalis* in the asexual generation is naked, so this character in the microgametes is not so much of a departure from the usual conditions.

The formation of large numbers of minute individnals in the spring, and their encystment and extrusion from the rectnm of the host into the water, is donbtless an adaptation to the fact that the most favorable time for infecting new hosts is a brief one in the spring while the adnlt frogs and toads are found almost exclusively in the water, and while the vegetarian tadpoles are browsing over the bottom of the ponds where the cysts lie. As my experiments have shown, adult frogs can be infected by *Opalina* cysts, but the feeding habits of the adult are such that infection of this sort must be very infrequent. Infection of the tadpoles, on the other hand, is very easy.

The period of very rapid division in *Opalina* is followed by sexual union, as is usual among *Ciliata*, the restriction of copulation to the spring being probably due to the occurrence of such rapid divisions only in the spring.

The formation of microgametes is by no means nnknown among the *Ciliata* — witness the Vorticellas. In the Vorticellas we also find complete fusion of the gametes.

The resemblance between the reproductive processes in *Opalina* and those in *Plasmodroma* seems superficial, the gametes in *Opalina* being true ciliates and the chief peculiarity being the restriction, for ecologic reasons, of the period of rapid division and copulation to a brief time in the spring, and the consequent false appearance of distinct asexual and sexual generations. There is no true alternation of generations in *Opalina*, any more than there is in Paramaecinm.

The qnestion as to whether *Opalina* is a primitive or a highly modified genus of the *Ciliata* also needs considering. Its parasitic

habit does not argue against its primitive nature. The secluded habitat of parasitic life, like that of deep sea life, has enabled many species to retain lowly character without extermination. The question of the primitive or secondary character of *Opalina* must be studied therefore chiefly through an examination of the structure of the animal itself in comparison with other forms.

In its cilia *Opalina* in as highly developed as most holotrichous *Ciliata*. The absence of a gullet seems probably secondary, an adaptation to parasitism, since it uses predigested dialyzable food from the rectum of its host and has no need of ingesting solid food. Even *Mastigophora*, which are doubtless more primitive than *Opalina*, have a gullet which is functionally comparable with that of *Ciliata*. Those species of parasitic *Ciliata* which retain the mouth have probably adopted the parasitic habit more recently than *Opalina*. *Chromidina elegans*, which in certain conditions of its nuclei resembles the multinucleated *Opalinae*, has what has been interpreted as a vestigial mouth Gonder (1905). So also has *Anoplophrya brasili* (Léger & Duboscq 1904*b*). Lang (1901) classes *Opalina* with the *Hymenostomidae* among the *Ciliata holotricha*, describing the suborder *Hymenostomidae* as possessing an undulating membrane and as having the mouth always open. Of course *Opalina* has neither undulating membrane nor mouth, yet Lang's phrase, "mouth always open", suggests a very interesting interpretation, which, however, he doubtless did not intend, namely, that the month (ingestion tube) of *Opalina* has disappeared by becoming shallower until finally it has joined the even contour of the outer surface of the body, being no longer distinguishable from the latter. This seems not at all unlikely in view of Gonder's description of the mouth of *Chromidina* as a shallow pit over whose walls run rows of cilia continuous with and similar to those on the surface of the body, and also in view of Léger & Duboscq's description of a vestigial mouth in *Anoplophrya brasili*.

The presence of functional vegetative and reproductive chromatin in the same nucleus in *Opalina* seems surely more primitive than their segregation in separate nuclei in higher *Ciliata*. In *Opalina*, spherules of vegetative chromatin are formed in the nucleus, are then dissolved and pass into the cytoplasm, where very likely they take part in forming the refractive spherules. In *Hoplitophrya* the functional vegetative chromatin is in a macronucleus distinct from the micronucleus. In this macronucleus refractive spherules are formed. (This process has not yet been described, Text Fig. VIII,

page 270, shows the refractive spherules in the macronucleus and in the groups of granules into which it fragments.) In higher *Ciliata* distinct vegetative and reproductive nuclei are present, but there are but few indications that the refractive spherules in the cytoplasm, are formed by the macronucleus. Each nucleus of *Opalina* is functionally comparably to both micronucleus and macronucleus of higher *Ciliata*, and is in much more primitive condition.

The formation of chromatin spheres in *Opalina* and their extrusion from the nucleus before copulation seems comparable to the formation of vegetative chromidia in *Plasmodroma* and is probably primitive. The degeneration of the macronucleus in higher *Ciliata* is probably somewhat comparable.

The conditions of maturation in *Opalina* seem simpler than those in higher *Ciliata*. In the maturation of higher *Ciliata* the nuclear divisions unaccompanied by cell-division have generally been discussed from the standpoint of cytophysiology or of the mechanism of heredity.[1]) The conditions in *Opalina* seem to indicate that the degeneration of the nuclei in maturing *Paramaecium* and other higher *Ciliata* is secondary and is connected with the suppression of two divisions of the cell body. In *Opalina* there is no such suppression of divison of the cell body and there is no degeneration of nuclei or cells in connection with maturation. In the division of the macrogamete mother-cells in *Opalina* both daughter cells become functional gametes; similar relations obtain in the formation of Opalina's microgametes. In *Paramaecium* three of the four nuclei (that is three of the four daughter cells) resulting from the maturation divisions in the similar gametes degenerate. In *Metazoa* spermatogenesis follows the type found in *Opalina*, maturation producing four functional gametes, but in the maturation of the eggs of *Metazoa* usually one functional gamete and three degenerate gametes (polar bodies) arise. The fact that the polar bodies are capable of fertilization and development (*cf.* Francotte 1897) seems to show beyond doubt that the usual interpretation of the polar bodies as degenerate gametes is correct.

Complete fusion of the gametes as in *Opalina* and the Vorticellas is doubtless more primitive than such temporary partial union, with exchange of parts of the nuclear material, as we see in *Paramaecium*.

[1]) Boveri (1892*b*) is the only one, so far as I know, who has discussed the phenomena of maturation in *Ciliata* from the comparative morphological point of view and has recognised that the two maturation divisions here, as in the maturation of the eggs of *Metazoa*, give rise to rudimentary individuals.

Usually in higher *Ciliata* the conjugating individuals are nearly or exactly equal in size. It might seem that *Opalina*, having gametes of unequal size, is in this regard less primitive, but the fact that in the Vorticellas, whose gametes completely fuse, the gametes are often very unequal in size, and also the conditions in *Opalina*, argue in favor of a belief that anisogametes are for the *Ciliata* more primitive than isogametes. CALKINS & CULL reach a similar conclusion. They say (1907, p. 405). "There is little evidence to indicate the lines of evolution that have been followed in the development of the participants in conjugation. The view that is usually adopted, without supporting evidence, is that the isogamous type like that of *Paramaecium*, was primitive and has developed into an anisogamous type with sexually differentiated gametes (e. g. HARTOG 1906). It is our belief that the reverse has been the case and that the *Paramaecium* type of conjugation has arisen from a type with sexually differentiated gametes, with intermediate stages in forms like the *Vorticellidae*, where the size difference is great in *Lagenophrys ampulla*, less marked in *Epistylis*, and still less in *Vorticella*; and in *Trachylinidae*, where in *Lionotus fasciotas* the two organisms are alike save for a slight difference in size (CALKINS 1902). In the *Vorticellidae* the macrogamete fuses with the microgamete and there is no mutual fertilization, but in *Paramaecium* and probably in *Lionotus*, mutual fertilization takes place. The case of *Lionotus* is to be interpreted as a reminiscence of anisogamy, and we would expect in this case, that the smaller conjugant, if fertilized, would have a reduced vitality. In *Paramaecium*, finally, there is no morphological evidence of the relation to an earlier anisogamous condition, but there is well-marked physiological evidence in the lesser vitality of one of the ex-conjugants, apparent in 72 % of all conjugations in which the history of both was followed (CULL 1907)." We can, I think, at least say that its anisogamous and slightly heterogamous copulation does not argue strongly, if at all, against the comparatively primitive character of *Opalina*.[1])

Finally the character of the excretory organs in *Opalina* — merely enlarged and confluent vacuoles of the ordinary cytoplasmic

[1]) The very interesting *Pycnothrix monocystoides* described by SCHUBOTZ (1908), which is apparently a holotrochous Ciliate, seems to have unequal gametes, but the exact systematic position of this remarkable form cannot be determined until its life history is more fully known, so that we cannot now say whither its condition argues in favor of the primitive nature of anisogamy for the *Ciliata*, or not.

foam — is very lowly. As parasitic life does not seem in general to produce degeneration of the excretory organs, the lowly character of the excretory organs of *Opalina* is probably primitive. Their resemblance to the excretory organs of *Hoplitophrya* argues for the relationship of the two genera.

It seems, therefore that *Opalina* is a member of the *Ciliata* and that in many respects it is quite primitive.

Léger and Duboscq (1904 *b*) suggest that, because of its habitat in a *marine* fish, *O. saturnalis* is probably the most primitive *Opalina* known, all others being parasitic in terrestrial or fresh-water forms. I have suggested above that the restriction of the rapid division and subsequent conjugation in *Opalina* to a brief time in the spring is an adaption to the habits of its hosts. It would be interesting to know of *O. saturnalis* 1) if it shows a similar restricted period of rapid division followed by conjugation, and 2) if the habits of *Box boops* are such as to make such a restriction in the rapid reproduction period of its parasite a decided advantage. If the habits of *Box boops* are not such as to render infection much more easy at one period, and if, in spite of this fact, *O. saturnalis* still has a restricted period of rapid reproduction, then probably the adoption of *Box boops* as a host is recent, *O. saturnalis* showing a peculiarity in its reproduction, which was originally adopted in adaptation to conditions such as we find among the *Amphibia*. I do not know the habits of *Box boops*. Léger & Duboscq found the cysts of *O. saturnalis* in the month of September, but do not say whether they are found only at that season. We must, therefore, leave unanswered this interesting question as to the comparatively recent adoption of *Box boops* as a host for *Opalina*.

It seems probable that the Opalinas were originally uninucleated, that the binucleated condition was brought about by the suppresion of one division of the body when the nucleus divided, and that the multinucleated condition is still more secondary bring due to further suppression of divisions of the body. In Text Fig. II, page 206, I have grouped the several species of *Opalina* in three groups which seem to me to indicate their probable relationship: group 1) binucleated species, all circular in cross section; group 2) multinucleated species, circular in cross section; group 3) multinucleated flattened species. Compare also the list of species on page 207 in which the same arrangement is followed. *Opalina ranarum*, the form which has been most studied, is probably one of the most highly modified species.

Léger & Duboscq (1904 a) separate the family *Opalinae*, including the genera *Opalina*, *Opalinopsis*, and *Foettingeria*, from the *Anoplophryinae* including the genera *Anoplophrya* and *Hoplitophrya*; a classification which seems reasonable. Instead, however, of believing with Léger & Duboscq that the resemblance between these two families is a superficial one due to convergence caused by parasitism, I think that the two families show real, though not close relationship, the *Anoplophryinae* being the nearest relatives of the *Opalinidae*, unless Schubotz' new *Pycnothrix monocystoides* stands still nearer. The difference between the two groups, is, however, probably too great to allow them with propriety to be placed in the same family, *Opalinidae*, as is usual. The absence of a distinct macronucleus in the *Opalininae* is the chief distinction between this family and other *Ciliata*; but, as I have shown, the distinction is not a fundamental one, the macronuclear elements being present in the nuclei of *Opalina*.

Abnormalities.

Some very interesting abnormal phenomena have been observed in the course of the work described in the previous pages. Brief reference should be made to some of these.

Under unfavorable conditions the animals enter on changes which in some respects resemble the phenomena preceeding or accompanying sexual reproduction. We have already noted that rearing *Opalinae* outside the host tends to make them divide. Even in the fall, when division is very infrequent in freshly taken material, it is found quite readily among animals that have been kept from one to three days in cultures. This recalls the fact that in the spring, when the period of sexual reproduction is approaching, division becomes much more rapid.

The major part of the chromatin in the nuclei of animals kept long in cultures tends to aggregate into compact masses (Pl. XXI, Fig. 94, lower nucleus of Fig. 95). This drawing together of the chromatin may go so far as to form a single ball. These phenomena recall the gathering of the vegetative chromatin into two masses before encystment, previous to their extrusion into the cytoplasm. R. Hertwig (1898) has shown that in starved individuals of *Actinosphaerium* the chromatin condenses into a single mass, while

in richly fed animals it is divided into fine granules scattered through the nucleus. In one case I found in the rectum of a *Hyla viridis* only six individuals of *O. obtrigona* all of which were abnormal, showing in their degenerating nuclei preliminary phenomena which very closely parallel the phenomena accompanying extrusion of the vegetative chromatin in normal animals at the time of formation of the infection cysts (Figs. 99—118, Pl. XXI).

Although keeping *Opalinae* outside the host tends to instigate division, life under the unfavorable conditions in the cultures tends to so weaken the animals that they generally do not succeed in completing the divisions. Division seems normally to be aided by the cilia, the swimming movements of the daughter animals tending to draw them apart. The cilia beat less vigorously in the animals weakened by life in the cultures, and, perhaps chiefly on this account, division is rarely completed. In animals which have thus failed to complete their division, the nuclei are very often almost completely divided so that four daughter nuclei are present, bound together in pairs by their connecting threads. Frequently one sees but one of the daughter nuclei of the anterior parent nucleus in one cell, the other daughter of the anterior nucleus remaining in the other cell along with the two daughters of the posterior nucleus, so that this cell contains three nuclei, two united to each other by a thread and a third united by a thread to the single nucleus in the other cell (Fig. 98, Pl. XXI). In nine such instances I have seen the odd nucleus in the trinucleated daughter cell either already fused with or in the process of fusing with the anterior of the two nuclei which properly belong to the cell. This recalls the fusion of nuclei in the zygote.

In material of *O. intestinalis* and *O. caudata* one finds, rarely during the fall and early winter, and a little more frequently in early spring, individuals with single nuclei of very large size (Figs. 92, Pl. XX; 96, Pl. XXI). In the character of their mitotic spindle and in the distribution of their chromatin these huge nuclei very closely resemble the large dividing syncaria in the zygotes in the tadpole (*cf.* Figs. 222, 224, Pl. XXV). The apparently abnormal uninucleated forms are found in freshly taken material. I have no evidence as to the mode of their origin or their relation to normal nuclei. They may possibly be compound nuclei resulting from the union of two nuclei, or from the failure of a nucleus to divide.

The very thick, stocky individuals of *O. caudata* often seen in the spring, have been described [1]) (Fig. 88, Pl. XX). They may be

[1]) page 250.

abnormal, though there is little to indicate that they are so. With them are often found clearly abnormal forms. Fig. 89 shows one such very stocky individual of *O. caudata* which had four nuclei each in a telophase stage of division. Two of these are seen in the figure in end view and so do not show that they are in mitosis. Fig. 90 shows an individual whose two nuclei have almost completely degenerated. I have found one individual of *O. intestinalis* with absolutely no trace of a nucleus. These were clearly pathologic forms and not in any way comparable to the non-nucleated individuals of *Actinosphaerium*, which HERTWIG (1899) describes as reforming their nuclei from chromidia. Fig. 91 shows an *O. caudata* whose two nuclei are in a condition characteristic of multinucleated *Opalinae*, but very rare and I think abnormal in *O. caudata*. I have never found nuclei of this sort in *O. intestinalis*.

In one lot of material of *O. intestinalis* from a fifty-four hour infection there were many individuals showing abnormal divisions similar to what COHN has described for the same species as budding [1]) (Figs. 228—235, Pl. XXV). When first seen these animals falsely seem to be zygotes in which fusion of the gametes is not yet complete. All the animals in this culture died within an hour. Fig. 229 shows an individual just hatched from the cyst which lies collapsed near by. The little spine-like tip of the body shown in Fig. 230 suggests that this individual was an imperfect microgamete (*cf.* Figs. 163, Pl. XXIII; 261, Pl. XXVI).

The abnormal nuclei in the six degenerating individuals of *O. obtrigona*, referred to above, were very interesting and deserve further description. Figs. 99—101, Pl. XXI, recall some of LÖWENTHAL's figures of nuclei of cysts of *O. ranarum* (Text Fig. X, *b*, page 280). Similar nuclei are quite usual in the full-grown forms of the multinucleated species, as well as in their cysts. The chromatin is found in two conditions 1) in a fine superficial net which slight nodal thickenings, this is hardly distinguishable from the achromatic foam; and 2) in from two to six, or more, large disc-shaped or hemispherical masses pressed close to the nuclear membrane. Figs. 102 and 103 show somewhat similar superficial chromatin masses in which the chromatin is in the form of a darkly stained network with much lighter meshes. The difference in the appearance in the two sorts of chromatin discs in probably not wholly due to difference in staining. Very heavy staining and long

[1]) COHN 1904, Figs. 14 and 15.

extraction of the stain seems always to bring out the netted appearance in some of the discs (generally the larger and thinner ones) and not in others which seem more nearly normal. In Fig. 102 and 103 each nucleus is seen to contain a central mass of granules whose later behaviour indicates that they are probably chiefly achromatic. Fig. 105 shows a radiate arrangement of these achromatic granules, the chromatin having gathered into a sphere which shows the characteristic netted appearance. Figs. 106—109 show that there may be one or two of these spheres. When two are in the same nucleus one may be much smaller (Fig. 107, *a*). In many of the figures, especially in Figs. 106 and 107, one sees that the center of each chromatin sphere is filled with a refractive body which does not stain with DELAFIELD's haematoxylin. The chromatin net lies like a cap partially, or almost completely, enclosing this central body. The presence of these referactive bodies in such intimate association with the aggregated chromatin recalls the formation of refractive spherules in the macronucleus or its fragments in *Hoplitophrya* and the possibly similar phenomena in *Loxodes rostrum* (JOSEPH 1907), and gives a little more probability to the suggestion that in *O. intestinalis* and *O. caudata* the chromatin spherules after they leave the nucleus and reach the cytoplasm, may aid in the formation of the refractive spherules of the endosarc. BOVERI (1907, see his plate XXIII) has described in degenerating nuclei of disperm Echinoderm larvae compound chromatin and refractive bodies very closely resembling those here described in the degenerating nuclei of *O. obtrigona.*

Figs. 110 to 118 show a very interesting arrangement of the achromatic granules in the form of two polar groups with lines of granules connecting them and often with a more or less evident radiate arrangement of the granules around the polar groups. One or two of the netted chromatin spheres lie on the outer side of the granular spindle-fibres, at the equator of the spindle. Fig. 110 shows the chromatin sphere still adhering to the nuclear membrane. In Fig. 111 we see the two chromatin spheres withdrawn from the nuclear membrane and lying upon the spindle. The polar groups of granules, and the lines of granules composing the spindle, at first sight suggest comparison with a mitotic spindle with large polar centrosomes. It seems to me not improbable that they are essentially similar to the structures in some protozoan nuclei which have been regarded as spindle and centrosomes. (Compare R. HERTWIG 1899, *Paramaecium* micronucleus; SCHAUDINN 1894, *Amoeba crystalligera*;

Keuten 1895, *Euglena viridis*; Calkins 1901, *Clepsidrina*; Hartmann & v. Prowazek 1907, numerous *Plasmodroma*.)

These are not functional mitotic spindles in these degenerating nuclei of *O. obtrigona*. The nuclei, though they become elliptical, do not divide, but soon go to pieces, leaving spaces in the cytoplasm where they lay (Fig. 118). During the process of degeneration the nuclear membrane becomes fainter and fainter and ultimately entirely disappears. Sometime the chromatin sphere, including both the chromatin net and the central refractive body, is extruded into the cytoplasm, leaving within the degenerating nuclens only one or more masses of debris representing the achromatic structures (Figs. 116 and 117). In other cases one finds the degenerate chromatin sphere lying in the space from which the nucleus has dissappeared.

The chromatin sphere itself resembles those of the cysts (*cf.* Fig. 133, Pl. XXII), and its extrusion is propably comparable to that of the latter. That is, under unfavorable conditions, the degenerating nuclei undertake a part of the activities which usually preceede copulation. I have not found a perfectly clear spindle-like arrangement of the achromatic grannles in the nuclei of the cysts or of the minute individuals in the spring which were preparing to extrude the vegetative chromatin, but most of my material of these forms was stained with acetic-carmine which does not give sharp pictures of the finest details. In sections of cysts stained with Delafield's haematoxylin, one sees in the center of the nuclei gronps of granules (Figs. 134, 136—139, Pl. XXII) resembling those in the earlier stages of degeneration in the nuclei of *O. obtrigona* (Figs. 105—109, Pl. XXI). Up to this point the two sets of phenomena, normal and abnormal, seem quite comparable. The granular spindles and polar masses do not seem to be paralleled in the normal nnclei at any stage of the life cycle. Their resemblance, however, to what is found normally in some *Plasmodroma*, e. g. *Amoeba cristalligera*, is such as to suggest that these abnormal phenomena in degenerating nuclei of *O. obtrigona* are a reminiscence of archaic normal conditions.

The abnormal phenomena described in this chapter are probably dne to nnfavorable conditions of life. Their resemblance to some of the phenomena usually preceeding copnlation suggests comparison with the well-known fact that many animals, e. g. *Rotifera*, *Cladocera*, which reproduce asexually under favorable conditions, are induced by unfavorable conditions to introduce sexual phenomena.

Infection Experiments.

Under natural conditions the several species of *Opalina* are found only in certain definite hosts, as noted in the table on page 207. In the hope of reaching a better understanding of this restricted distribution, many artificial infections were made with the cysts of *O. intestinalis*, *O. caudata* and *O. dimidiata* upon the larvae of *Rana esculenta*, *Bufo vulgaris* and *Bombinator pachypus* and upon the adults of several species of frogs and toads and *Triton cristatus*. Attempts were made also to infect the same larvae and some of the same adults with adult *Opalinae* of four species, *O. intestinalis*, *O. caudata*, *O. dimidiata* and *O. obtrigona*.

Opalina intestinalis cysts cause infection of the tadpoles of *Rana esculenta* and *Bufo vulgaris* as readily as of the tadpoles of *Bombinator pachypus*. Under natural conditions *Rana esculenta* only very rarely contains this parasite. It has never been reported from *Bufo vulgaris*. In both of these hosts the *Opalinae* form normal gametes which copulate. After four weeks the infection appeared normal when studied from living material. Preserved material from older infections has not get been examined.

Adult *Hyla arborea* and *Rana temporaria*, as well as tadpoles of the latter species, are also readily infected if forcibly fed with the cysts, the young *Opalinae* in the rectum being apparently entirely normal. The later history of these infections was not followed to see if copulation occurred. *O. intestinalis* has never been reported from *Hyla arborea* or *Rana temporaria*.

Tadpoles of *Bombinator pachypus*, *Bufo vulgaris*, and *Rana esculenta*, when placed with foeces of either species of *Bombinator* containing adult *O. intestinalis*, ingest many of the parasites with the foeces. Many others of the parasites pass into the nostrils with the respiratory current. Many of these ingested adult *Opalinae* are digested by the tadpoles of *Bufo vulgaris*, but some pass uninjured through the whole alimentary canals to the recta and there establish thriving colonies. Tadpoles of *Bombinator pachypus* digest a smaller proportion, and tadpoles of *Rana esculenta* digest almost none of the adult *Opalinae*, their recta becoming very richly infected with large *Opalinae*. There is no subsequent degeneration of the *Opalinae* in any of these infections, at least within four weeks. Adult *Hyla viridis* and adult *Rana temporaria* are also readily infected if forcibly fed with adult *O. intestinalis*.

Attempts to infect two adult individuals of *Triton cristatus* with cysts and adults of *O. intestinalis* failed, possibly because the newts did not swallow the *Opalinae*. The negative result cannot be trusted to show that such infection is difficult to secure. CONTE & VANEY report *Opalina intestinalis* from *Triton taeniatus*.

Opalina caudata gave exactly similar results with only the additional fact that adult *Rana esculenta* are abundantly infected from the cysts. Doubtless *O. intestinalis* cysts would infect adult *Rana esculenta*, but no such experiment was made. *Opalina caudata* has never been reported from *Rana esculenta*, nor from *Rana temporaria*, *Bufo vulgaris*, nor *Hyla viridis*.

Opalina dimidiata cysts cause abundant infection of the tadpoles of *Bufo vulgaris*, *Rana temporaria* and *Bombinator pachypus*. Adult *O. dimidiata* cause abundant infection in tadpoles of the same species and in tadpoles of *Rana esculenta*. *Rana esculenta* is the usual host for *O. dimidiata*. *Bufo vulgaris* is also frequently infected with this species, but it has never been reported from *Rana temporaria*.

Adult *Opalina obtrigona* cause abundant infection of tadpoles of *Bufo vulgaris*, *Rana esculenta* and *Bombinator pachypus*, though this parasite has never been reported from these species.

Table showing results of infection experiments.

[Asterisks indicate infections different from those known to occur in nature.]

O. intestinalis cysts	cause infection of	*Bombinator pachypus* tadpoles.
		Bufo vulgaris tadpoles.*
		" " metamorphosing tadpoles.*
		Rana esculenta tadpoles.
		Rana temporaria tadpoles.*
		" " adult.*
		Hyla viridis adult.*
" " adults*	" " "	the same tadpoles and adults.
O. caudata cysts	" " "	*Bombinator pachypus* tadpoles.
		Bufo vulgaris tadpoles.*
		" " metamorphosing tadpoles.*
		Rana esculenta tadpoles.*
		" " adult.*
		Rana temporaria tadpoles.*
		" " adult.*
		Hyla viridis adult.*
" " adults*	" " "	the same tadpoles as the cysts.

O. dimidiata cysts cause infection of				*Rana esculenta* tadpoles.
				Bufo vulgaris tadpoles.
				Rana temporaria tadpoles.*
				Bombinator pachypus tadpoles.*
" "	adults*	"	"	" the same tadpoles as the cysts.
O. obtrigona	adults*	"	"	" *Bufo vulgaris* tadpoles.*
				Rana esculenta tadpoles.*
				Bombinator pachypus tadpoles.*

In the preliminary notice of this work there were two errors: 1) adult *Rana esculenta* were infected by cysts of *O. caudata*, not of *O. intestinalis* as there stated; 2) the *Bufo vulgaris* called "young toads" in that paper were not adult, but were metamorphosing tadpoles with fully formed legs, with the tails only beginning to diminish, and with months of the larval type.

In the light of the results of these infection experiments, the restricted distribution of the parasites in the several hosts is very difficnlt to understand. It seems probable that any species of frog or toad can be infected by cysts or adults of any species of *Opalina* (except, of course, *O. saturnalis*). Why, then, is the distribution of the parasites so restricted? Why, for example, do not *O. dimidiata*, *O. intestinalis* and *O. caudata* all naturally occur in both *Rana esculenta* and *Bombinator pachypus*? The tadpoles of *Rana esculenta* and *Bombinator pachypus* live together in the same ponds and streams. Why does one species become infected only with *O. dimidiata* and the other species only with *O. intestinalis* and *O. caudata*, when all three kinds of cysts are present in the same ponds at the same time of year and must donbtless often be ingested by both species of tadpoles? The question deserves more attention than I had time to give it last spring. If, upon my return to America, I find conditions there favorable for experiment upon this point, I shall study it further. I hope also the matter will be further studied upon Europaean forms, the species mentioned above being especially favorable for study.

A Description of *Opalina zelleri*, NERESHEIMER.

In his fine paper upon the Opalinas, published in 1877, ZELLER describes finding, along with *O. dimidiata*, in the rectnm of *Rana esculenta*, certain individuals much more stocky than the ordinary *O. dimidiata*. They were especially characterized by having the body folded posteriorly, with deep furrows between the folds, ordinary

Opalinae dimidiatae having the posterior end of the body pointed. ZELLER was uncertain whether to regard these stocky individuals as *Opalinae dimidiatae* of a peculiar form, or as belonging to a new species.

DELAGE & HEROUARD (1896), without having seen these Opalinas, interpreted ZELLER's figure as indicating the presence of vestigial excretory organs, an interpretation which I have shown to be mistaken (METCALF 1908*b*).

NERESHEIMER (1907) again found these peculiar *Opalinae* in *Rana esculenta*, and, without adding to ZELLER's description, gave them the name *O. zelleri*, believing them to belong to a distinct species.

In the same year I independently (but later) gave them the same name, it being only natural to name them after their discoverer.

I have seen these forms but twice, ZELLER apparently saw them several times, thongh he does not say definitely. In both instances when I saw them they were with *Opalinae* which undoubtly belonged to the species *dimidiata*. ZELLER reports the two forms as occuring together. NERESHEIMER does not say how often he saw these peculiar forms, or whether *O. dimidiata* was present with them. All the *Opalinae zelleri* I have seen were large, all the small individuals present with them, as well as many of the large ones, being typical *O. dimidiata*. The fact that very much swollen and stocky individuals of *O. caudata* are frequent in the late winter and in the spring, and the fact that I once fonnd a few very thick individuals of *O. ranarum*, make one suspect that the forms called *zelleri* may be merely similar stocky individuals of *O. dimidiata*. Until this question can be definitely settled, it is convenient, and is apparently justifiable, to give these forms a specific name.

Opalina zelleri (Text Fig. II, p. 206) is the largest Opalina known, when we consider its breadth and thickness as well as its length. A large example has a length of 0.25 mm and a breadth of 0.13 mm. In cross section the animal is circular except that there are present upon the body four to eight longitudinal ridges with intervening furrows, which show, of course, in cross section. The anterior end oı the body is bent to one side, as in all other Opalinas. The animal is, however, so stocky that the bend is not quite so noticeable as iu slenderer forms. In *O. ranarum* the correspouding bend in the body is present, but the decided flattening of the body, and its consequent great breadth, somewhat obscure the bend. The anterior end of

O. zelleri is slightly compressed to the form of a very thick wedge. If this compression could be carried much further until the whole body was thin and flat, its anterior end, and indeed its whole form, would resemble that of *O. ranarum*, for frequently *O. ranarum* has the contour of the posterior end of the body concave.

At the posterior end of the body the longitudinal ridges show rounded ends, there being a terminal depression of considerable depth between their posterior ends. *O. dimidiata* is pointed posteriorly, often very sharply pointed and slender, differing most markedly from *O. zelleri*. It was the superficial furrows between the ridges at the posterior end of the body, rather poorly drawn by ZELLER, which DELAGE & HEROUARD interpreted as internal canals, remnants of a system of water canals.

The broad rounded longitudinal ridges are usually five or six in number, though one finds individuals showing four or eight ridges. They are constant, not changing as the animal swims. They are slightly spiral, following the same general direction as the spiral lines of cilia.

Opalinae of any cylindrical species may often, when living under adverse conditions, show a decided spiral twisting of the body, which is then raised into ridges. These ridges are constant, not changing as the animals swim. They may possibly be, in a general way, comparable to those of *O. zelleri*.

Always at least one and often two of the longitudinal ridges show at the posterior end a rounded protrusion, giving a more pointed appearance than that of the other ridges. It is possible that this rounded point marks the morphological posterior end of the body and that when two such points are present they indicate nascent longitudinal division of the body. These points remind one slightly of the short sharp protrusions from the posterior ends of very stocky *Opalinae caudatae* (Fig. 88, Pl. XX), though I have never seen two points upon an animal of the latter species.

The minute structure of *O. zelleri*, as seen in sections, agrees so exactly with that of *O. dimidiata*, as to need no description. The anterior end of the body, as in all other species of *Opalina*, has denser endoplasma and more numerous endosarc spherules than the rest of the body.

This form deserves further study, but it is rare. I found it only twice, once on the 22d of June and again on an unrecorded date at about the same time. No cysts were present with the Opalinas in the rectum. ZELLER describes its reproduction as like

that of *O. dimidiata*, but it is difficult to see how he knew that the reproductive stages studied belonged to *O. zelleri* and not to *O. dimidiata* which was living in the same host. I have once seen an individual of *O. zelleri* in longitudinal divison, the phenomena being as in *O. dimidiata*.

I have no constant opinion as to the distinctness of *O. zelleri* from *O. dimidiata*.

Chronological Review of the Literature of Opalina.[1])

Opalina was first mentioned by Leeuwenhoek in 1685. In his *Opera omnia* (1722) he quotes the earlier record of finding innumerable *animalculae* of various sizes and forms in the foeces of the frog. One of these figured seems in all probability to have been *O. ranarum* (Text Fig. XII, *B*). Another may have been *O. dimidiata* (Text Fig. XII, *A*) [not *O. intestinalis* as Kent (1881—1882) supposed].[2])

Text Fig. XII.
Leeuwenhoek's figures of *animalculae* from the rectum of frogs: *A* may be *O. dimidiata*; *B* is almost surely *O. ranarum*.

One hundred years later than Leeuwenhoek, Bloch (1782, p. 36, Taf. XXIII) described and figured two forms from the alimentary canal of the frog, which he called *Hirudo intestinalis* and *Chaos intestinalis cordiformis*. The former was probably *O. dimidiata* or

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[1]) Only papers and books which include observations upon *Opalina*, or discussions based definitely upon conditions in *Opalina*, are included in this review. General discussions which do not especially mention *Opalina* are omitted, so also are most text books which refer but briefly to observations upon *Opalina* made by others than the authors of the books in question. I have endeavored, with these exceptions, to make the review as complete as possible, but doubtless I have failed to find numerous references to the genus. I should cordially appreciate the kindness of any one who would direct my attention to references to *Opalina* not mentioned in this review.

[2]) Throughout this review of the literature my own comments are included within brackets.

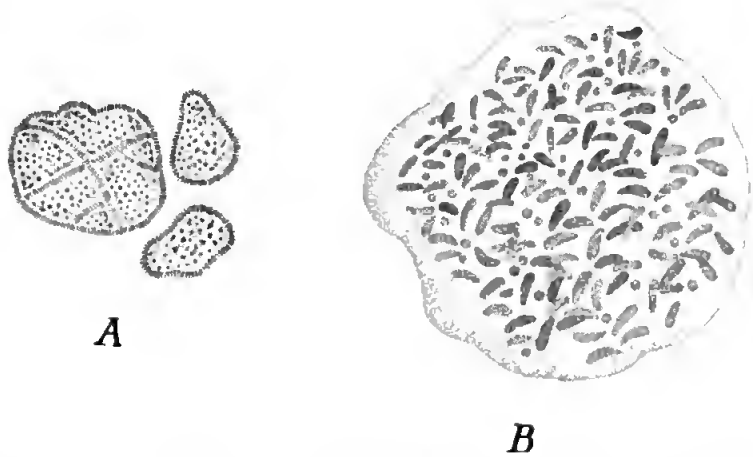
O. intestinalis (Text Fig. XIII, *A*). A late stage of division was observed in Bloch's preparations by his friend "*Herr Oberprediger* Herbst" and was interpreted by Bloch as copulation (upper two animals of Text Fig. XIII, *A*). As the animals were united by their pointed ends, these were regarded as posterior and it was assumed that the months must be at the opposite broader ends. The second form, Chaos, may possibly have been *O. ranarum* (Text Fig. XIII, *B* and *C*). Bloch's artist saw many small particles come out of the posterior (?) end of a quiet individual, which soon died, phenomena which Bloch interpreted as the birth of young. [Doubtless the animal was going to pieces.]

Text Fig. XIII.

Bloch's figures of *Opalina* (?). *A*, three of his nine figures of "*Hirudo intestinalis (der Eingeweideblutigel)*". The upper pair [in a late stage of division] show what he interpreted as copulation. *B*, two of his seven figures of "*Chaos intestinalis cordiformis (das herzförmige Infusionstierchen)*". *C*, an animal of the same species "giving birth to young". *B* and *C*, probably represent *Balantidium* and not *Opalina*.

Göze (1782, p. 429 - 433, Taf. 34), in the same year, makes a more important contribution to our knowledge of *Opalina*. He says *Chaos* includes several sorts of intestinal worms which are found in the "*Landfrosch*" [*Rana temporaria*], the "*Wasserfrosch*" [*R. esculenta*], the "*Mittelfrosch*" [*Bombinator pachypus?*], in "*Landkröten*" [*Bufo cinereus?*] and "*Wasserkröten*" [*Pelobates fuscus?*]. He says they live naturally in the recta of these amphibia and have not merely wandered in from the water because: 1) they are never found in water; 2) they are found in land-toads as well as in water-toads; 3) they are found only in the anterior end of the rectum, just behind the constriction which separates it from the small intestine, never in the small intestine or in the back part of the rectum; 4) one finds three or four species always of constant shape; 5) if frogs are kept a quarter of a year in water the rectum becomes entirely empty of the animals, and still none of these animals are found in the water, though many other *Infusoria* are present. He says that one finds frogs or toads with either very few or none of the *Chaos* in the recta, that the little animals are more abundant

in April and May; that they decrease in the hot summer months; that in December and January they are entirely absent. On March 22d numerous "*Flimmerwalzen*" [apparently *O. intestinalis*], were found in the rectum of the "*Mittelfrosch*" during the winter sleep, but they were not so large as in summer. The "*Flimmerwalzen*" (Text Fig. XIV, *B*) are found only in the "*Mittelfrosch*", never in the "*Landfrosch*" or "*Wasserfrosch*". When magnified 370 diameters they appear 1 inch long and $^1/_{12}$ of an inch broad. On p. 311 he gives the name *Leucophra* to these forms. Certain much larger "*Flimmerquadrate*" [probably *O. ranarum*] are mentioned and figured (Text Fig. XIV, *A*).



Text Fig. XIV.

Goeze's figures of *Opalina* (?). *A*, three of his eight figures of "*Flimmerquadrate*" [apparently *O. ranarum*]; *B*, a group of "*Flimmerwalzen* (*Leucophra*)" [probably *O. intestinalis* or *O. caudata*] from the rectum of the "*Mittelfrosch*".

[O. F. Müller's (1786) *Leucophra globulifera* thought by Ehrenberg (1821) to have been an *Opalina*, seems clearly not to have belonged to this genus].

Schrank (1803) gives a brief description (p. 68) of *Paramaecium incubus*, which he regards as perhaps the same as Bloch's *Hirudo intestinalis*, which was probably *Opalina intestinalis* or *O. dimidiata*. [His description however seems to apply to *Balantidium entozoon* and not to an *Opalina*.]

[Bory de St. Vincent's (1824) reference to *Leucophra globulifera*, thought by Ehrenberg to apply to *Bursaria [Opalina] ranarum*, is really to a different form, as O. F. Müller's (1786) original description of *Leucophra globulifera* shows.]

Ehrenberg (1831) (p. 110) describes very briefly *Bursaria*

[Opalina] ranarum, and (p. 111) briefly describes *Bursaria intestinalis*, [which according to his description cannot be an *Opalina*].

Ehrenberg (1835, p. 164) in a discussion of the "male gland" of *Protozoa*, says that that of *Bursaria [Opalina] ranarum* is band-shaped or has a "*Seidenschnurform*".

Purkinje & Valentin (1835) mention a form which they believed may perhaps be the same as Ehrenberg's *Bursaria ranarum*. To this form they give the name *Opalina ranarum*, the first use of the name *Opalina*. "*Propter colorum superficei splendorum et varietatem sub sole pleno adparentem Opalinam eam vocavimus.*"

Von Siebold, in 1835, mentions the occurrence in the spring in *Rana temporaria* of a great number of completely ciliated, light gray animalcula, and refers to the regularly undulating stripes over the whole body, due, as he [correctly] says, to the serial wave--like movement of the cilia. [The reference is clearly to *O. ranarum*.]

Ehrenberg (1838) gives drawings of his *Bursaria intestinalis*[1]) and *B. ranarum*, which beyond doubt are respectively of *O. intestinalis* and *O. ranarum*. His description of the former species shows that he confused with it *O. dimidiata*. He says it is abundant in February, near Berlin, in *Bufo cinereus*, *Rana temporaria* [incorrect] and *R. esculenta*. The nucleus is called a male gland, and a mouth is [of course erroneously] described as present at the pointed [posterior] end of the body. Numerous digestive vacuoles [probably nuclei of *O. dimidiata*] are described. The same interpretation is given to the nuclei of *Bursaria* [*Opalina*] *ranarum*. The abundant refractive spherules of both species are called egg-granules. Transverse division of *O. intestinalis* is mentioned and figured. *Bursaria* [*Opalina*] *ranarum* is decribed as large, flat, with 32—33 longitudinal rows of cilia; a mouth is described at the pointed anterior end, and an anus at the broad posterior end; a small curved male gland is also described. Neither species was found to ingest pigment granules given to it, so the position of the egestion opening was only donbtfully identified. [Of course *Opalina* has neither mouth nor anus.] The dimensions of *B. intestinalis* are given as: length $^1/_{240}$—$^1/_{120}$ of an inch, diameter of the eggs $^1/_{4000}$ of an inch; of *B. ranarum*, length $^1/_{256}$—$^1/_{72}$ of an inch. Ehrenberg's figures give the first entirely certain identification of any species of *Opalina*, there being no doubt as to the species from which they were made, but *O. intestinalis*, though well

[1]) He makes no reference to his previous description of *Bursaria intestinalis* (1831) which does not apply to any *Opalina*.

figured, is not distinguished in the description from *O. dimidiata* [which has the same shape but contains many nuclei].

Dujardin (1841) makes very brief reference to the Opalinas, giving an unrecognisable figure [probably not of an *Opalina*].

Max Schultze (1851) says (p. 68) that it seems to him very probable that the Opalinas form no true independent genus, but are rather developmental stages or nurses ("*Entwicklungsstufen oder Ammen*") of other animals. [The word *Ammen* is difficult to understand in this connection.] He notes (p. 69) the absence of a contractile vacuole in *O. ranarum*.

Perty (1852) correctly describes the form of *O. ranarum* from the alimentary canal of *Rana temporaria*. He says that the mouth and body cavity are scarcely recognisable; that the whole surface in life seems evenly ciliated, and that the longitudinal striation in dead individuals is due to delicate folds, not to cilia. The appearance of the waves of motion of the cilia is aptly compared to that of the waves which pass across a wheat field in the wind. No figures are given. *Bursaria* [*Opalina*] *intestinalis* is mentioned but not described.

Stein (1856) mentions (p. 56) *O. ranarum* and also suggests that *Bursaria intestinalis* may be an *Opalina*. On p. 37 he diagnoses the genus *Opalina* (in the broader sense) saying that the cilia are in rows over the whole surface of the body, that these animals are distinct from all other Infusoria in having no mouth and therefore taking their nourishment in liquid form through the whole surface of the body; in having for the most part no contractile water vacuole; and in being often without nucleus. He considers it doubtful whether they are to be regarded as true *Infusoria*, or as developmental stages of endoparasitic worms. They are said to reproduce almost always by transverse division. *O. ranarum* is said to be most divergent from other ciliate *Infusoria* since it has no nucleus or contractile vacuole and has never been seen in division.

Leydig (1857) doubts the position of the Opalinas as *Infusoria* arguing from the many nuclei of the multinucleated forms and from the "beautifully cellular structure" of the outer plasma of *O. intestinalis* that they may be multicellular forms.

Pagenstecher (1857) regards *Opalina* as probably a stage in the development of a Trematode. He figures a form which seems to be *O. ranarum*.

Kühne (1859, p. 823) stimulated *Opalina* (species not mentioned) and other *Ciliata* with strong induction electric currents and saw

vigorous movements arise, followed by protrusions from the body as if it were broken, and finally the body became completely flat. "With moderate currents, at the first stimulation the animals drew back strongly, then lay entirely quiet in a sort of tetanus of all the muscles, if they were not carried forward by the action of the cilia, upon which the current seemed to have absolutely no influence." If the stimulus was strengthened, constrictions soon appeared and then breaks at different places at the edge of the body. In this condition many animals swam about for a considerable time if the stimulus was removed. After long stimulation with very strong currents the whole animal liquefied, forming a shapless flat mass ("*unförmigen Brei*") in which for a long time the cilia here and there would continue moving.

Stein (1859) mentions (p. 72) *Opalina* as belonging to the holotrichous *Infusoria*; he again refers (p. 75) to the divergence of this genus from the rest of the *Infusoria* in its lack of mouth and anus and in absorbing liquid food through the surface of the body: he describes (p. 91) the presence of many vacuoles with definite contour, filled with liquid containing granules. [This reference is apparently to the nuclei.] Division or budding had not up to that time been observed in *O. ranarum* (p. 94). He says he had sought in vain for a nucleus in *O. ranarum* (p. 94).

Stein (1860, p. 54) again emphasises the divergence of the Opalinas from the other holotrichous *Infusoria*. He says the former genus *Opalina* divides itself into several genera: *Discophrya* including the forms with a sucking disc at the anterior end of the body; *Hoplitophrya* including the forms bearing horny hooks at the anterior end of the body; *Anoplophrya* including forms, nearly related to *Hoplitophrya* but without discs or hooks, which have simple nuclei in the axis of the body and contractile vacuoles of different forms; and *Opalina* including *O. ranarum*, the form longest known, and *O. dimidiata*,[1]) a nearly related, slenderer, more elongated form, also living in the alimentary canal of the frog. These two species have no contractile vacuoles and no ordinary nuclei, but instead have numerous small nucleus-like structures scattered through the whole parenchyma.

Pritchard (1861) describes at considerable length the *Opalinidae*, giving however but little attention to *O. ranarum* the only true *Opalina* he recognises. He says of *O. ranarum* that the mouth

[1]) I have not found Stein's original description of *O. dimidiata*.

described by EHRENBERG is no true mouth but a mere fold of the surface as may be seen after the body has been distended by adding a little dilute solution of iodine, alcohol, or acetic acid (STEIN); that no nucleus was found by STEIN; that contractile vacuoles are wanting; that the cilia are disposed in longitudinal lines; that the species is common in the intestine and bladder of frogs; that "the absence of a mouth affords evidence of the merely transitive nature of *Opalinoea*", that "these simple beings are not independent but the mere embryonic or transitional phases of other animals", that "they are probably larvae of various worms", "consequently this group of beings is at best but provisional, serving only the purposes of definition and nomenclature"; that „neither the intimate structure nor the developmental history of the *Opalinoea* is sufficiently well understood for them to be arranged in well-defined genera; that *O. tritonis* (PERTY) "is very like *O. ranarum* and requires further examination"; that *O. nucleus*, *O. entozoon* and *O. intestinalis* „... are nothing more than different phases of growth and development of *Opalina ranarum*". Two unnamed and unrecognisable figures are given.

KÖLLIKER (1864, p. 24) recognises the many nuclei of *O. ranarum* as true nuclei and for the first time mentions the cysts, which he describes as multinucleated. He regards the cysts as eggs, and thinks that the fact that *Opalina* develops from eggs confirms MAX SCHULTZE's view that they are developmental stages of *metazoa*.

QUENNERSTEDT (1865) discusses the organization of the *Infusoria*, making but brief reference to *Opalina*. In his description of species he treats *O. ranarum*, giving fairly good figures.

STEIN (1867) opposes (p. 10) LEYDIG's belief that the Opalinas are multicellular, saying that *O. intestinalis* is clearly not so, and that the numerous clear vesicles of *O. ranarum*, *O. dimidiata* and *O. obtrigona*, which are demonstrated with acetic or chromic acid, are not nuclei, but are vacuoles of liquid containing granules. The structure of the *Opalinae* therefore, does not confirm belief in the multicellular nature of the *Protozoa*. The binucleated Opalinas are classed as numbers of his genus *Anoplophrya*. On p. 311 brief reference to the synonomy and occurrence of these forms is made.

CLAPARÈDE & LACHMANN (1868, p. 373) class the *Opalinae*, with many of the other forms now placed as members of the family *Opalinidae*, as an appendage to the ciliate *Infusoria*.

LANKESTER (1870) excludes from the genus the forms now called *Opalina*, reserving this name for the species "so frequently found in

both marine and frish-water Annelids". He says "the simple structureless body of these first named parasites has really very little in common with Opalina, properly so-called — an abundance of highly refringent granules being the only differentiated portions of its substance, no trace of the nucleus and contracted vesicles, nor of the furrowed cuticle of true Opalina being observable. It is not impossible that these swimming flakes of sarcode — for they are nothing more — may undergo subsequent metamorphosis of the most extreme character."

Engelmann published in Dutch (1875) and in German (1876) the first account of the growth of the young Opalinas in the rectum of the tadpole. He reared tadpoles from the egg in glass dishes. He does not say how they were fed or how they became infected, though he mentions remnants of plants as present in their alimentary canals. [Probably the tadpoles were naturally infected from the material in the dishes in which they were kept, else Engelmann would have mentioned artificially infecting them from the material in the recta of the frogs.] He found uninucleated cysts in the recta of the tadpoles and he also describes stages in the development of the little Opalinas from the uninucleated to the multinucleated condition. [His figures suggest tha the may have seen both micro- and macrogametes, though he did not recognise them as such, or observe copulation. Engelmann says he studied *O. ranarum* from tadpoles of *Rana esculenta*. As this parasite has never before or since been reported from this host it seems possible that cross infected material was studied. The minute Opalinas figured seem clearly to be *O. ranarum*, for many of them are not slender enough for the corresponding stages of *O. dimidiata* [1]), while they resemble Zeller's figures of the minute *O. ranarum* in the tadpoles. His observation of uninucleated cysts suggests either that possibly Engelmann saw encysted zygotes, as Neresheimer thinks, or that infection cysts of *O. intestinalis* or *O. caudata* may have been present also, for uninucleated infection cysts are common only in these two of the Europaean *Opalinae* though they are not rare in *O. ranarum* (cf. p. 281). As the tadpoles were reared from eggs, there can be little doubt of their correct identification, since the time of year when the eggs were found, and their size and color, as well as the size and color of the tadpoles themselves, would distinguish them from

[1]) Neresheimer (1907) thinks that they were *O. dimidiata*, but I have not found the minute individuals of this species presenting this appearance.

those of *Rana temporaria*. In the chapter on infection experiments, page 314, I have shown that cross infections are very easily secured. In view of this fact it seems hardly safe to accept ENGELMANN's report of *O. ranarum* from *Rana esculenta* as surely establishing that that species naturally occurs in this host.] ENGELMANN's work definitely settled the fact that the nuclei of the multinucleated *Opalinae* are true nuclei. He regards *Anoplophrya* and *Hoplitophrya* as transitional forms between *Opalina* and other *Infusoria*. He sought unsuccessfully to find the manner of origin of the cysts in the rectum of the frog.

ZELLER, the following year (1877) published a very accurate paper describing for five species (*O. ranarum*, *O. obtrigona*, *O. dimidiata*, *O. intestinalis* and *O. caudata*, n. s.) 1) the rapid transverse and oblique [really longitudinal] divisions in the spring, within the frog's rectum, by which the Opalinas become minute; 2) the cysts in the rectum of the frog and the process of encystment; 3) the cysts in the rectum of the tadpole; 4) the character of the animals hatched from the cysts; 5) their growth to adult character. Good descriptions of the form and structure of the adults are given. ZELLER is the first to describe the nucleoli and their behaviour in mitosis and gives also the first good description of the disc-shaped refractive spherules. In studying the multinucleated species of *Opalina* he found the cysts in the rectum of the frog to be multinucleated (usually 4 nuclei); in the recta of the tadpoles he found both multinucleated and uninucleated cysts, the latter with large nuclei, as ENGELMANN described. He figures a minute "abnormal" individual of *O. ranarum* from the rectum of the tadpole [which was probably a microgamete or a microgamete mother-cell (Text Fig. XV), but he did not so interpret it, nor did he observe copulation]. He figured and briefly described a large form occurring with *O. dimidiata* in *Rana esculenta*, which he said might be either a new species or a form of *O. dimidiata*. [NERESHEIMER has since named this form *O. zelleri*.] This, the finest of all the papers upon *Opalina* will long serve as the starting point with all students of the genus.

Text Fig. XV.
ZELLER's figure of a minute *O. ranarum* from a tadpole of *Rana temporaria*. It was possibly a microgamete or a microgamete mother-cell, though the tail bears cilia.

CERTES (1880) discusses the presence of glycogen in the *Infusoria*, either in the form of refractive spherules or in solution in the endo-

plasm. *Opalina* is referred to as agreeing with the other *Infusoria* in never having the glycogen granules in the nuclei.

BALBIANI (1881) makes brief reference to observations of ENGELMANN and of ZELLER upon the division of the nucleus in *Opalina*.

KENT (1881—1882) gives good diagnoses of the five species of *Opalina* then known. He quotes ENGELMANN's and ZELLER's descriptions of the phenomena of encystment and the growth of the young animals in the tadpoles. Many of ZELLER's and ENGELMANN's figures are well copied. The hosts of *O. intestinalis* are given as *Pelobates fuscus* and *Rana esculenta* [in both of which it is very rare, its usual hosts being *Bombinator pachypus* and *B. igneus*].

KRUKENBERG (1882) in a discussion of digestion refers to *Opalina* among the animals which do not ingest solid food.

DE LANNESAN (1882) gives a very brief description of *Opalina* and says that the *Opalinae* are doubtless descended from non-parasitic forms which became parasitic and gradually lost mouth and anus. Brief reference is made to the cysts and to the reproduction.

STOKES (1884) describes *O. flava* n. s., from *Scaphiopus holbrooki*, the Hermit spade-foot Toad of eastern North America.

NUSSBAUM (1884) a preliminary notice of NUSSBAUM 1886.

BARFURTH (1885) includes *O. ranarum* in a general discussion of glycogen in the bodies of animals. He showed that, when treated with "*Jodgummi*" or with iodine glycerine, some individuals remained merely yellow, others showed in certain restricted areas of the body a red brown color "characteristic of glycogen", the color sometimes being diffuse, sometimes following the lines of the cilia. Under higher magnification irregular masses of glycogen were found staining brown, while near them were many light yellow strongly refractive drops of another substance ("fat") [probably the refractive spherules of the endosarc].

VON KÖLLIKER (1885) says (p. 23) that division in multinucleate forms like *Opalina*, which takes place without coöperation of the nuclei, is not comparable to true division among the protozoa, for the fragments do not grow to the size of the parent, but continue their division until they become very minute particles comparable to spores. The process may be described as cell-formation without division of the nucleus. [The distinction is superficial, not fundamental. Nuclear division, of course, occurs, but the properly concomitant divisions of the body are for a time suppressed, to appear later, in the spring, when the animals are preparing for encystment.]

Gruber (1885a) in a discussion of multinucleate protozoa, refers briefly to *Opalina*, saying that the fragmentation of the multinucleate Opalinas in the spring, by which a generally uninucleate condition is reached before encystment, is not comparable to ordinary binary fission. [The encepted forms usually have two to four nuclei.]

Gruber (1885*b*) in a discussion of artificial division in infusoria, refers briefly to Nussbaum's (1884) work, mentioning the fact that *Opalina* fragments into dissimilar pieces which later by regeneration reach normal form.

Bütschli (1886) describes and figures the alveolar structure of the endoplasma of *O. ranarum* and says that the ectoplasma shows similar structure.

Peitzner (1886) gives a [too schematic] account of the division of the nnclei in *O. ranarum*. He interprets the refractive spherules as algae. He showed clearly that the nnclei of *O. ranarum* divide mitotically. The true nucleolus was not seen. The absence of centrosomes and the persistence of the nuclear membrane were observed. Splitting of the chromosomes was [mistakenly] said to occnr during a typical eqnatorial plate stage.

Nussbaum (1886) gives a brief outline of the life history of *O. ranarum* after Engelmann and Zeller. He says the least particle of foecal matter canses a culture of *Opalina* quickly to die. [Others, myself included, have found that cellures with foecal matter live longer than those without.] He describes form, mode of swimming, and structure. He says that division apparently stops during the winter sleep of the host: that animals ready for encystment have four or more nuclei. [I find frequently one, two, or three, as well ar four or more]: division is described at considerable length; the products of division are not always alike; sometimes there is division into three pieces; at the temperature of the room division occupies forty to forty-five minutes; division of the body is independent of the division of the nuclei, but division of the nucleus does not occur dnring division of the body. [I have not found the last statement correct for my preparations]; the direction of the mitotic spindle with reference to the planes of the body is very various; the nuclei show no interrelation in their divisions, dividing at different times without reference to one another: he found multinucleated and uninncleated cepts in the recta of tadpoles and thought it probable that the latter are derived from the former by fusion of nuclei: the nuclei of the young *Opalinae* in the tadpoles divide mitotically: he confirms Zeller that some *Opalinae* in the

frog's rectnm remain large in the spring and do not rapidly divide: the cysts in the tadpole hatch in either the intestine or the rectum, some within an hour after ingestion, some remainiug unhatched as much as seven days: the cysts never hatch in water bnt will hatch in the aqueous humor of the frog: experiments in artificial division were unsuccessful for the pieces, like the whole animals, died.

Balbiani (1887) refers at some length to the Opalinas; naming the hosts of the five species then known [*Bombinator* should have been included as a host of *O. intestinalis* (*cf* Zeller 1877)]; diagnosing the genus and describing the shape of all the species except *O. intestinalis*; brief citations are made of the work of Zeller, Engelmann, and Nussbaum, upon the rapid division in the spring and encystment; he mentions having, frequently himself seen, in *O. ranarum*, conjugation preceeding the rapid multiplication in the spring [donbtless it was obliqne division already correctly described by Zeller].

Entz (1888) [whose paper I have been unable to obtain] is qnoted as saying that *Opalina* npon a partly shaded slide will swim out of the lighted area into the shaded area [a result not confirmed by other students].

Bütschli (1887—1889) in his great work upon the *Protozoa* in Bronn's *Klassen und Ordnungen des Thierreichs*, gives [not quite complete] literature references to that date. He describes the genus, giving fignres of *O. ranarum*, *O. dimidiata* and *O. intestinalis*. He [mistakenly] snggests that the oblique division described by Zeller was propably conjugation. The figures of mitosis, apparently taken from Pfitzner, are inaccnrate. He [mistakenly] says (p. 1500) that the nnclei lie close under the cnticle, irregulary distributed in a single layer. He says the nuclei are comparable to micronuclei, not macronuclei [They are probably comparable to both, each nucleus being both nutritive and generative].

Fabre-Domergue (1888) mentions with approval Bütschli's (1886) "first" [1]) description of alveolar protoplasm in *Ciliata*, observed in the ectosarc [Bütschli says endosarc] of *O. ranarum*.

Verworn (1889) found that *Opalina* [probably *O. ranarum*] gave no reaction to stimulation by light. The center of a drop of water was brilliantly illuminated while the rest remained dark. There was no difference in the behaviour of the Opalinas in the two areas

[1]) Leydig (1857) had already mentioned the "beautifully cellular structure" of the ectosarc of *Opalina*.

and they were equally abundant in the two regions. Light causes no injury to *Opalina*. A frog was opened in faint light and the Opalinas in the rectum were placed in two cultures, one remaining in the dark, the other being placed in bright daylight. No subsequent difference between the two cultures was observed. He found that Opalinas in a culture swim indifferently toward the warmer or toward the cooler area.

In a second paper (1890) VERWORN showed that *O. ranarum*, stimulated by an electric current, swims toward the anode.

PARKER (1891 and subsequent editions) describes briefly the structure and life history [so far as then known] of *O. ranarium*. [The only error is the statement (after ENGELMANN) that] the little *Opalinae*, which hatch from the infection cysts in the alimentary canal of the tadpole are uninucleated.

PERRIER (1893) refers to the difference between ectosarc and endosarc in *O. ranarum*, to the presence of paraplasmatic bodies [refractive spherules] in the cytoplasm, to the process of encystment, to the fact that nuclei and cytoplasm divide independently, to the process of mitosis which is described according to PFITZNER's observations [and therefore inaccurately].

VERWORN (1896) mentions again the anodic galvanotropism of *O. ranarum* and says further that under stimulation from a strong current the side of the body toward the kathode becomes clearer and more strongly refractive; that the granules of protoplasm and the nuclei withdraw more and more from that edge of the body; that small hyaline vesicles soon appear there; that the cilia of that side are then destroyed; and that the contour of that part of the body then becomes uneven. There follows immediately a granular disintegration of the side of the body toward the kathode. VERWORN believes that the anodic galvanotropism of *O. ranarum* is due to a contractile irritation of the kathodic side of the body [later shown by DALE (1901) and WALLENGREN (1903) to be a mistaken interpretation].

DELAGE & HEROUARD (1896) [mistakenly] interpret ZELLER's figure of the form [which NERESHEIMER has named] *O. zelleri*, as indicating the presence of remnants of an excretory organ.

LOEB & BUDGETT (1897) quote VERWORN's description of the fragmentation of the kathodic side of the body of *O. ranarum* under strong electric stimulation, ascribing this to the action of acid; they believe that this anodic reaction of *Opalina* is due to the fact that it is always studied in physiological sodium chloride solution. [PÜTTER, 1900, has shown the error of this assumption.]

Parker & Haswell (1897) say "in *Opalina* numerous nuclear bodies are present which divide by mitosis, and therefore resemble micronuclei: if they are to be considered as such, this genus must be held to differ from the other *Ciliata* in the total absence of a meganucleus". They refer to the processes of reproduction in the spring as described by Zeller and give figures from Zelller, Kent and Pfitzner (mitosis).

Von Prowazek (1898) says that the protoplasm of *O. ranarum* stains *intra vitam* rosy red with neutral red, the unstained nuclei then being more evident. [I have not found a diffuse protoplasmic stain with very weak solutions of this reagent.]

Tönniges (1898) recognised the alveolar structure of the protoplasm of *O. ranarum* and distinguished the ectosarc and endosarc. He describes the cilia as perforating the pellicle [*vs.* Bütschli 1887—1889 p. 1325. The cilia propably consist of a prolongation of the pellicula containing an axial fibril which extends at its base through the pellicula, to the basal granule. Both Bütschli's and Tönniges' statements seem to be correct but incomplete.] and as arising from the nodal points of a network of very delicate fibrils lying beneath the cuticle, ascribing the motion of the cilia to the contraction of these fibrils. He describes well the alveolar structure of the refractive spherules of the endoplasma. He says that they divide by constriction [probably an error]; that they are not excretory and are probably not parasitic, but are to be interpreted as a diffuse macronucleus.

In a further communication the following year (1899), in regard to *O. ranarum*, he notes that the division of the body has no discernable relation to the division of the nuclei; he describes irregular divisions of the body; he says that the several nuclei within the multinucleated cysts fuse into one [not confirmed by later students]. The nuclear membrane is described as showing alveolar structure [not confirmed by my study]. Amitotic division is said to occur side by side with mitotic division in the fullgrown forms [not confirmed by later students]. The true nucleolus was not observed. Pfitzner's work was confirmed in that no centrosomes were found and the nuclear membrane was seen to persist during the mitosis. The chromatin is described as superficial, lying just beneath the nuclear membrane. No longitudinal splitting of the chromosomes was seen.

Birukoff (1899) discusses Verworn's observations upon positive galvanotropism in *O. ranarum*.

Boveri (1900) discusses the evolution of centrosomes and mitotic spindle, illustrating from the nucleus of *O. caudata* one stage in this hypothetical evolution. He notes (p. 185) that the division of the nuclei and the division of the cytoplasm are relatively independent phenomena in the multinucleated *Opalinae*, and (p. 187) that, in the binucleated *Opalinae*, the binucleated condition is apparent, not real, being due to delay in division of the body after the division of the nuclei.

Pütter (1900) opposes Loeb & Budgett's (1897) statement that the anodic reaction of *Opalina*, differing from all other *Ciliata*, is due to its always being experimented upon in sodium chloride solution. He found that when *Opalina* and *Balantidium* from the same host were experimented upon together in the same culture, *Opalina* showed anodic reaction while the reaction of *Balantidium* was kathodic.

Lang (1901) classes *Opalina* in the suborder *Hymenostomidae* among the *Ciliata holotricha*. In the same suborder, which he characterizes as having the mouth always open and as possessing an undulating membrane, he places *Colpoda*, *Colpidium*, *Urocentrum*, *Paramaecium*. *Anoplophrya*, *Frontonia*, *Leucophrys*, *Ophryoglena*, and *Pleuronema*. [*Opalina* of course has no undulating membrane and no mouth.]

Doflein (1901) gives a suscinct account of the structure and development of *Opalina*, [as liable to mislead, may be noted the statements that] the ectoplasma is homogeneous and that the endoplasma is granulated; and, [as a probable error] quoted from Przesmycki, the statement that the animals in the cysts often divide into several offspring.

Dale (1901) refers to Verworn's observation of positive galvanotaxis in *O. ranarum*; he notes that this species is found in the anterior end of the rectum of the frog; he describes experiments upon its chemotaxis and galvanotaxis which he summarizes as follows —

	Alkalinated	Neutralized	Acidified
Chemotaxis	Attraction to acid Repulsion from alkali	Attraction to acid Repulsion from alkali	Attraction to alkali Repulsion from acid
Galvanotaxis	Collects at anode	Collects at anode	Collects at kathode;

he describes the normal movements of the cilia and their reaction to chemical and electrical stimuli; he describes the modification of galvanotaxis by changes in concentration of the media; and finally considers theoretically the phenomena.

CONTE & VANEY (1902) mention the occurrence of *O. intestinalis* in *Triton taeniatus*, the first report of *Opalina* from a tailed Batrachian. They say the refractive spherules arise in the nucleus and wander out through the nuclear membrane into the cytoplasm. There are regarded as comparable to zymogen granules and yolk nuclei.

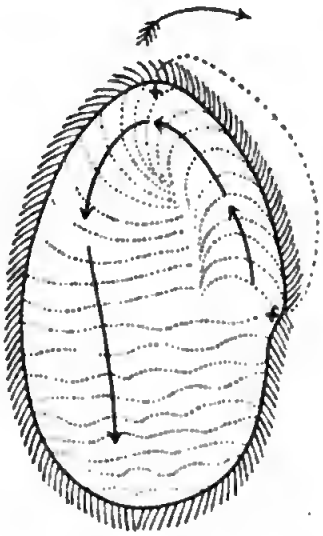
KUNSTLER & GINESTE (1902) give some anatomical notes upon *O. dimidiata* emphasizing especially the presence in the endoplasma of certain "vesicles" which contain each a central granule, which reproduce by division and which therefore have an individuality of their own. [Instead of one granule, these bodies, the endosarc spherules, contain many. They apparently do not divide. Instead of being constituent parts of the living protoplasm they seem to be nutritive material, paraglycogen, so that KUNSTLER & GINESTE's conclusions in regard to them seem inadmissible.]

KÖLSCH (1902) studies minutely the drops of liquid which are extruded from *O. ranarum* and *O. dimidiata* when under pressure. He believes that the pressure causes partial liquefaction of the pellicle, that the culture fluid (sodium chloride solution) is thus allowed to enter the body, and that this fluid unites chemically with the protoplasm forming "paramylin". He confirms VERWORN's description of anodic galvanotropism in *O. ranarum* and describes the peculiar but constant curve through which it swims slowly toward the anode.

HICKSON (1903) makes numerous [inaccurate] references to *Opalina*. He says (p. 364) "The mouthless *Opalina* found in the bladder [!] of frogs may owe its many peculiarities of form to its entozoic habits"; *Opalina* is included with other *Ciliata* in the [mistaken because incomplete] statement (p. 368), quoted from BÜTSCHLI, that the cilia spring from the pellicula and are continuous with it; reference is made to the nuclei of *Opalina* as follows (p. 378): "If the current views concerning the nuclei of *Opalina* are trustworthy, this genus should no longer be regarded as a member of the *Heterocaryota* [*Ciliata*]. *Opalina* possesses, according to PFITZNER and others, a large number of meganuclei, but no micronuclei. [PFITZNER (p. 466) regards the nuclei of *Opalina* as homologous with the micronuclei of *Paramaecium*.] Thin sections of *Opalina* that are suitably stained show, in addition to the numerous macronuclei, a large number of small bodies containing chromatin. They are probably micronuclei. [The accompanying figure shows them to be refractive spherules of the endosarc.] The meganuclei divide sometimes amitotically [probably not true], and it is probable that they always do so [mistaken]. The mitotic figures discovered by PFITZNER are clearly seen in a

large number of sections examined, but they are smaller than the meganuclei [no] which, as in other forms, increase considerably [very slightly] in size before division." [Each nucleus of *Opalina* seems to be functionally comparable to both micro- and macronucleus of higher *Ciliata*, but to be homologous with each, both the nuclei of *Ciliata* being phylogenetically complete nuclei.]

WALLENGREN (1903) describes the form of the body of *O. ranarum* (Text Fig. XVI) [which has its very broad anterior end bent "to the right"]; the arrangement of its cilia; the normal movement of the cilia in which the cilia that lie along the "anterior half of the right side" [corresponding to the morphological anterior end] beat forward [morphologically backward and toward the left] while all the others beat backward, the animal thus turning over to the right as it swims; he describes and analyzes the reactions of the cilia under electric stimulation, which cause the animal with a weak current to swim forward toward the anode, with a stronger current to swim forward toward the kathode, with a still stronger current to swim backward or sidways toward the anode.



Text Fig. XVI.

WALLENGREN's figure of *O. ranarum*, illustrating the direction of the motion of the cilia waves (plain arrows) and the direction in which the animal turns (feathered arrow). The dotted lines across the body do not indicate the lines of insertion of the cilia but the waves of contraction of the cilia. What I interpret as the morphological anterior end stretches form + to + and is indicated by the dotted index line.

MAIER (1903) describes carefully for *O. ranarum* the pellicula, and the cilia and their basal granules. He denies the connection of the cilia with a network of subcuticular fibrils such as TÖNNIGES describes, ascribing the appearance of the transverse lines observed to ridges in the pellicle. [My study confirms TÖNNIGES as to the presence of a sub-pellicular network in connection with the cilia, though I would not ascribe the movement of the cilia to the contraction of the fibrils of the network. The network seems more likely to be useful for the coordination of the movements of the cilia.] MAIER opposes TÖNNIGES' description of the alveolar struc-

ture of the refractive spherules, saying they are homogeneous. [BEZZENBERGER and I confirm TÖNNIGES.] MAIER opposes TÖNNIGES' description of the ectoplasma as containing large alveoles, saying that its alveoles are as small as those of the endoplasma. [My work confirms TÖNNIGES.]

VENEZIANI (1904) divided cultures of *O. ranarum* into two exactly equal parts and placed a tube containing $^1/_{10}$ gram of active radium bromide in one dish and none in the other. In ten experiments when the culture medium was 0.5 % or 0.6 % sodium chloride solution, and in four experiments when the animals very kept in ordinary water (not distilled), the Opalinas in the culture containing the tube of radium bromide remained active longer than in the corresponding unstimulated cultures. The author says it is doubtful whether the longer continued activity of the Opalinas is due to the direct effect of the radium upon their protoplasm, or to same modification of the density or chemical composition of the culture media.

STATKEWITSCH (1904) finds that *O. ranarum* does not react at all to weak constant and induction electric currents (0.5—1 MA), with stronger currents (3 - 4 MA) they generally swim slowly toward the kathode. With rather strong currents (2.3 or 4 MA) they often swim slowly toward the anode; sometimes they first approach the anode and later turn and approach the kathode. Often with a weak current they start toward the kathode, but, without reaching it, turn back and swim in various directions through the culture. In the latter cases they probably become accustomed to the stimulation. The character of the reaction depends on the strength of the current.

In another paper (1905) STATKEWITSCH mentions *O. ranarum* in a discussion of the reactions of cilia in the *Ciliata* to electric currents.

BEZZENBERGER (1904) describes five new species of *Opalina* from Asiatic frogs and toads (*O. macronucleata*, *O. lanceolata*, *O. coracoidea*, *O. lata* and *O. longa*), giving many anatomical details. He figures mitosis in *O. macronucleata* and *O. lanceolata* (*cf.* Text Fig. V, page 250). For *O. longa* he describes very peculiar elongated rod-shaped basal granules of the cilia, reaching from the pellicula through the whole ectoplasma and as far again into the endoplasma. The ectoplasma is described as having a zone entirely without demonstrable structure. [His figure was evidently drawn from very poorly preserved material in which it was probably not possible to recognise the real structure of pellicula, cilia, basal granules, or ectoplasma.] The proto-

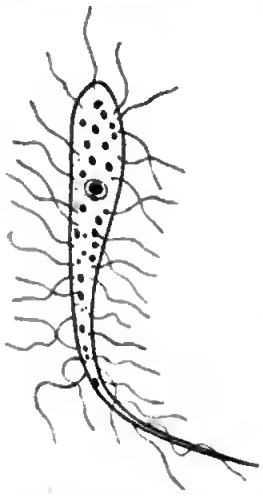
plasm of *Opalina* is spoken of as containing intestinal contents. [It is, I think, always free from food particles or foecal matter, as STEIN had already shown.] He confirms TÖNNIGES' statement that the refractive spherules contain granules, but finds no sign of alveolar structure in them. [My work confirms TÖNNIGES in the latter regard.] He saw no indications of division of the refractive spherules. The longitudinal striae, between the rows of cilia, he describes as compesed of rows of granules. [My study indicates that this always hazy appearance of granules (Fig. 2, Pl. XIV) may be an optical effect produced where the longitudinal ridges (MAIER) of the pellicula pass above the transverse fibrillae (TÖNNIGES) of the subpellicular network which is connected with the basal granules of the cilia.]

LÖWENTHAL (1904) describes for *O. ranarum* the formation of the chromatin spheres in the nuclei before encystment (Text Fig. X, page 280). He distinguishes the more strongly staining sphere from the less deeply staining [and, according to my own observations, granular] mass, saying that the former arises from the latter. The deeply staining compact sphere he regards as homologous to a micronucleus (sexual), the weakly staining residue to a macronucleus (nutritive). His figures show what he believes to be the sequence of phenomena. [The darkly staining spheres are extruded from the nucleus, as NERESHEIMER and I have shown, and go to pieces in the cytoplasm. They are probably composed of nutritive chromatin.]

COHN (1904) gives an account of *O. intestinalis*, which is either inaccurate in most points, or is based wholly or in part on abnormal animals or on some other form or forms. Some of the features described [which do not fit normal *O. intestinalis*] are: that the body is often triangular and flattened; that the refractive spherules disappear after twenty-four hours if the animals be kept without food in a hanging drop in a moist chamber; that the alveoles of the cytoplasmic foam grow smaller from the center of the body toward the periphery; that small forms are never binucleated and large forms never uninucleated. The individuals figured in conjugation are evidently not Opalinas. The budding described appears, to have been pseudoencystment following fragmentation.

LÉGER & DUBOSCQ (1904*a* and *b*) give a fine account of the structure of an interesting new species, *O. saturnalis*, which is found in the rectum of *Box boops*, a fish from the Mediterranean Sea. This is the only *Opalina* which is reported from a host which is not an Amphibian. The authors describe elongated and stocky forms [as in *O. caudata*]; "lecithin (?)" bodies [my "ectosarc spherules"] are

described in the outer, coarsely alveolated layer of the body; the endosarc is said to show no special inclusions [but bodies apparently resembling the ordinary refractive spherules of the endosarc are figured]; the phenomena of mitosis are described with very clear figures (*cf.* Text Fig. IV, page 249); longitudinal division is described and figured; uninucleated infection cysts are shown; true copulation was not observed; an individual resembling a microgamete or microgamete mother-cell is described and figured (Text Fig. XVII) without being recognised as a gamete; *O. saturnalis*, on the ground of its occurrence in a marine fish, is regarded as the most primitive of the *Opalinae*. The authors say the family *Opalininae* (including the genus *Opalina*, *Opalinopsis* and *Foettingeria*) should be sharply distinguished from the *Anoplophryinae* (including *Anoplophrya* and *Hoplitophrya*, which they would unite to form one genus *Herpetophrya*) and that the two families should not be regarded as closely related, the resemblance between them being a superficial one due to convergence caused by parasitism. The authors describe for *O. ranarum* three sorts of cysts 1) the well known infection cysts "exogenous", 2) "endogenous" cysts — in an ordinay large individual a bit of the protoplasm containing one to four nuclei separates itself from the rest of the protoplasm and forms a cyst around itself, being then extruded from the body —, 3) conjugation cysts — two individuals like those of the ordinary cysts come together by their anterior ends, lie for a long time rubbing against each other and turning, and then form a cyst enclosing them both, each animal occupying half of the cyst. [These remarkable phenomena of the formation of the second and third kinds of cysts have not been observed by other students. Léger & Duboscq's very brief unillustrated description can hardly be accepted without confirmation.]



Text Fig. XVII.
An individual of *O. saturnalis* figured by Léger & Duboscq. Doubtless it was a microgamete or a microgamete mother-cell.
× 600 diameters.

Fauré-Fremiet (1904), in a brief discussion of the structure of protoplasm, refers to Kunstler's [Kunstler & Gineste's] idea of the vesicular structure of protoplasm. He says that the "vesicles" of Kunstler [in part refractive spherules of the endosarc in *O. dimidiata* and other *Protozoa*] are not inclusions, are not reserve food, are not

excretory vesicles. [Several different unrelated structures seem to be included under the term "vesicles" as here used. The endosarc spherules of *Opalina* seem to be composed, chiefly at least, of paraglobulin and to be a reserve food supply. The often reiterated conceptions of KUNSTLER & GINESTE and FAURÉ-FREMIET seem therefore to be founded on an insecure foundation.]

KUNSTLER & GINESTE (1905) discuss the refractive spherules of the endoplasma in *O. dimidiata*; they say that they divide by constriction, a central granule in each dividing first [not confirmed by NERESHEIMER or myself]. They estimate eight thousand of these spherules to be present in an *O. dimidiata* $112^1/_2$ μ long by $37^1/_2$ μ broad. These spherules are regarded as a secretory apparatus. [They are apparently paraglycogen.]

SCHOUTEDEN (1905) in a brief note reports finding longitudinal division (ZELLER's oblique division) in *O. ranarum* very frequent in the spring. Division took from 50 to 90 minutes. In isolated individuals he saw the longitudinal division begin and complete itself, thus confirming ZELLER's description of the division (as COHN had done before) and refuting BÜTSCHLI's suggestion that ZELLER had probably mistaken conjugation for division.

PÜTTER (1905) finds that after an hour in sodium chloride solution *O. ranarum* begins to show signs of injury, cilia movements becoming slower. The animals are at first clear and transparent, as they become abnormal they get darker. The abnormal condition and final death may be caused by the unnatural environment, or may be due to the noxious effect of free oxygen in the cultures. *Opalinae* in culture media containing no free oxygen live longer than those in control cultures in which free oxygen is present. A better culture medium than sodium chloride solution is a solution made of

sodium chloride 0.8 %	 100	parts
sodium and potassium tartrate 30 %	5	„
distilled water	 400	„

In this fluid, free from oxygen, *Opalinae*, if fed, live up to three weeks. Without food they live from one to seven days, showing how long they can live upon the energy already stored in their bodies, for there is in the fluid no source of energy for the *Opalinae*. The stored energy in the bodies of the *Opalinae* is not in the form of "*Polysachariden*", for with iodine we do not get the characteristic color reaction. [This statement needs modification. Compare BARFURTH (1885) and my statement of the reaction to iodine—page 216.] The stored energy is not in the form of fat, so probably it must be

some form of proteid. The appearance of myelin after solution (KÖLSCH) would favor the presence of Lecithin. [Nutrition in a form similar to glycogen seems to be present.] The *Opalinae* live longer in this oxygen-free culture fluid if certain substances be added. Addition of egg albumin, maltose (?), and uric acid especially a mixture of uric acid and dextrin, produce this result. It is not certain whether in the case of egg albumin the *Opalinae* form an extracellular enzyme which digests it, or whether the egg albumin is acted upon by anaërobic bacteria and is changed to a liquid dialyzable form. In the case of uric acid it is doubtful if the effect is an indirect one, or if the acid is used as food. PÜTTER says that after a few days in the cultures rather numerous instances of conjugation [doubtless really longitudinal division] and of transverse division appear.

FAURÉ-FREMIET (1905 *a* and *b*, 1906 *a* and *b* discusses the refractive spherules in the *Protozoa*, referring with approval to KUNSTLER's [KUNSTLER & GINESTE's] interpretation of these bodies in *Opalina* as a secretory apparatus. He distinguishes [upon grounds that are not clear] between "spheroplasts of internal secretion" and "spheroplasts of external secretion". The spherules of *Opalina* are said to belong to the latter group. They are regarded as fundamental elements of the cell, comparable to the "leucites" of plants and to the nucleus, in this regard.

HARTOG (1906) notes Opalina's positive galvanotaxis and refers to DALE's experiments which show that the direction of motion varies with the nature and concentration of the medium; "It would thus be a reaction to the ion liberated in contact with the one or the other extremity of the being". *Opalina* is classed among the *Flagellata* in the group *Protomastigaceae*. The author says "The numerous similar long flagella of the *Trichonymphidae* afford a transition in the genus *Pyrsonympha* to the short abundant cilia of *Opalina*, usually referred to the Ciliate Infusoria"; and again "The *Opalinidae* have also an investment of cilia, which are short and give the aspect of a Ciliate to the animal. But despite the outward resemblance, the nuclei, of which there may be as many as 200, are all similar, and consequently this group cannot be placed among the Infusoria at all".

KUNSTLER & GINESTE (1906 *a* and *c*) describe for *O. dimidiata*, a mouth and spiral oesophagus, a cup-shaped depression, in front of the mouth, into which opens an excretory tube whose branches ramify through the body, and retractile papillae at the posterior end

of the body in the midst of which is an anal aperture leading from a short rectal tube. The position of the mouth indicates the true ventral surface, the animals having bilateral symmetry. [In careful study of living animals, and of preparations of total objects and sections, of *O. dimidiata* and other species, I have found no trace of any of the organs mentioned, and cannot believe them to be present.]

Kunstler & Gineste (1906 *b*) describe the protoplasm of abnormal *O.* [*dimidiata?*] (kept too long in pure water, or from frog kept too long in captivity [?]) as resembling a continuous gelatinous substance, the appearance of a net, seen in the protoplasm of normal animals, being no longer discernable.

Schneider (1906), after studying iron-haematoxylin sections of *O. ranarum*, quotes with approval (p. 48) Maier's statement that the ectosarc is homogeneous [apparently referring to only what I have called the subcuticular layer, since he later mentions the presence of large spaces filled with a thick substance, which were doubtless the large alveoles of the ectosarc]. He describes (p. 49, Fig. 14 a—c) the appearance of threads, coarser and finer, which one sees in iron-haematoxylin preparations, distinguishing coarser branching fibres, connected with the basal granules of the cilia, from more delicate ones forming a network not connected with the cilia. At the nodal points of this delicate network one sees thickenings. The coarser threads lie chiefly in the ectosarc, but extend also into the endosarc, the finer threads lie throughout both ectosarc and endosarc. Lying upon the latter [error] are found the disc-shaped granules [refractive spherules] described by Zeller. Schneider strongly opposes Bütschli's conception of the cytoplasm of the *Infusoria* as alveolar, saying that the threads described form clearly a framework within the cytoplasm, such as is present in the ciliated cells of *Metazoa*. [Had Schneider studied sections of *Opalina*, especially *O. intestinalis* or *O. caudata*, which had been stained with Ehrlich's triacid mixture and others stained with methyl violet, he could hardly doubt the alveolar nature of both ectosarc and endosarc. The threads he describes seem to me chiefly optical sections of the walls of the alveoles.] Schneider describes (p. 50) and figures (his Fig. 14 *c* and *d*) the appearance [described by Tönniges, Maier and Bezzenberger. *Cf.* my Plate I, Fig. 2] of longitudinal markings between the rows of cilia, and other similar transverse markings at a more internal level. He says the latter have no connection with the cilia. [Tönniges, and, in the present paper, I also, describe the transverse fibrils as uniting the bases of the cilia.] Schneider

describes (p. 62) the drops of liquid which exude from the ectosarc of *Opalina* when pressed; the fact that they do not mix with the water; and the further fact that on relieving the animal from pressure the drops are again absorbed by the body. These he regards as drops of hyaloplasma. Schneider says (p. 71) that the protoplasmic currents, so general in the *Infusoria*, are entirely wanting in *Opalina*. His discussion of the hyalo plasma (p. 83—111) is based in part upon his studies of *Opalina*. He strongly opposes Bütschli's belief in the prevalence of alveolar structure in living protoplasm. [It seems to me that few objects could be found more satisfactory than *O. intestinalis* and *O. caudata* for demonstrating the alveolar structure of protoplasm.]

Jennings (1906) describes the "avoiding reaction" in *O. ranarum*, and its behaviour with reference to acid and alkaline media showing that Opalina's reactions to stimuli, like those of other Protozoa, are always negative or null, never positive; he also quotes, with figures, Wallengren's analysis of the reaction of the cilia of this species to electric stimuli of different intensities.

Neresheimer (1906 preliminary notice, and 1907) is the first to recognise the presence of gametes in *Opalina* and is the first to describe the extrusion from the nuclei of the chromatin spheres previously described as micronuclei by Loewenthal. He saw no splitting of the chromosomes, confirming Tönniges and Bezzenberger against Pfitzner. He found the chromosomes in the asexual forms of *O. ranarum* and *O. dimidiata* to be apparently 12 in number. He describes in detail phenomena preceeding encystment in the rectum of the frog; the process of encystment and the character of the infection cysts; the hatching of the cysts in the rectum of the tadpole; the formation of isogametes and their copulation; encystment following copulation; a spindle-like shape of the male and female pronuclei in the copulation cysts, and of the large syncaria of the zygotes. He gives the name *O. zelleri* to the large stocky form which Zeller found with *O. dimidiata* in *Rana esculenta*, having himself seen the same form in the same host. On the basis of its manner of reproduction, he regards *Opalina* as related to the *Plasmodroma* rather than to the *Ciliophora*. [In many points the results of my study are opposed to Neresheimer. Some of his statements and beliefs to which I am unable to subscribe are: — his detailed description of the formation of generative chromidia, the degeneration of the old nuclei, and the formation of new sexual nuclei from the generative chromidia and refractive spherules, all of which is

said to preceede the formation of the infection cysts in the rectum of the frog; the complete disappearance of the refractive sphernles before the formation of the infection cysts; the presence of always two and only two chromatin spheres in the nuclei before the formation of the infection cysts, and their extrnsion, one before encystment, and one afterwards in the water or in the rectum of the tadpole (The definitiness of these phenomena seems to me uncertain); the presence of a double number of chromosomes in the sexual nnclei, 24 instead of 12 for *O. ranarum* and *O. dimidiata*; isogamous copulation; encystment following copulation (This seems to me donbtful); his interpretation of certain phenomena as abnormal budding (probably heterogamous copulation); his statement that the refractive sphernles are homogenons; his belief that *Opalina* is related to the *Plasmadroma* rather than the *Ciliophora*; and his belief that all healthy frogs contain Opalinas.]

Dobell (1907) observed *O. ranarum* and says he "confirms fully" Neresheimer's description of the nuclear phenomena preceeding encystment, i. e., "(1) formation of chromidia, (2) synthesis of fresh, nuclei from these chromidia, (3) reduction of chromatin and (4) encystment" [(1) and (2) I have not succeeded in finding in *O. ranarum*, or in any multinucleated *Opalina*; they do not occur in *O. intestinalis* or *O. caudata*; (3) seems to have no connection with true "reduction"]. He observed and interprets as degenerative 1) the irregular divisions preceeding encystment [described by Tönniges for *O. ranarum*; see my Text Fig. III, page 241]; 2) the loss of cilia from animals kept "some days" outside the host; 3) the presence of refractive spherules in the cytoplasm, which "ultimately run together forming large masses lying in the cells" [the refractive spherules are, of course, present in normal Opalinas; I have never seen them fused to form large masses]; 4) nnclear degeneration accompanied by amitotic division, equal or unequal: the degenerating nuclei are said to extrude their chromatin in the form of chromidia and entirely disappear; "as a rnle most of this chromatin is cast ont of the organism, which then dies and breaks up, but occassionally only a part of the chromatin in cast ont and perishes, the remaining granules" running together to form "two nuclei, consisting of solid chromatin", which "then approach one another and fuse".

Metcalf (1907 *a*) a preliminary notice of the present paper.

Hartmann (1907) accepts Neresheimer's (1907) conclusion that *Opalina* should be removed from the *Ciliophora* to the *Plasmodroma*, bnt does not assign it a definite position in the latter group.

Metcalf (1907 *b* and *c*) describes large excretory organs in *O. intestinalis*, *O. caudata* and *O. dimidiata*, and a very rudimentary excretory organ in *O. obtrigona*. No excretory organs were found in *O. ranarum* or *O. zelleri*, that which Delage & Herouard interpreted as remnants of excretory canals in the latter species not being so. The excretory organ is very simple, being merely a series of enlarged and connected alveoles of the ordinary cytoplasmic foam. The primitive character of the excretory organs and their resemblance to those of *Hoplitophrya* is emphasized.

Loewenthal (1908) [1]) found cysts of *O. ranarum* in young partly grown *Ranae temporariae* [showing that sexual maturity of the frog and the approach of its breeding season is not a necessary stimulus and probably does not furnish any stimulus to cause encystment of the parasites.] Cysts are sometimes found containing two Opalinas. This condition does not surely indicate division within the cyst, but in one observed instance arose by a fusion of at first independent cysts. This is regarded as very likely pathologic. Neresheimer's (1907 and 1908) description of the extrusion of chromatin spheres from the nuclei of encysted Opalinas is confirmed. Loewenthal gives up his former (1904) interpretation of these bodies as micronuclei and accepts Neresheimer's comparison of the phenomena with the formation of polar bodies [This I have opposed]. Stained with Giemsa's solution the spheres [1]) are clear blue [a reaction usually thought to indicate achromatic nature] while the smaller granules are red. If the nuclei are stained with methyl green in weak acetic acid, only the spheres are green [a reaction generally accepted as almost definitive for chromatin]. Treated with acetic acid the spheres become more highly refractive, if ammonia be added they become invisible but are not dissolved, both reactions indicating that the spheres are chromatin [?; the nucleoli show similar reactions]. On the basis of the reaction to Giemsa's stain the substance of the spheres is called cyanochromatin and the finer granules in the nucleus erythrochromatin, the two sorts of chromatin being regarded as functionally different, corresponding the cyanochromatin to Schaudinn's somatic chromatin, the erythrochromatin to his sexual

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[1]) Through the kindness of Dr. Hartmann I have been able to read the manuscript of this interesting paper, sent to him for publication, and to include a reference to it here.

[1]) Loewenthal cells them "*Körperchen*".

chromatin. In "*Paramaecia*" [probably *Balantidium*] from the rectum of the frog, and in other *Ciliata*, GIEMSA's staining colors the micronucleus red, the macronucleus blue. [The finer nuclear granules in *Opalina* I have regarded as chiefly achromatic, being influenced largely be their behaviour in degenerating nuclei of *O. obtrigona*, and by the fact that after staining with safranin and light green they are green, not red.]

Appendix. ***Staining reactio***

Stains and Reagents	Cilia	Pellicula	Basal granules of Cilia	Ectosarc films and granules	Ectosarc spherules
Intra vitam.					
1 Neutral red	O	O	O	O	dark red
2 Methylen blue	O	O	O	O	blue
3 Toluidin blue	O	O	O	O	blue
4 Congo red	O	O	O	O	O
5 Indigo-carmine	O	O	O	O	O
6 Methyl violet	O	O	O	O	O
7 Dahlia	O	O	O	O	O
8 Bismarck brown	O	O	O	O	O
9 Gentian violet	O	O	O	O	O
10 Thionin	O	O	O	O	O
After fixation with corosive-sublimate-acetic acid.					
11 Methylen blue	O	faint blue	green	green	green
12 Methyl violet	violet	very pale violet	violet	violet	pale violet
13 Gentian violet	pale violet	pale violet	dark violet	violet	violet, the smaller are darker
14 Thionin	O	O	green	green	green
15 Dahlia	very faint purple	very faint purplish gray.	purple	purple	purple
16 Fuchsin	pale red	good red	pale red	films faint pink, granules yellow with faint pink flush.	yellow with faint pink flush
17 Rubin S.	pale red	red	red	red	pale red
18 Orange G.	yellow	yellow	yellow	yellow	yellow
19 Bismarck brown	brown	brown	brown	brown	brown
20 Kernschwarz	brown	brown	brown	brown	brown
21 Safranin	red	red	red	red	red
22 Safranin and light green	green	green	green	green	green
23 Ehrlich's triacid mixture	? very faint	green	blue	blue	O
24 Biondi-Ehrlich-Heidenhain's mixture	? very faint	green	blue	blue	O
25 Borax carmine	faint red	? very faint	red	red	O
26 Paracarmine	faint red	? very faint	red	red	O
27 Delafield's haematoxylin	blue	blue gray	blue	blue	muddy yellow, i. e. unstained
28 Heidenhain's iron-haematoxylin	O	O	black	black	larger, gray smaller, black
29 Ehrlich's indulin-aurantia-eosin	blue	blue		very faint blue, almost unstained	O

n Opalina. (O = unstained.)

Endosarc films and granules	Endosarc spherules	Endosarc, Granules in posterior end of Excretory organ	Nuclear Membrane	Nucleus, Achromatic films and granules	Nucleolus	Nucleus, Chromatin including fibres	
O	dark red	O	O	O	O	O	1
O	O	O	O	O	O	O	2
O	O	O	O	O	O	O	3
O	O	O	O	O	O	O	4
O	O	O	O	O	O	O	5
O	stain strongly	O	O	O	O	O	6
O	stain well	O	O	O	O	O	7
O	O	O	O	O	O	O	8
O	violet	O	O	O	O	O	9
O	O	O	O	O	O	O	10
blue	O		?	very faint blue	very faint blue, almost unstained.	faint blue	11
violet	O		dark violet	violet	very faint blue, almost unstained.	red violet	12
violet	O		violet	violet	O	violet	13
blue	O		dark blue	faint blue, almost unstained.	O	blue	14
purple	O		purple	faint purple	O	purple	15
faint pink	good red		red	films pink. granules red	red	pink	16
pale red	good red	good red	red	red	red	red	17
yellow	yellow	yellow	yellow	yellow	yellow	yellow	18
brown	brown	brown	brown	brown	brown	brown	19
brown	brown	brown	brown	dark brown	brown	dark brown	20
red	fainter red	red	red	red		red	21
green	very pale red		green	green	green	red	22
blue	red purple		blue	blue		purple	23
blue	red	blue	blue	blue	blue	blue	24
red	O	dark red	red	red	O	red	25
red	O	red	red	red	O	red	26
blue	O	dirty dark dull blue	dark blue	dark blue	light grayish blue	very dark blue	27
black	black	black	black	black	black	black	28
blue	red		blue	blue		red	29

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Explanation of Plates.

All figures are camera drawings unless otherwise indicated. The degree of accuracy of all figures is told. When nothing is said as to accuracy, everything shown is carefully drawn with the camera; omissions are not always noted. Drawings from acetic-carmine preparations cannot show fine details for the stain does not bring out the finer structure.

Plate XIV.

Fig. 1. A schematic drawing of an optical longitudinal section of *O. intestinalis*, showing cilia, basal granules of cilia, ectosarc with ectosarc spherules (gray), endosarc with endosarc spherules (black), axial excretory organ, two nuclei (connected by a thread) each containing eight chromosomes. In the posterior nucleus is seen the vacuolated nucleolus.

Fig. 2. Part of a tangential section (superficial) of *O. ranarum*. Two rows of basal granules of the cilia are shown. Above each of these rows is a fibril which probably connects the outer ends of the basal granules. Transverse fibrils, at a little lower level than the last, run between the basal granules. The two longitudinal striae between the rows of basal granules are probably ridges in the pellicula. Where they cross over the transverse fibrils one sees a misleading hazy appearance of granules which do not exist. Coros. subl.-acetic acid, iron haematoxylin. × 4100 diameters.

Fig. 3. Part of an oblique section of *O. intestinalis*, showing cilia (diagrammatically drawn), pellicula, basal granules of cilia, five alveoles of the ectosarc, two ectosarc spherules with contained granules, and two endosarc spherules with contained granules. Coros. subl.-acetic acid, iron haematoxylin. × 2000 diameters.

Fig. 4. A cross section of *O. intestinalis*, showing the endosarc spherules (black) and the ectosarc spherules (gray). No attempt to indicate protoplasmic structure is made, merely the large alveoles of the ectosarc being drawn. Coros. subl.-acetic acid, iron haematoxylin (but little extracted). × 1485 diameters.

Fig. 5. Part of a longitudinal section of *O. intestinalis*, showing cilia (slightly diagrammatic), basal granules of cilia, granular spherules in ectosarc and endosarc, cytoplasmic granules, nucleus with three large granular masses of chromatin and granular achromatic foam, also three chromatin spherules seeming about to be extruded from the nucleus. The small faintly stained spherical body in the nucleus is probably a partly dissolved chromatin spherule. The arrows in-

dicate the boundary between ectosarc and endosarc. Coros. subl.-acetic acid, iron haematoxylin (well extracted). × 2000 diameters.

Fig. 6. Part of a cross section of *O. obtrigona*, showing cilia (semi-diagrammatic), pellicula, rows of basal granules of cilia, large alveoles of ectosarc containing finely granular ectosarc spherules (gray), granular endosarc spherules (more darkly stained) each in an alveole (some of these endosarc spherules lie in strands of endoplasma which have pushed out into the ectosarc), three nuclei in two of which one sees masses of chromatin lying against the nuclear membrane, while all show the superficial network of chromatin with nodal thickenings. The achromatic structures in the nuclei are not drawn, and the fine-meshed cytoplasm is but conventionally shown as granular. Some of the rows of basal granules and cilia are double because the rows are dividing where the body broadens, so as to cover the broader body surface. Coros. subl.-acetic acid, iron haematoxylin (well extracted). × 2000 diameters.

Fig. 7. Part of an oblique section of *O. intestinalis*, showing pellicula, well stained ectosarc spherules (some granular), unstained endosarc spherules looking like vacuoles, and nucleus. The boundaries between the different alveoles of the ectosarc were not well shown, nor was the structure of the endosarc clear. Coros. subl.-acetic acid, dahlia. × 2000 diameters.

Fig. 8. Part of an oblique section of *O. intestinalis*, showing pellicula (its ridges are drawn at the left of the figure), basal granules of cilia, sub-pellicular layer of ectosarc, and the alveolar layer of ectosarc, each alveole containing one ectosarc spherule. Coros. subl.-acetic acid, methyl violet. × 2000 diameters.

Fig. 9. Three ectosarc spherules from a section of *O. intestinalis*. Coros. subl.-acetic acid, iron haematoxylin (not much extracted). × 2000 diameters.

Fig. 10. Ten endosarc spherules from the same animal as in Fig. 9. The last two spherules show the not infrequent dumb-bell shape. The last spherule shows superficial granules. Coros. subl.-acetic acid, iron haematoxylin (not much extracted). × 2000 diameters.

Fig. 11. An endosarc spherule from a section of *O. obtrigona*. This slender dumbbell-shaped spherule shows the nearest approach to division I have found in the endosarc spherules of *Opalina*. Coros. subl.-acetic acid, iron haematoxylin (not well extracted). × 2860 diameters.

Fig. 12. Five endosarc spherules from a section of *O. ranarum*, showing internal (alveolar?) structure. In each instance the left side of the figure represents the side of the spherule toward the outer surface of the body. Coros. subl.-acetic acid, iron haematoxylin (long extracted). × 2860 diameters.

Fig. 13. Five endosarc spherules from a section of *O. intestinalis*. All are from the same animal, yet one sees that they are differently stained, indicating difference of condition. Coros. subl.-acetic acid, iron haematoxylin (not much extracted). × 2000 diameters.

Fig. 14. Six ectosarc spherules from the same animal as in Fig. 13. This animal was considerably shrunken and the spherules of the ectosarc and endosarc were also. Coros. subl.-acetic acid, iron haematoxylin (not much extracted). × 2000 diameters.

Fig. 15. A bit of unusually coarse-meshed endoplasmic foam from a section of *O. caudata*. Coros. subl.-acetic acid, Delafield's haematoxylin. × 1485 diameters.

Fig. 16. A bit of endosarc from a section of *O. intestinalis*, showing that each endosarc spherule lies in an alveolus. The structure of the endoplasma is not well shown. Coros. subl.-acetic acid, EHRLICH's triacid mixture. × 2000 diameters.

Fig. 17. An optical section of the posterior end of a slender *O. dimidiata*, showing part of the excretory organ. One nucleus, in mitosis, lies surrounded by the excretory vacuoles. Only the upper half of this nucleus is drawn. Ten (?) or twelve rows of chromatin granules (chromosomes) were present. The general cytoplasmic foam is not shown. Coros. subl.-acetic acid, DELAFIELD's haematoxylin. × 1420 diameters.

Plate XV.

Opalina intestinalis (Figs. 18—31) and *O. dimidiata* (Fig. 31 *a*).

Fig. 18. Part of a section stained with methylen blue. Pellicula pale blue, ectosarc films and spherules green, endoplasma blue (structure very obscure and not shown), endosarc spherules unstained appearing like vacuoles, nuclear membrane blue, nuclear contents not shown, except the nucleolus (pale greenish blue with a dark blue vacuolated cap). Coros. subl.-acetic acid, methylen blue. × 2000 diameters.

Fig. 19. Another nucleolus from the same preparation as Fig. 18, but with two dark blue unvacuolated caps. × 2000 diameters.

Fig. 20. An individual stained intra-vitam with tolnidin blue. The ectosarc spherules are stained, the endosarc spherules unstained. The band of ectosarc spherules shown crossing the cell in front of the posterior nucleus is superficial and is half of a complete ring. Very darkly stained bodies of a problematic nature are shown. The nuclei were entirely unstained until after all motion of the cilia ceased. Then certain masses (of chromatin?) began to stain as indicated. This individual was in an early stage of transverse division. × 500 diameters.

Figs. 21 and 22. Two sections showing the whole of one nucleus, stained with safranin and light green. The heavily stained red spherules are chromatin spherules. The chromosomes are paler red and granular. (The granules were not drawn with the camera. They should be one-half larger and one-third less numerous.) The achromatic granules and the large nucleolus are green. Neither the chromatin network nor the achromatic films were clearly enough seen to draw. The former was composed of extremely delicate threads. Coros. subl.-acetic acid. × 2000 diameters.

Fig. 23. Part of a section stained with safranin and light green, showing cilia, outer contour of pellicula, basal granules of cilia, ectosarc with coarser and finer fibrillae or films (green, semi-diagrammatic) and green-stained spherules, endosarc with pink-stained spherules and coarser and finer fibrillae and films (These are stained green in this preparation. If left a moment longer in absolute alcohol they would be red. It was impossible to draw these with entire accuracy), nucleus with chromatin masses and threads stained red, and the achromatic structures green. The three faint red bodies in the nucleus are probably dissolving chromatin spherules. All the nuclear structures shown (accurately drawn) lie near the upper, anent, surface of the nucleus. The nucleolus is not in this section. Coros. subl.-acetic acid. × 2000 diameters.

Fig. 24. A longitudinal section of a nucleus in a late telophase of mitosis. Only a portion of the achromatic granules present are drawn. The nucleolus is not in this section. Coros. subl.-acetic acid, safranin and light green. × 2000 diameters.

Fig. 25. A section of another nucleus similarly preserved and stained. The chief lines of achromatic granules at the upper surface of the section are accurately shown; the other granules are drawn free hand. × 2000 diameters.

Figs. 26 and 27. Sections of other nuclei in which the fibrillar structures were more clearly seen. In Fig. 27 the achromatic granules and films lying beneath the chromatin net in the center of the nucleus are not drawn. Coros. sublim.-acetic acid, safranin and light green. × 2000 diameters.

Figs. 28—31. Four sections showing the whole of one nucleus and, in Fig. 29, part of the cytoplasm. The chromatin spherules are numerous; the nucleolus is vacuolated. Except the very delicate chromatin net, which was difficult to see, all the chromatin structures present are drawn. In Fig. 29 the thickness of the pellicula (green) is uncertain, for the section was a little oblique. The interruption of the pellicula, as shown, was undoubted. Ectosarc films, granules, fibrils (?) and spherules, green; endosarc spherules pink; endosarc granules, films and fibrils (?), green. × 2000 diameters.

Fig. 31*a*. On optical section of a macrogamete or macrogamete parent cell of *O. dimidiata* from a tadpole of *Bufo vulgaris*, infected 19½ hours. The ectosarc spherules are yellow, the endosarc spherules red. The nuclei are not drawn. Acetic-carmine. × 1010 diameters.

Plate XVI.

Living Opalina intestinalis.

Except in Fig. 34, the anterior end of each nucleus in toward the top of the plate.

Fig. 32. The anterior end of a daughter cell whose nucleus is in a telophase of mitosis. Only the nuclear structure is shown. This was the posterior nucleus of the parent cell, as is indicated by its pointed anterior end. Eight chromosomes are seen in the anterior end of the nucleus; in the posterior end but seven can be seen, the eighth lying in the lower half of the nucleus below the right hand chromosome of the upper group of four. All the structures in the nucleus were remarkably clear, as clear as in the best stained preparations. The achromatic granules of only the upper half of the nucleus are shown, except that in the anterior end of the nucleus two rows of granules at a lower level are also drawn. × 1010 diameters.

Fig. 33. A nucleus drawn five to forty-five minutes after active swimming movements of the animal ceased, the cilia being then but slightly active. The granules of only the upper third of the nucleus are drawn. A few endosarc spherules and large granules of the cytoplasm are drawn for comparison. Eight chromosomes are seen in the anterior nucleus; three of those in the posterior nucleus are hidden. This nucleus was later almost completely isolated from the body, being held at one end by part of the broken body. It withstood uninjured the most violent currents produced by intermittent pressure on the cover-glass and after three days was entirely unchanged, except that the chromosomes seemed more nearly spherical and the achromatic granules were less refractive, some having disappeared. × 1485 diameters.

Fig. 34. A pair of nuclei from an animal with active cilia, which was held immovable by pressure between a hair and the cover-glass. Eighteen hours after all movements of the cilia ceased the nuclei were in the same condition. Eight (or nine) distinct chromatin masses are in the anterior nucleus (one chromosome

has constricted or is constricting into two); in the other nucleus the chromosomes are already united into a ribbon. The difference in condition in the two nuclei is greater than usual, but is apparently not abnormal. The achromatic granules, in this case, were not carefully counted or drawn with entire accuracy. × 1010 diameters.

Fig. 35. A pair of nuclei connected by a bent and somewhat spiral thread. In the anterior nucleus seventeen chromatin (?) masses (one may be the nucleolus) are seen, also several spindle fibers which were remarkably clear. This is an early prophase of mitosis. × 1010 diameters.

Fig. 36. The posterior nucleus of a binucleated individual, in a late anaphase of mitosis. Eight chromosomes are in each end. But few of the spindle fibres were clear enough to draw and even these showed only faintly. The achromatic granules of only the upper half of the nucleus are shown. These were unusually large and were irregular in shape, a fact difficult to show in a drawing at this scale. × 1485 diameters.

Fig. 37. A pair of nuclei connected by a very long and irregularly bent thread. Fourteen to sixteen chromatin masses are shown in the anterior nucleus, in the posterior nucleus the chromatin ribbon is still incompletely fragmented. Almost none of the achromatic granules in the anterior nucleus are drawn. In the posterior nucleus only the larger achromatic granules are shown. × 1010 diameters.

Plate XVII.

Opalina intestinalis.

Longitudinal division Figs. 38—43, transverse division Fig. 44.

In drawings at this scale the nuclear phenomena cannot be accurately shown.

Fig. 38. A very early stage of division, the anterior end of the body showing merely a slight indentation. The nuclei are in a late anaphase of mitosis. Coros. subl.-acetic acid, borax carmine. × 460 diameters.

Fig. 39. A little later stage of division. The nuclei are in a late anaphase of mitosis. Coros. subl.-acetic acid, borax carmine. × 460 diameters.

Fig. 40. A still later stage of division. The nuclei are in a late anaphase of mitosis. Both anterior and posterior ends of the body are dividing. Coros. subl.-acetic acid, borax carmine. × 460 diameters.

Fig. 41. A dividing individual, slightly abnormal, having been kept four days in Lock's fluid before killing. The nuclei are in a late telophase of mitosis. The body should before this have completely divided. The general form of the body, however, well represents the usual normal manner of division. Coros. subl.-acetic acid, Delafield's haematoxylin. × 460 diameters.

Fig. 42. An almost completely divided individual. The nuclei are in an early telophase. This individual had been kept three days in sodium chloride solution before killing. Probably under normal conditions division would have been completed before the nuclei reached this stage. The general form is exactly similar to what is found in entirely normal animals in a late stage of division. Coros. subl.-acetic acid, Delafield's haematoxylin. × 460 diameters.

Fig. 43. A daughter cell fresh from division, as is shown by the irregular contour of one side of the body where it was connected with its sister cell (compare Fig. 41). The nucleus is in a rather late anaphase, as is usual in such young daughter cells. This cell has received the posterior nucleus from the parent,

as is shown by its position (compare Figs. 40—42). Coros. subl.-acetic acid, borax carmine. × 460 diameters.

Fig. 44. An individual in transverse division. The nuclei are in an anaphase of mitosis. Coros. subl.-acetic acid, DELAFIELD's haematoxylin. × 460 diameters.

Plate XVIII.

Opalina intestinalis.

All figures are reduced one-fifth, to the magnification indicated.

Fig. 45. An individual with two nuclei each in a prophase of mitosis. Coros. subl.-acetic acid, DELAFIELD's haematoxylin. Drawn at 1010 diameters, reduced to 808 diameters.

Figs. 46—48. Three optical sections, through the upper, middle and lower thirds respectively, of the posterior nucleus shown in Fig. 45. In Fig. 46 most of the fibrils shown belong to the chromatin network. In Fig 47 the varicose fibrils in the center of the figure are optical sections of the films of the achromatic foam. In Fig. 48 only the chromatin masses and a few of the chromatin fibres are shown. × 1600 diameters.

Fig. 49. An individual with two nuclei in an early stage of mitosis. Especially in the posterior nucleus, one sees that the chromosomes are arranging themselves in two adequatorial rows preparatory to migration to the poles of the nucleus. The distinct and rather coarse fibers are fibers of the chromatin spindle. Most of the achromatic structures and the finer chromatin threads are omitted. Coros. subl.-acetic acid, DELAFIELD's haematoxylin. × 808 diameters.

Figs. 50—52. Three optical sections, through the upper, middle and lower thirds respectively of the posterior nucleus shown in Fig. 49. The coarse varicose longitudinal chromatin fibres of the spindle are well seen in Fig. 50. Fig. 51 shows the emarginate form of the ends of the chromatin spindle (compare Fig. 49). Fig. 52 shows that not all of the chromosomes have yet been formed from the chromatin ribbon (spireme). The achromatic structures lying in the deeper layer of the upper third of the nucleus are omitted from Fig 50. In Figs. 50 and 51 one sees that the chief longitudinal fibres of the chromatin spindle are attached to each pole of the nuclear membrane. The nucleolus lies in the lower part of Fig. 52. It is darkly shaded, not because it was heavily stained, but to make it seem to lie near the top of the section. × 1600 diameters.

Fig. 53. An individual with its nuclei each in an anaphase of mitosis. Eight chromosomes were present in each end of each nucleus. Most of the achromatic structures and the finer chromatin fibres are omitted in each nucleus. Coros. subl.-acetic acid, DELAFIELD's haematoxylin. × 808 diameters.

Fig. 54. A daughter cell with its single nucleus in a late anaphase of mitosis. Some of the chromosomes are constricted transversely into two unequal portions (compare Fig. 53). All structures are omitted except the chromosomes and the thickest chromatin fibres. Coros. subl.-acetic acid, DELAFIELD's haematoxylin. × 808 diameters.

Fig. 55. A nucleus, in an anaphase of mitosis, in which the fibres of the chromatin spindle were more delicate, more numerous and less distinctly longitudinal than usual. Except in the center of the nucleus, the achromatic structures are omitted. Here some of the chromatin fibres are omitted, leaving a "window" through which the vacuolated nucleolus and the films of the achromatic foam are seen. The nucleolus is seen to lie in an enlarged alveolus of the achromatic foam,

whose fibres radiate from the surface of the nucleolus. Where they touch the nucleolus triangular nodal thickenings of the films are seen. The chromosomes are granular. Coros. subl.-acetic acid, DELAFIELD's haematoxylin. × 1600 diameters.

Fig. 56. A nucleolus with central vacuole and minute peripheral vacuoles. Coros. subl.-acetic acid, DELAFIELD's haematoxylin. × 1600 diameters.

Fig. 57. A nucleus showing characteristic enlargement of the ends of the longitudinal fibres of the chromatin spindle, only a small part of the structures in the nucleus are drawn. Coros. subl.-acetic acid, DELAFIELD's haematoxylin. × 1600 diameters.

Fig 58. A nucleus in an early telophase of mitosis. In one end of the nucleus two of the chromosomes have fused by sending out a broad band of chromatin which unites them. There are eight chromosomes in this end. At the other end two of the chromosomes are constricted each into two. None of these chromosomes are yet united as in the opposite end of the nucleus. At the centre of the nucleus are a few alveoles of the achromatic foam which stain more deeply than the rest and are probably filled with dissolved chromatin from the "chromatin spherules". With the exception of these alveoles, none of the achromatic structures in this nucleus are shown, and only the larger fibres of the chromatin net are drawn. Coros. subl.-acetic acid, DELAFIELD's haematoxylin. × 1600 diameters.

Fig. 59. A very thin transverse section of a nucleus, showing the chromosomes just beneath the nuclear membrane, also lines of achromatic granules, and four partially dissolved masses of chromatin ("chromatin spherules"). The finer details of the achromatic structures were not clear enough to draw. Coros. subl.-acetic acid, borax carmine. × 1188 diameters.

Plate XIX.

Opalina intestinalis.

All figures are reduced one-fifth, to the magnification indicated.

Fig. 60. A daughter cell with the nucleus in a late telophase of mitosis Coros. subl.-acetic acid, DELAFIELD's haematoxylin. × 808 diameters.

Figs. 61—62. Two optical sections, through the upper and lower halves respectively, of the nucleus shown in Fig. 60. The chromosomes are seen to be united together by broad thin bands of chromatin. The transverse line near the upper end of Fig. 61 is a fold in the nuclear membrane. The finer chromatin fibres are not drawn nor are the fibres of the achromatic foam. Only a few of the achromatic granules are shown. The nucleolus is not drawn. Coros. subl.-acetic acid, DELAFIELD's haematoxylin. × 1600 diameters.

Fig. 63. An older daughter cell whose nucleus is nearly separated into two daugther nuclei, one of which shows eight distinct chromosomes, while in the other the chromosomes are mostly united as in Fig. 61. The drawing shows the chromosomes, the polar fibres connecting one set of these with the pole, and several faintly stained bodies, nearer the constricted center of the nucleus, which seem to be dissolving "chromatin spherules". Coros. subl.-acetic acid, DELAFIELD's haematoxylin. × 808 diameters

Fig. 64. Outlines of the nucleus and anterior end of the body of another individual. × 404 diameters.

Fig. 65. The same nucleus as that shown in Fig. 64. It is in a late telophase of mitosis, the daughter nuclei, being nearly distinct. Eight chromosomes were present in each daughter nucleus, as was shown when the animal was

slightly rolled. These are all distinct. A few dissolving chromatin spherules are shown near the constricted end of each daughter nucleus. The nucleolus is not drawn. Coros. subl.-acetic acid, DELAFIELD's haematoxylin. × 1600 diameters.

Fig. 66. Outlines of the nuclei and anterior end of the body of another individual. × 404 diameters.

Fig. 67. The posterior of the two nuclei shown in Fig. 66. Only the granular chromatin ribbon and a little of the achromatic foam is shown. Coros. subl.-acetic acid, DELAFIELD's haematoxylin. × 1600 diameters.

Fig. 68. The anterior end of a binucleated individual, showing in each nucleus the chromatin ribbon beginning to break up into the chromosomes preparatory to the next mitosis. The achromatic granules are conventionally drawn. The nucleolus is not shown. Coros. subl.-acetic acid, DELAFIELD's haematoxylin. × 808 diameters.

Fig. 69. A thin optical section through the posterior nucleus shown in Fig. 68. All the granules in the chromosomes and the achromatic granules and film-lines are accurately drawn, except that the level of some of the achromatic granules is not correctly shown. × 1600 diameters.

Fig. 70. The anterior end of an individual in whose nuclei the chromatin ribbon has apparently already constricted to form separate chromatin masses, although, from the short thread connecting them, the nuclei seem to be young. The chromatin masses of only the upper half of the nuclei are drawn. The spherical nucleolus is seen in the posterior nucleus. Coros. subl.-acetic acid, DELAFIELD's haematoxylin. × 808 diameters.

Fig. 71. The anterior end of an individual with two nuclei. The chromatin masses of only the upper half of the nuclei are drawn. The spherical nucleolus is seen in the posterior nucleus. Chromatin spherules are forming from the chromatin masses. Coros. subl.-acetic acid, DELAFIELD's haematoxylin. × 808 diameters.

Fig. 72. The anterior end of an individual with two nuclei in which the chromatin spherules are for the most part already separated from the chromatin masses. The old nucleolus is seen in the posterior nucleus. A new nucleolus is forming near the pointed end of the anterior nucleus. Coros. subl.-acetic acid, DELAFIELD's haematoxylin. × 808 diameters.

Fig. 73. The old nucleolus and a single chromatin mass from a nucleus in a condition slightly earlier than that shown in Fig. 72. The chromatin mass is granular, as the chromosomes always are. The chromatin spherules, much larger than the chromatin granules, are about to be cast off. Coros. subl.-acetic acid, DELAFIELD's haematoxylin. × 1600 diameters.

Fig. 74. An injured daughter cell (posterior end broken) whose nucleus shows the only exception I have found to the rule that in this species the old nucleolus remains in the posterior daughter nucleus. In this case it is in the anterior daughter nucleus. No attempt is made in this small scale drawing to represent all the chromosomes. Coros. subl.-acetic acid, DELAFIELD's haematoxylin. × 368 diameters.

Plate XX.

Opalina intestinalis and *O. caudata*.

All figures are reduced one-fifth, to the magnification indicated.

Figs. 75—80. *Opalina intestinalis*.

Fig. 75. The posterior nucleus of a binucleated individual, entering upon mitosis. Numerous faintly stained partially dissolved chromatin spherules are

shown. The achromatic structures (except the nucleolus), the finer threads of the chromatin net, and the details of form of the chromosomes are omitted from the figure. Coros. subl.-acetic acid, DELAFIELD's haematoxylin. × 1600 diameters.

Fig. 76. An individual with its nuclei in what may perhaps be called the resting condition. Only the chromatin masses and the larger vacuoles of the achromatic foam are shown. Coros. subl.-acetic acid, DELAFIELD's haematoxylin × 808 diameters.

Fig. 77. The posterior nucleus shown in Fig. 76. Only the achromatic foam and the chromatin masses are shown, the nucleolus and the chromatin net being omitted. × 1600 diameters.

Fig. 78. An anterior nucleus drawn to show a weakly stained chromatin spherule in a tubular process from the anterior end of the nucleus. The chromatin masses also are drawn. Coros. subl.-acetic acid, DELAFIELD's haematoxylin. × 1600 diameters.

Fig. 79. A nucleus similar to that drawn in Fig. 78, showing several partially dissolved (weakly stained) chromatin spherules, one of which lies in a projection from the anterior end of the nucleus. All achromatic structures and the chromatin net are omitted from the drawing. Coros. subl.-acetic acid, DELAFIELD's haematoxylin. × 1600 diameters.

Fig. 80. A dumbbell-shaped nucleus of a daughter cell. A few partially dissolved chromatin spherules are seen in each end of the nucleus. The chromatin net, the nucleolus, and all the other achromatic structures except a few granules are omitted from the drawing. Coros. subl.-acetic acid, DELAFIELD's haematoxylin. × 1600 diameters.

Figs. 81—92. *Opalina caudata.*

Fig. 81. An individual with each of its nuclei in a late anaphase of mitosis. Six chromosomes are seen in each end of each nucleus. The spherical bodies at the centers of the nuclei are the nucleoli. The achromatic foam and the finer fibres of the chromatin net are omitted from the figure. Coros. subl.-acetic acid. borax carmine. × 246 diameters.

Fig. 82. A daughter cell showing the nucleolus in the anterior daughter nucleus, and six chromosomes in each nucleus; some of these are already united preparatory to forming the chromatin ribbon. Coros. subl.-acetic acid, borax carmine. × 262 diameters.

Fig. 83. A section of a nucleus showing granular chromatin masses, some fibres of the chromatin network with darkly stained nodal thickenings, and some films and granules (lightly stained) of the achromatic foam. Observe that the granules in the chromatin masses are of various sizes and shapes. Coros. subl.-acetic acid, iron haematoxylin. × 1188 diameters.

Fig. 84. Part of a section of a nucleus, showing one granular chromatin mass, and a few other granules probably achromatic. Near the nucleus is shown a single granular endosarc spherule. Coros. subl.-acetic acid, iron haematoxylin. × 1188 diameters.

Fig. 85. Part of a longitudinal section, showing one nucleus and a little of the adjacent cytoplasm. This nucleus was in an anaphase of mitosis. In order to show more clearly the chromatin granules upon the chromatin net these are drawn much darker than the chromosomes and the fibres of the chromatin net.

In reality all were stained equally dark. Coros. subl.-acetic acid, iron haematoxylin. × 808 diameters.

Fig. 86. A longitudinal section of a nucleus in a telophase of mitosis. Four of the chromosomes are seen to have at their edges rows of more or less elongated granules. These granules are not arranged in pairs. Most of the longitudinal fibres of chromatin are seen to be very granular. The finer chromatin threads and most of the achromatic structures are omitted. In this nucleus it was impossible to sharply distinguish between chromatin granules and achromatic granules. Coros. subl.-acetic acid, iron haematoxylin. × 1600 diameters.

Fig. 87. An abnormal individual showing a great lateral swelling in which the endosarc was evenly granular, and the ectosarc apparently structureless. There were no endosarc spherules in the swollen area. They were abundant in the rest of the body, but are not drawn. The finer structures of the nuclei were not clear enough to draw. Coros. subl.-acetic acid, Delafield's haematoxylin. × 404 diameters.

Fig. 88. A very stocky individual. In the nuclei only the chromosomes and chief chromatin fibres of the upper surface are shown. Coros. subl.-acetic acid, Delafield's haematoxylin. × 556 diameters.

Fig. 89. A very stocky and abnormal individual, with four nuclei each in a telophase of mitosis. Two of these, seen in end view, do not show their mitotic condition. In the right band nucleus the chromatin is abnormally compact. In the nucleus in the middle most of the granules drawn are probably achromatic. In the other two nuclei only the chromosomes and the chief fibres of the chromatic spindle are drawn. Coros. subl.-acetic acid, Delafield's haematoxylin. × 556 diameters.

Fig. 90. An individual with almost completely degenerated nuclei. Coros.-subl.-acetic acid, Delafield's haematoxylin. × 400 diameters.

Fig. 91. A probably abnormal individual with nuclei in a condition characteristic of the multi-nucleated *Opalinae*, but never, I think, found in normal individuals of *O. caudata*. Coros. subl.-acetic acid, Delafield's haematoxylin. × 808 diameters.

Fig. 92. An individual with abnormal nucleus. Coros. subl.-acetic acid, borax carmine. × 404 diameters.

Plate XXI.

Abnormalities, *Opalina intestinalis* and *O. obtrigona*.

All figures are reduced one-fifth, to the magnification indicated.

Figs. 93—98. *Opalina intestinalis*.

Fig. 93. Outlines of the nuclei and the anterior end of a young individual. × 404 diameters.

Fig. 94. The same nuclei as those shown in Fig. 93. The aggregation of the chromatin into large irregular masses is abnormal, although the animal was killed immediately after removal from the host. Coros. subl.-acetic acid, Delafield's haematoxylin. × 1600 diameters.

Fig. 95. The anterior end of an individual where posterior nucleus is abnormal, as is shown by the aggregation of the chromatin into a few dense compact masses. This was first observed upon staining with acetic-carmine, after which the animal was stained with Delafield's haematoxylin and drawn. In the anterior

nucleus only the chromatin masses are drawn. Kept alive three days in 0,6% NaCl solution, acetic-carmine four hours, 0,6% NaCl solution ½ day, coros. subl.-acetic acid, Delafield's haematoxylin. × 404 diameters

Fig. 96. A small individual with a single abnormal nucleus. (Compare Plate XX Fig. 92.) Flemming's stronger fluid, Mayer's haemalum. × 368 diameters

Fig. 97. An individual kept alive three days in 0,6% NaCl solution. The shape of the body and the position of the nuclei are unusual and may be abnormal. In the nuclei, which were in a late anaphase of mitosis, the chromatin masses are not carefully drawn. At the posterior end of the body one sees a depression which marks the position of the excretory aperture, an ovoid mass of the excretory granules, and the enlarged posterior vesicle of the excretory organ. The method of preparation was the same as for the individual shown in Fig. 95. × 368 diameters

Fig. 98. A dividing individual, still active after living 5½ days in 0,6% NaCl solution. Division began the third day and the form of the body reached the condition shown upon that day; the animal remained apparently unchanged two days more and was then killed, stained and drawn. Three nuclei are in one daughter cell. The anterior daughter of one nuclear pair and the posterior daughter of the other are about to fuse. (In other instances more advanced stages of this fusion were found.) The four daughter nuclei seem normal; each has eight chromosomes; each posterior daughter nucleus contains a nucleolus. Coros. subl.-acetic acid, Delafield's haematoxylin. × 368 diameters.

Figs. 99—118. Abnormal *Opalina obtrigona*.

(Coros. subl.-acetic acid, Delafield's haematoxylin. All figures × 1600 diameters, except Fig. 111 which is a free hand sketch on a slightly smaller scale.

Figs. 99—101. Nuclei seeming almost if not quite normal. The network with thickened nodes is superficial and is probably chromatin, but the achromatic foam (not drawn), filling the whole nucleus, presents much the same appearance. Each nucleus shows from two to six discoid masses of chromatin upon the nuclear membrane. The bodies which are lightly shaded are chromatin discs on the far side of the nucleus.

Figs. 102 and 103. Nuclei in which the chromatin plates upon the nuclear membrane are reticulate. In the center of each nucleus is a mass of granules probably chiefly achromatic. A little of the superficial, chromatin (?) net is shown and a little of the achromatic foam is drawn in Fig. 103. It is hardly distinguishable from the superficial net, except by its more central position and the smaller size of its meshes.

Fig. 104. A nucleus in which the chromatin in chiefly in two reticulate masses, in one of which a central refractive body is seen. The reticulation of the lower mass is not shown.

Figs. 105—109. Optical sections through nuclei showing similar conditions. In each is a central mass of granules probably chiefly achromatic and one or two bodies consisting of a central refractive sphere surrounded by a layer of chromatin which shows a denser net with more faintly staining interspaces. Bits of the achromatic foam are indicated in some of the figures.

Fig. 110. A nucleus in which the achromatic granules are in two masses connected by lines of similar granules. One sphere, with its chromatin either more compact or not well differentiated by the stain, is shown, as is also the achromatic foam.

Figs. 111—115. Optical sections of other nuclei in similar conditions. Note that in Fig. 111 there are two chromatin-covered spheres upon the pseudo-spindle.

Figs. 116 and 117. Nuclei which have almost completely degenerated, showing only one or two masses of debris within the still intact nuclear membrane.

Fig. 118. An optical section through a part of the body showing two nuclei and the remains of eight or nine others. In some cases merely an empty space marks the former position of a nucleus; in other cases one sees small spheres, the remains of the chromatin-covered spheres; in other cases there are left merely masses of debris representing the achromatic structures.

Plate XXII.

Opalina intestinalis. Figs. 137—139 *O. caudata.*

All figures reduced one-third, to the magnification indicated.

Fig. 119. A small individual with eight chromosomes, from a tadpole of *Bombinator pachypus*, infected 5 days. This *Opalina* passed unencysted through the alimentary canal of the tadpole. Acetic-carmine. × 673 diameters.

Fig. 120. A small individual with four chromosomes, from the same tadpole. It passed unencysted through the alimentary canal of the tadpole. The reduced number of chromosomes is seen in animals of this size and smaller. Acetic-carmine. × 673 diameters.

Figs. 121—128. Optical sections of minute individuals about ready for encystment, from the rectum of an adult *Bombinator pachypus*. Figs. 121 and 122 show respectively an early and a late stage of the last division before encystment. Observe that the nuclei are not in mitosis. In Figs. 122 and 123 the posterior end of the body shows a very delicate pellicula and numerous slight lobulations. In each nucleus the chromatin spheres are shown, and in most of them the group of achromatic granules is drawn. The cytoplasmic structure is either omitted or conventionally drawn. The ectosarc spherules are shown in Figs. 123 and 128. In Figs. 121, 126 and 127 probably the larger spherules belong to the ectosarc, the smaller to the endosarc. Acetic-carmine. × 673 diameters.

Fig. 129. An optical section of an individual which had begun to encyst. Endosarc spherules (shaded, more abundant in the anterior part of the body) and a few ectosarc spherules (unshaded, in the posterior end of the body) are shown. The nuclear and cytoplasmic structure is not carefully drawn. Acetic-carmine. × 673 diameters.

Figs. 130—132. Optical sections of cysts from the rectum of an adult *Bombinator pachypus*. In Fig. 130 the unusually small endosarc spherules are drawn. Fig. 131 shows one chromatin sphere in the nucleus, Fig. 132 shows three. Acetic-carmine. × 673 diameters.

Fig. 133. The nucleus of another cyst from the same preparation, showing only the reticulate character of the superficial chromatin in the chromatin sphere. Acetic-carmine. × 1010 diameters.

Fig. 134. An optical section of a newly formed cyst with very delicate wall, from the same preparation. There are three chromatin spheres in the nucleus. The ectosarc spherules are at the outermost edge of the ectosarc. Acetic-carmine. × 673 diameters.

Fig. 135. An optical section of another cyst from the same preparation. Most, perhaps all, of the ectosarc spherules have been extruded and lie between the body and the wall of the cyst. Acetic-carmine. × 990 diameters.

Fig. 136. An optical section of another cyst from the same preparation, showing in the nucleus one large and one small chromatin sphere and a central mass of granules. Between the cell body and the cyst wall is a mass of debris probably derived from degenerated ectosarc spherules. Acetic-carmine. × 673 diameters.

Figs. 137—139. *Opalina caudata.* Sections (2 μ) of cysts from the rectum of an adult *Bombinator pachypus.* All of the nuclear structures present are accurately drawn. In Figs. 137 and 138 all of the chromatin seems to be gathered into the chromatin spheres. In Fig. 139, in addition to the chromatin sphere, ten small chromatin masses are seen. Others were present in the adjacent sections. Coros. subl.-acetic acid, Delafield's haematoxylin. × 1334 diameters.

Figs. 140—143. Animals hatching from the cysts in the rectum of a tadpole of *Bombinator pachypus* five hours or less after ingestion of the cysts. Ectosarc spherules are shown (shaded) in Fig. 140. In the same figure granular debris is seen in the cyst. The boundary between ectosarc and endosarc is indicated by dotted lines. Most of the cilia were distroyed by the acetic acid and the currents caused in making the preparation; all which were seen are drawn. The details of nuclear structure were not well shown. Acetic-carmine. × 673 diameters.

Figs. 144 and 145. Macrogametes or macrogamete parent-cells [1]) from the rectum of a tadpole of *Bombinator pachypus*, infected 6 days. Few cilia were drawn in the original sketch for Fig. 144, the rest having been filled in later. The endosarc spherules are shown in Fig. 145. No nucleus was visible in this darkly stained animal. Possibly it had degenerated. Acetic-carmine. × 673 diameters.

Fig. 146. A living dividing individual from a tadpole of *Bufo vulgaris*, infected 60 hours. Endosarc spherules (shaded) and ectosarc spherules (unshaded) are shown. The nuclei were not clear. A second, more anterior, seemed to be present at a lower level, but I could not be sure of it. The long and sparce cilia make it probable that (after not less than two divisions) this individual would have given rise to microgametes. × 673 diameters.

Fig. 147. A free-hand sketch of a very active, dividing macrogamete mother-cell (it may be a parent-cell) from a tadpole of *Bufo vulgaris*, infected 36 hours. The two daughter cells are of the same size. They were slightly flattened, one being seen more in edge view. Extruded excretory granules were seen dragging behind one daughter cell.

Figs. 148 and 149. Optical sections of macrogametes or macrogamete parent-cells from a tadpole of *Bombinator pachypus*, infected 136 hours. But few cilia were in the original sketch for Fig. 148, the rest having been supplied later. Each nucleus shows four chromosomes. Acetic-carmine. × 673 diameters.

Fig. 150. An optical section of a macrogamete from a tadpole of *Bombinator pachypus*, infected 43 hours. Acetic-carmine. × 673 diameters.

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[1]) I use the word parent-cell to indicate a cell which after one or more divisions will produce gametes. The word mother-cell is used for a cell whose next division will give rise to gametes. I realize that the phraseology is not satisfactory, but with the understanding that parent is entended to include grand parent or still earlier generations, it may do for the purposes of the present paper.

Fig. 151. An optical section of a macrogamete from a tadpole of *Bombinator pachypus*, infected 6 days. Acetic-carmine. × 673 diameters.

Fig. 152. A section (4 μ) of a macrogamete or macrogamete parent-cell in the rectum of a tadpole of *Bombinator pachypus*, infected 24 hours. Coros. sublimate-acetic acid, DELAFIELD's haematoxylin. × 1334 diameters.

Fig 153. A living dividing gamete parent-cell from a tadpole of *Bufo vulgaris*, infected 42 hours. Extruded excretory granules are seen at the posterior end of the body. The long and sparce cilia make it probable that (after not less than two divisions) this cell will give rise to microgametes. × 673 diameters.

Plate XXIII.

Opalina intestinalis.

All figures are reduced one-third, to the magnification indicated.

Fig. 154. A microgamete parent-cell from a tadpole of *Bombinator pachypus*, infected 70 hours. The cilia are taken from a sketch of the living animal; the body form and nucleus were drawn after treatment with acetic-carmine. × 673 diameters.

Figs. 155–158. Microgamete parent-cells from a tadpole of *Bombinator pachypus*, infected 70 hours. In Fig. 155 parts of the excretory organ are seen. Acetic-carmine. × 673 diameters.

Fig. 159. A living microgamete parent-cell in division, from a tadpole of *Bombinator pachypus*, infected 70 hours. The cilia are too short. × 1010 diameters.

Fig. 160. A living cell ready to metamorphose into a microgamete, from a tadpole of *Bombinator pachypus*, infected 42 hours. I suspect that the posterior two or three cilia were attached further forward than is shown, since the naked end of the tail is longer in the fully formed microgamete than in this animal as here drawn. × 673 diameters.

Figs. 161 and 162. Microgametes (the first mature, the second probably not so) whose tails are contracted by acetic-carmine; from tadpoles of *Bombinator pachypus*, infected 91 hours (Fig. 161) and 70 hours (Fig. 162). In Fig. 161 the endosarc spherules are drawn. × 673 diameters.

Fig. 163. A living microgamete from a tadpole of *Bufo vulgaris*, infected 42 hours. Accurately drawn, except that the nuclear structure, which was not clear, is omitted. The endosarc spherules are shown. × 673 diameters.

Fig. 164. An early stage of copulation. From life. The animals were too active for drawing with the camera. The record of the infection from which these animals were obtained is lost. Some cilia have been added to the microgamete in the original sketch, which represented rather too thin an optical section. (*Cf.* METCALF 1907*a*, Fig 3.)

Figs. 165—167 Later stages of copulation in different individuals from a tadpole of *Bombinator pachypus*, infected 88 hours. Free hand drawings from life.

Fig. 168. A copulating pair from a tadpole of *Bombinator pachypus* infected 88 hours. The nucleus of the male seemed to contain two chromatin masses of unequal size, but the staining was not sufficiently clear to determine accurately the structure. Probably the larger mass was composed of three chromosomes lying close together. Acetic-carmine. × 673 diameters.

Fig. 169. An early stage of copulation, from a tadpole of *Bombinator pachypus*, infected 70 hours. Acetic-carmine. × 693 diameters.

Fig. 170. A copulating pair from a tadpole of *Bombinator pachypus*, infected 88 hours.

Fig. 171. A copulating pair from a tadpole of *Bufo vulgaris*, infected 45 hours. The microgamete was the smallest I have seen of this species. Its nucleus was not visible. Drawn from life.

Fig. 172. A copulating pair from a tadpole of *Bufo vulgaris*, infected 60 hours. Acetic-carmine. Magnification not recorded.

Fig. 173. A copulating pair from a tadpole of *Bombinator pachypus*, infected 7 days. The macrogamete is much larger than usual. Acetic acid. × 673 diameters.

Figs. 174—177. Successive stages of copulation, drawn from life: Fig. 174 at 12^{45} P.M., Fig. 175 at 1^{40} P.M., Fig. 176 at 1^{45} P.M., Fig. 177 at 1^{49} P.M. From a tadpole of *Bombinator pachypus*, infected 70 hours.

Fig. 178. A copulating pair from a tadpole of *Bombinator pachypus* infected 70 hours. Acetic-carmine. × 673 diameters.

Figs. 179 and 180. Two stages in the attempted copulation of a pair of gametes from a tadpole of *Bombinator pachypus*, infected 70 hours. The microgamete was unusually large. Later it separated from the macrogamete and formed a pseudocyst. Drawn from life.

Fig. 181. A copulating pair from a tadpole of *Bombinator pachypus*, infected 91 hours. The microgamete is attached by the whole anterior half of its body, the tail remaining free. This is the only instance in which such a condition was seen. The preparation was inadvertently stained before I learned if the conjugation would become complete. From life. × 673 diameters.

Fig. 182. A copulating pair from a tadpole of *Bombinator pachypus*, infected 7 days. There are four chromosomes in the nucleus of the male and four in one end of the dividing nucleus of the female. In the other end of the nucleus of the female only two chromosomes were visible. Acetic-carmine, magnification not recorded.

Plate XXIV.

Opalina intestinalis.

All figures are reduced one-third to the magnification indicated.

Figs. 183—185*a*. Copulating pairs in sections of the rectum of a tadpole of *Bombinator pachypus*, infected 60 hours. Figs. 185 and 185*a*, are from successive sections. The macrogamete shown in Fig. 183 was broken as indicated. Coros. subl.-acetic acid, Delafield's haematoxylin. × 990 diameters.

Fig. 186. A copulating pair from a tadpole of *Bombinator pachypus*, infected 88 hours. One of the two anterior nuclei may be from the male. If so it passed by the posterior nucleus to reach its present position. Acetic-carmine. × 673 diameters.

Fig. 187. A zygote, with the nuclei still unfused, from a tadpole of *Bombinator pachypus*, infected $7^1/_2$ days. Acetic acid. × 673 diameters.

Fig. 188. A zygote, with the nuclei ready to fuse, from a tadpole of *Bombinator pachypus*, infected 7 days. Acetic acid. × 673 diameters.

Fig. 189. A similar zygote from a tadpole of *Bombinator pachypus*, infected 91 hours. Acetic-carmine. × 990 diameters.

Fig. 190. A section of a zygote from a tadpole of *Bombinator pachypus* infected 7 days. The two nuclei are still unfused. Coros. subl.-acetic acid, Delafield's haematoxylin. × 673 diameters.

Fig. 191. A section of a zygote from a tadpole of *Bombinator pachypus*, infected 64 hours. The membrane between the two nuclei is broken down in the middle. Coros. subl.-acetic acid, DELAFIELD's haematoxylin. × 990 diameters.

Fig. 192. A zygote, with nuclei marly fused, from a tadpole of *Bombinator pachypus*, infected 7½ days. The nuclear structure was not clearly seen. Acetic acid. × 673 diameters.

Fig. 193. A zygote from a tadpole of *Bombinator pachypus*, infected 7½ days. Acetic acid. × 673 diameters.

Figs. 194—196. Zygotes from tadpoles of *Bombinator pachypus*, infected respectirely 4¾ days, 5 days, 4¾ days. Each nucleus (syncarion) shows eight chromosomes. In the nucleus shown in Fig. 196 were two structures, of different refractive quality from the chromosomes, which may have been nucleoli or vacuoles, probably the latter. Figs. 194 and 196 acetic acid; Fig. 195 acetic-carmine. × 990 diameters.

Figs. 197—200. Zygotes formed by the fertilization of binucleated macrogametes. In each case the nucleus from the male lies between the two macrogamete nuclei. In Figs. 198—200 it is evident that the nucleus from the male will fuse, or is fusing (Fig. 200), with the anterior of the macrogamete nuclei. The first is an acetic acid preparation, the others are acetic-carmine preparation, from tadpoles of *Bombinator pachypus*, infected respectirely 7 days, 136 hours, 88 hours, 88 hours. Figs. 197, 199, 200 × 673 diameters; magnification of Fig. 198 not recorded.

Figs. 201—203. Binucleated zygotes, the anterior nuclei all being syncaria as is shown by their size and the number of their chromosomes. From tadpoles of *Bombinator pachypus*, infected 7 days. Acetic acid. × 673 diameters.

Fig. 204. A zygote formed by the union of a male with a binucleated female whose nuclei were in a telophase of mitosis. The nucleus from the male is also in mitosis. The radiations at its two ends are merely films of the cytoplasmic foam (compare the next figure). From an acetic-carmine preparation; this animal, however, lay in a part of the slide where only the acetic acid, and not the carmine, had taken effect. The preparation was very clear. From a tadpole of *Bombinator pachypus*, infected 106 hours. × 673 diameters.

Fig. 205. An optical section through the same animal, showing on a larger scale the nucleus from the male and the cytoplasm surrounding it. The nucleus lies in a vacuole of the cytoplasm, which it completely fills, its membrane being covered by a film of cytoplasm from which radiate other films. Where the radiating films join the film covering the nuclear membrane, granules are seen similar to those in the rest of the cytoplasm. The drawing — an ink copy of the original pencil drawing — is inaccurate, for these granules upon the nuclear contour should be shown as lying wholly outside (though abutting upon) the membrane. × 990 diameters.

Fig. 206. A daughter cell from the division of a zygote similar to that shown in Fig. 204; from a tadpole of *Bombinator pachypus*, infected 7 days. Acetic acid. × 673 diameters.

Fig. 207. An nuclear acetic-carmine preparation from a tadpole of *Bombinator pachypus*, infected 91 hours. × 673 diameters.

Fig. 208. An acetic-acid preparation from a tadpole of *Bombinator pachypus*, infected 7 days. A spindle-shaped microgamete nucleus lay near what seemed to be four daughter nuclei. × 673 diameters.

Fig. 209. A living animal from a tadpole of *Bombinator pachypus*, infected 88 hours. The nuclei were not perfectly clear. In the original free-hand sketch they are annotated with a question mark.

Plate XXV.

Opalina intestinalis.

All figures are reduced one-third, to the magnification indicated.

Figs. 210—218. Successive stages of copulation and pseudoencystment of a pair of gametes from a tadpole of *Bufo vulgaris*. Fig. 210 is a free-hand sketch from memory made immediately after the male become attached; the other figures × 673 diameters. From life.

Figs. 219—221. Pseudocysts from tadpoles of *Bombinator pachypus* (Figs. 219 and 221) and *Bufo vulgaris* (Fig. 220). In Fig. 219 four granular chromosomes are seen in each end of the dividing nucleus. The granules apparently were not arranged in pairs, their number in two of the chromosomes being uneven. In Fig. 220 each macrogamete nucleus had four chromosomes the uppermost two of which in each case showed distinct granules, so arranged however as not to be readily counted; the granules of the lower two chromosomes were less distinctly seen. The nucleus from the microgamete showed a single compact chromatin mass which contained a group of granules at one side. The excretory vacuole with faintly visible granules (in Brownian movement) is shown near the top of the figure. Fig. 219 from life, × 990 diameters; Fig. 220 from life, × 673 diameters; Fig. 221 acetic-carmine, × 673 diameters.

Figs. 222—225. Zygotes with peculiar spindle-shaped syncaria, from tadpoles of *Bombinator pachypus*, infected respectively 7½ days, 88 hours, 7 days, 113 hours. In Fig. 224 a vacuole of the excretory organ is shown in dotted outline. In Fig. 225 the large disc in the nucleus was probably not a nucleolus but a mass of chromatin. Figs. 222 and 224 acetic acid; Fig. 223 acetic-carmine; Fig. 225 from life. × 673 diameters.

Figs. 226 and 227. Zygotes whose daughter nuclei somewhat resemble the nuclei shown in Figs. 222 and 224; from a tadpole of *Bombinator pachypus*, infected 7½ days. Acetic acid. × 673 diameters.

Figs. 228—235. Abnormal individuals from tadpoles of *Bombinator pachypus*, infected 43 hours (Fig. 228), 54 hours (Figs. 229—233), 91 hours (Fig. 234), and 114 hours (Fig. 235). These tadpoles were infected from two different lots of cysts, Figs. 229—233 being from one series of infections, Figs. 228, 234 and 235 from another. From life. Figs. 234 and 235 free-hand sketches; the rest camera drawings × 673 diameters.

Fig. 236. A section of an infection cyst from a tadpole of *Bombinator pachypus*. Coros. subl.-acetic acid, Delafield's haematoxylin. No record was made of the infection or of the magnification of the drawing.

Plate XXVI.

Opalina intestinalis and *O. caudata.*

All figures are reduced one-third, to the magnification indicated.

Figs. 237—247. *Opalina intestinalis.*

Figs. 237—239. Three sections of a small individual which passed unencysted through the alimentary canal into the rectum of a tadpole of *Bombinator pachypus*.

Large reticulate masses of chromatin and some smaller fragments are seen extruded from the nucleus and lying in the cytoplasm. Apparently the full number of chromosomes (8) were in these nuclei. Vacuoles around some of these masses are indicated by dotted contours. From a 6 day infection. Coros. subl.-acetic acid, Delafield's haematoxylin. Restaining with iron haematoxylin gave the same results. × 673 diameters.

Fig. 240. The anterior end of another individual from the same preparations, showing a reticulate mass of chromatin slightly displaced, probably by the microtome knife, from its position in the nucleus. × 673 diameters.

Fig. 241. A combination of three drawings from sections of an individual which had passed unencysted through the alimentary canal and had been six days in the rectum of a tadpole of *Bombinator pachypus*. Vacuoles, probably of the excretory system, are shown in dotted outline. Six masses of chromatin are seen lying in the cytoplasm. One of these is in the form of a reticulate layer partly surrounding a central refractive sphere (*cf.* the figures on Plate XXI). The nuclei contained the reduced number of chromosomes (4). Coros. subl.-acetic acid, Delafield's haematoxylin. × 673 diameters.

Figs. 242—244. Successive sections from a small individual from a tadpole of *Bombinator pachypus*, infected 6 days. Extruded masses of chromatin are seen in the cytoplasm. The animal may or may not have come from a cyst so far as its size would indicate. It seems probable, however, that animals with this type of chromidia passed unencysted through the alimentary canal. Four chromosomes are in each nucleus. Coros. subl.-acetic acid, Delafield's haematoxylin. × 673 diameters.

Figs. 245—247. The three central sections from a series of five through an individual from a tadpole of *Bombinator pachypus*, infected 6 days. Extruded masses of chromatin are seen in the cytoplasm near each of the two nuclei. The other two sections showed no such chromidia. Coros. subl.-acetic acid, Delafield's haematoxylin. × 673 diameters.

Figs. 248—262. *Opalina caudata*.

Figs. 248—250. Individuals which passed unencysted through the alimentary canal into the rectum of a tadpole of *Bombinator pachypus*; 118 hours infection. Excretory vacuoles are seen in each. In Fig. 248 the boundary between ectosarc and endosarc is indicated by a dotted line. In this figure there are shown twelve chromatin masses (chromosomes) in each nucleus which must be ready to enter upon mitosis. The other animals show the reduced number of chromosomes (3). Acetic-carmine. × 673 diameters

Fig. 251. A section through a small individual from the rectum of an adult *Bombinator pachypus* which contained many infection cysts. In each nucleus are two chromatin spheres whose presence at this early stage is unusual. Coros. subl.-acetic acid, Delafield's haematoxylin. × 673 diameters.

Figs. 252—256. Infection cysts from aquaria in which *Bombinator pachypus* was kept (Fig. 254 shows a cyst from the rectum of one of these adult Bombinator). Fig. 252 shows one chromatin sphere in the nucleus; Fig. 253 shows two chromatin spheres extruded into the cytoplasm and none in the nucleus; Fig. 254 shows a binucleated cyst with one chromatin sphere in one nucleus and none in the other; Fig. 255 shows a binucleated animal which encysted while in division; in Fig. 256 two animals are shown inside one cyst (one cannot be certain that they are entirely

separate; they may be connected by such delicate strands as are found uniting the daughter cells in late stages of division). Figs. 252—254 acetic-carmine; Figs. 255 and 256 from life. × 440 diameters.

Figs. 257 and 258. Macrogametes (Fig. 257 possibly a macrogamete parent-cell) from a tadpole of *Bombinator pachypus* (?), infected 22 hours. The first figure shows the usual size, the second the smallest size found. The ectosarc spherules are indicated. Acetic-carmine. × 673 diameters.

Fig. 259. A microgamete from the same tadpole of *Bombinator pachypus* (?), infected 22 hours. Ectosarc spherules (unshaded) and endosarc spherules (shaded) are shown. In no other series of infections have I found apparently mature gametes before 42 hours after the beginning of infection. Acetic-carmine. × 990 diameters.

Fig. 260. A macrogamete (or macrogamete parent-cell?) from the same tadpole of *Bombinator pachypus* (?), infected 22 hours. The ectosarc spherules are shown. Acetic-carmine. × 673 diameters.

Fig. 261. A living but almost quiescent microgamete. × 673 diameters. Infection data were not noted.

Fig. 262. A free-hand drawing of a living copulating pair from a tadpole of *Bufo vulgaris*, infected 66 hours.

Plate XXVII

Opalina caudata and *Opalina dimidiata.*

All figures are reduced one-third, to the magnification indicated.

Figs. 263—276. *Opalina caudata.*

Fig. 263. A copulating pair from a tadpole of *Bufo vulgaris* infected 66 hours. The macrogamete is in division. Free-hand drawing.

Figs. 264—271. Examples of copulation in living animals from tadpoles of *Bombinator pachypus*, infected 60 hours. Fig. 265 shows a late stage of copulation; Figs. 269 and 270 show copulation while the macrogamete is in division; Fig. 271 shows two males attached to one female. Fig. 264 × 438 diameters; the other figures are free-hand drawings.

Figs. 272—274. Copulating pairs from a tadpole of *Bombinator pachypus* infected 60 hours. The endosarc spherules are drawn in Figs. 272 and 273. Each nucleus in Figs. 273 and 274 shows three chromosomes. The nature of the body just outside the microgamete nucleus in Fig 272 is uncertain. Acetic-carmine. × 673 diameters.

Fig. 275. A pseudocyst (macrogamete?) from a tadpole of *Rana esculenta*, infected with both *O. caudata* and *O. intestinalis* by being placed for an hour in a jar with adult *Bombinator pachypus*. Cast off cilia and extruded globules lie around the pseudocyst. Each nucleus has three chromosomes. × 673 diameters.

Fig. 276. Abnormal copulation of two individuals of nearly similar size, the smaller of which showed a nucleus in division. Acetic-carmine. Infection data and the magnification were not noted.

Figs. 277—298. *Opalina dimidiata.*

Figs. 277—281. Minute individuals from a culture in 0,6 % NaCl solution, kept three days after removal of the animals from the rectum of an adult *Rana esculenta*. Division continued during this time and many of the animals became minute, as shown, and were apparently ready for encystment. Fig. 277 shows

some of the endosarc spherules present; in the cytoplasm only the larger granules are carefully drawn; the living animal was treated directly with EHRLICH's acid haematoxylin. Figs. 278—280 are acetic-carmine preparations; Fig. 279 shows apparently a chromatin sphere being extruted from the anterior nucleus. Fig. 281, EHRLICH's acid haematoxylin. All figures × 673 diameters.

Figs. 282—284. Spheroidal individuals, not true cysts, from a tadpole of *Rana esculenta*, infected 24 hours. From these apparently pseudocysts some of the endosarc spherules are being extruded. My notes on these drawings, as also my memory of them, are insufficient to explain the conditions shown. Acetic-carmine. × 673 diameters.

Figs. 285—288. Infection cysts from tadpoles of *Bufo vulgaris*, naturally infected. The size of the cysts, their multinucleated condition, and the presence of free-swimming minute *Opalinae dimidiatae* in the same recta, show these to be cysts of *O. dimidiata*. Fig. 285, from a living cyst; the other figures from acetic-carmine preparations. × 673 diameters.

Figs. 289–291. Peculiarly shrunken individuals which passed unencysted through the alimentary canal and were lying in the rectum of a tadpole of *Rana esculenta*, infected 24 hours. Endosarc spherules (shaded) and ectosarc spherules (unshaded) are shown. Acetic-carmine. × 673 diameters.

Fig. 292. An individual from a large tadpole of *Rana esculenta*, infected 24 hours. Acetic-carmine. × 673 diameters.

Fig. 293. A living gamete parent-cell from a tadpole of *Bufo vulgaris*, infected 6 hours. The endosarc spherules are shown. × 673 diameters.

Fig. 294. A living gamete parent-cell in division; from a tadpole of *Bufo vulgaris*, infected 36 hours. The endosarc spherules are shown. The granules shown as black were highly refractive. The condition of the nuclei is of interest. × 673 diameters.

Figs. 295—298. Macrogametes from a tadpole of *Rana esculenta*. × 673 diameters.

Plate XXVIII.

Opalina dimidiata and *Opalina ranarum*.

All figures are reduced one-third, to the magnification indicated.

Figs. 299—324. *Opalina dimidiata*.

Figs. 299—302. Gamete parent-cells from tadpoles of *Rana esculenta*, infected respectively 32 hours, time?, 97½ hours, 32 hours. Fig. 302 shows the endosarc spherules and also the extrusion of chromatin spheres from each nucleus. Acetic-carmine. × 673 diameters.

Fig. 303. An individual from a tadpole (of a species not noted) infected 8 days. The structures in the elongated nuclei were not clearly seen. It is doubtful whether the animal was a gamete parent-cell with both nuclei in mitosis, or a zygote with its syncarion already divided into two. Acetic-carmine. × 673 diameters.

Fig. 304. An individual from a tadpole of *Rana esculenta*, infected 80½ hours. Its nucleus was in mitosis. The nature of the bodies at the two ends of the spindle was not clear; they were probably chromatin spheres similar to those shown extruded from the larger nucleus in Fig. 309. The animal was probably a microgamete mother cell. Acetic-carmine. × 673 diameters.

Figs 305 and 306. Macrogametes from a tadpole of *Rana esculenta*, infected 97½ hours. In the second figure endosarc spherules are shown, and also a chromatin sphere extruded from the nucleus into the cytoplasm. Acetic-carmine. × 673 diameters.

Figs. 307 309. Divisions in the formation of the microgametes; from a tadpole of *Rana esculenta*, infected 80½ hours. The number of chromosomes seemed in some nuclei five, in others six. Probably six is the correct reduced number of chromosomes for *O. dimidiata*. Some of the nuclei were not sufficiently clear to draw and are left empty. In Fig. 309 the division may be abnormal, but in this species the nuclei are so independent of one another that the lack of synchronism in their division in this case may not indicate abnormal condition. The endosarc spherules are shown in all figures From life. × 673 diameters.

Fig. 310. A living microgamete, with unusually long tail, from a tadpole of *Rana esculenta*, infected 8 days. There was no swelling at the tip of the tail such as is usually seen in microgametes in this and other species. × 673 diameters.

Fig. 311. An individual which seems to be a microgamete mother-cell from a tadpole of *Bufo vulgaris*, naturally infected for at least three weeks. The tail is some what contracted by acetic carmine. Copulation was found among the Opalinas in this tadpole, this being by far the oldest infection in which copulation or microgametes were seen. Acetic-carmine. × 673 diameters.

Fig 312. A living copulating pair from a tadpole of *Rana esculenta*, infected 98 hours. × 673 diameters.

Fig. 313. A pair of living individuals from a tadpole of *Rana esculenta*, infected 80½ hours, showing a dividing microgamete mother-cell attached to another cell of about the same size. The manner of the attachment and the condition of the nuclei indicate that this was either a chance connection or an abnormal attempted copulation. There was no change after three-quarters of an hour. Free-hand drawing.

Figs. 314—318. Zygotes from tadpoles of unnoted species (probably *Rana esculenta*, otherwise the fact would have been noted), infected 7 days, except the host for the animal shown in Fig. 117, which was infected 97½ hours. The nuclei were not sufficiently well stained to allow accurate drawing of the chromosomes or chromatin masses, which were so numerous as to obscure one another. The posterior nuclei shown in Figs. 315 and 316 are the syncaria. Possibly all the nuclei shown in Fig. 318 have come from the syncarion, the posterior daughter having completed a second division with which the anterior nucleus is still engaged; or the macrogamete may have contained originally three nuclei. The smaller size of the posterior nuclei makes the first interpretation much the more probable. The details of nuclear structure are not adequately shown. The chromatin masses were so numerous as to obscure one another. Acetic-carmine. × 673 diameters.

Fig. 319. An individual from the same preparation as Fig. 318. The anterior nucleus from its shape would seem not to be a syncarion. It may be abnormal. The chromatin is not accurately drawn. Acetic-carmine. × 673 diameters.

Fig. 320. Another individual from the same preparation, in which the anterior nucleus is the syncarion with the spindle form which persists until after at least one division. The chromatin could not be accurately drawn. Acetic-carmine. × 673 diameters

Figs. 321—324. Young individuals from tadpoles of *Bufo vulgaris*, naturally infected for an unknown period. Still larger individuals, with twice as many

nuclei irregularly arranged instead of in an axial row, were present in the same tadpoles. Acetic-carmine. × 673 diameters.

Figs. 325—327. *Opalina ranarum.*

Fig. 325. A minute individual ready for encystment, from the rectum of an adult *Rana temporaria.* The endosarc spherules (unshaded) are shown; cilia are not drawn because injured. The method of preparation was not noted, but the injury to the cilia makes it probable that acetic-carmine was used. × 673 diameters.

Fig. 326. A cyst from the same host. Cilia were present within the cyst but were too confused to draw. The method of preparation was not noted. × 673 diameters.

Fig. 327. A section of a zygote in the rectum of a tadpole of *Rana temporaria.* The nucleus is carefully drawn, as are also the endosarc spherules. Coros. subl.-acetic acid, iron haematoxylin. × 990 diameters.

Referate.

Breinl, A. and Hindle, E. — Contribution to the Morphology and Life History of *Piroplasma canis*. Annals of Tropical Medicine and Parasitology Vol. II No. 3 1908.

B. und H. wenden das Färbungsverfahren von BREINL und die von ersterem angegebene Modifizierung der Heidenhain'schen Eisenhämatoxylinfärbung bei *Piroplasma canis* an. Sie beginnen mit der Beschreibung der ersten auftretenden Formen und verfolgen die Entwicklung bis kurz vor dem Tode des infizierten Tieres.

(Die vorliegende Arbeit gibt den vollkommenen Beweis der Richtigkeit der SCHAUDINN'schen Auffassung dieser Protozoen als doppelkernige Zellen. Die Entstehung des zweiten Kernes aus dem größeren Kern (Hauptkern), die Teilung desselben vor der des größeren und die manchmal beobachtete Geißel sprechen für die Flagellatennatur dieser Protozoen und die Einreihung in die Ordnung der Binucleaten [HARTMANN]).

B. und H. beschreiben den größeren Kern als eine kleine kompakte, dunkel gefärbte Chromatinmasse, welche von einer Vacuole umgeben ist, die leicht gefärbte Substanz enthält. (Es handelt sich wahrscheinlich nicht um eine Vacuole, wie die Verf. sich ausdrücken, sondern vielmehr ist es der äußere Teil des Kernes selbst (Kernsaftzone), während der kleine kompakte Körper dem Caryosom der anderen Protozoen entspricht.) Die Teilungsart soll eine Amitose sein, der kleinere Kern (Blepharoplast) teilt sich zuerst.

Eine Anzahl Abbildungen erläutern die Erklärungen der Autoren.

ROSENBUSCH.

Salvin Moore, J. E. Breinl, A. and Hindle, E. (1908). — The Life History of *Trypanosoma lewisi*. Annals of Tropical Medicine and Parasitology Vol. II No. 3.

Moore, Breinl und Hindle beschreiben die Bildung der multinuclearen Formen, die cytologische Struktur der verschiedenen Übergangsformen und die angebliche Parthenogenese, die in ähnlicher Weise stattfinden soll wie der von M. und B. schon in einer anderen Arbeit beschriebene Vorgang bei *Trypanosoma gambiense, equiperdum* und *equinum* (s. Ref. in Bd. XII Heft 1 u. 2).

Die mittelgroßen Trypanosomen wachsen zu großen Formen an, dabei geschehen zwei Metamorphosen, eine, indem das Intranuclear centrosom (Caryosom) sowie der äußere Teil des Kernes einen Teil abspaltet, der sich nach dem Vorderende der Zelle begibt, dort degeneriert und verschwindet. Sodann werden Massen aus dem Extranuclear centrosom (Blepharoplast) abgespalten, die sich unter der Vacuole, die bei dem Extra nuclear centrosom liegt, zu einem zweiten Kern sammeln, der nach dem Nucleus hin rückt und längere Zeit in seiner Nähe bleibt. S. M., B. u. H. nehmen an, daß nähere Beziehungen zwischen diesen Extranuclear centrosom adventive und dem Nucleus zustande kommen, die sie als Autogamie deuten. (Abgesehen davon, daß derartige Beziehungen nicht erwiesen sind, können sie auf keinen Fall als Autogamie gedeutet werden. Vgl. Ref. in Bd. XII Heft 1 u. 2.) Nach dem angenommenen Befruchtungsvorgang kommt es zur Teilung der Kerne, die direkt oder nach vorangegangener Zweiteilung zur Bildung von multinucleären Formen führt. Die Autoren meinen, daß diese Zweiteilungsbilder Anlaß zu falscher Auslegung geben konnten, und vergleichen sie mit den von Prowazek als Copulatien abgebildeten Formen. Doch Ref. glaubt, daß zwischen den Bildern von M., B., H. und denen von Prowazek keine derartige Ähnlichkeit besteht, die eine solche Verwechselung möglich machen könnte; denn das wesentliche bei der von Prowazek angenommenen Copulation sind die Kernverhältnisse, und wenn auch die Untersuchung nicht mit einer guten Färbung gemacht wurde, so läßt sich doch erkennen, daß die Kernstruktur der miteinander verbundenen Trypanosomen sehr verschieden ist. Die multinucleären länglichen Formen spalten sich und bilden Rosetten von kleinen Trypanosomen — dieses Stadium vergleichen M., B. u. H. mit den von ihnen beschriebenen latenten Körpern der anderen Trypanosomen.

Sie wachsen heran und nehmen nach Bildung der mittelgroßen Formen die gleiche Entwicklung, wie es schon referiert wurde.

Die Teilung des Hauptkernes wird, wie in einer anderen Arbeit von M. u. B. näher beschrieben, als Amitose betrachtet.

Bei der Teilung des Extranuclear centrosom (Blepharoplast) beschreiben die Autoren die Anordnung von Stäbchen zu einer Scheibe, die sich weniger intensiv färbt als das ruhende Extranuclear centrosom; danach sammelt sich das färbbare Material an entgegengesetzten Seiten der Scheibe und bildet dann von neuem die zwei täbchenförmigen Extra nuclear centrosoms. (Nach unseren Untersuchungen ist die Teilung des Blepharoplasten eine vollkommene Mitose mit Spindel und Chromosomen.)

Die Geißel hat an ihrer Wurzel ein Knötchen, das aus dem Blepharoplast entsteht (Basalkorn).

Der Arbeit sind eine Anzahl schöner Abbildungen beigegeben.

F. Rosenbusch.

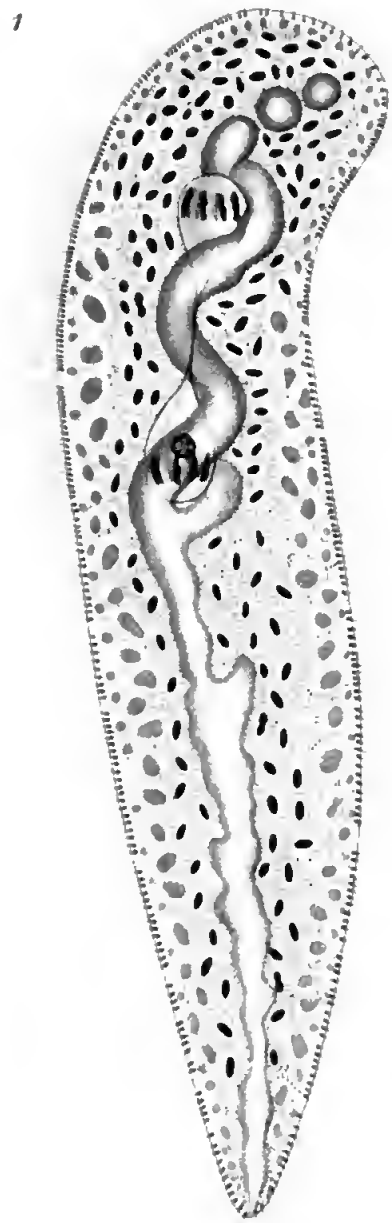
Berichtigung

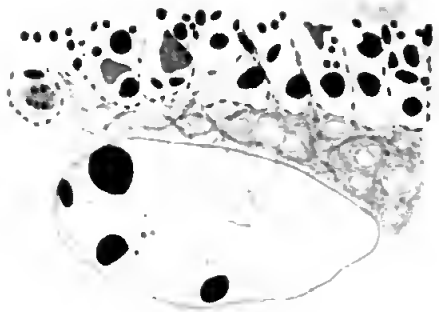
zum Referat über die Arbeit von **S. Moore** u. **A. Breinl** in Bd. XII Heft 1 u. 2 dieser Zeitschrift.

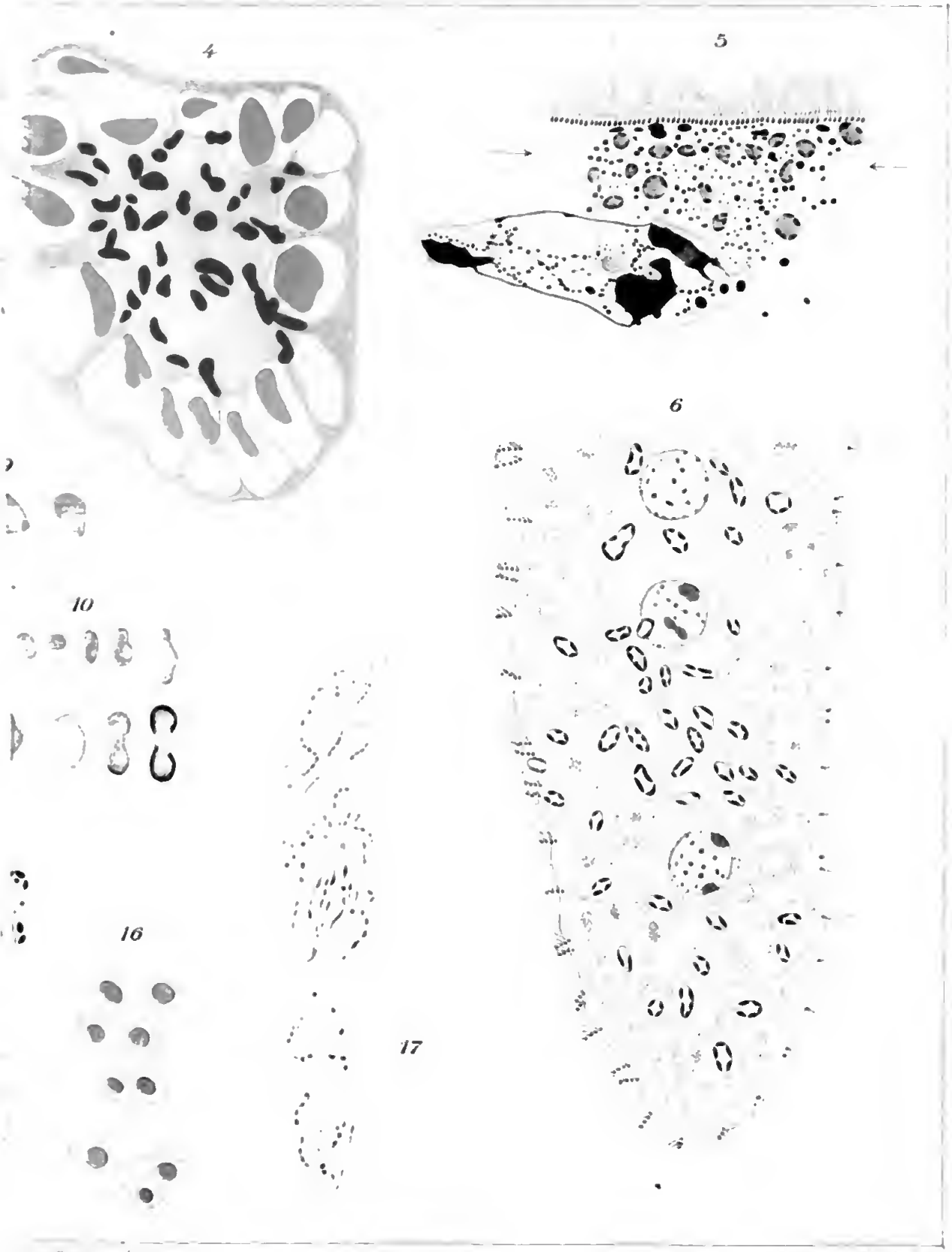
Die angegebene Modifizierung der Heidenhain'schen Eisenhämatoxylinmethode ist von mir irrtümlicherweise Moore zugeschrieben. Das Verdienst, diese Färbung für Trypanosomen ausgearbeitet zu haben, gebührt Breinl.

Rosenbusch.

Lippert & Co. (G. Pätz'sche Buchdr.), Naumburg a/S.







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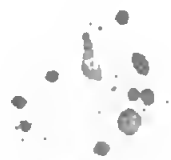

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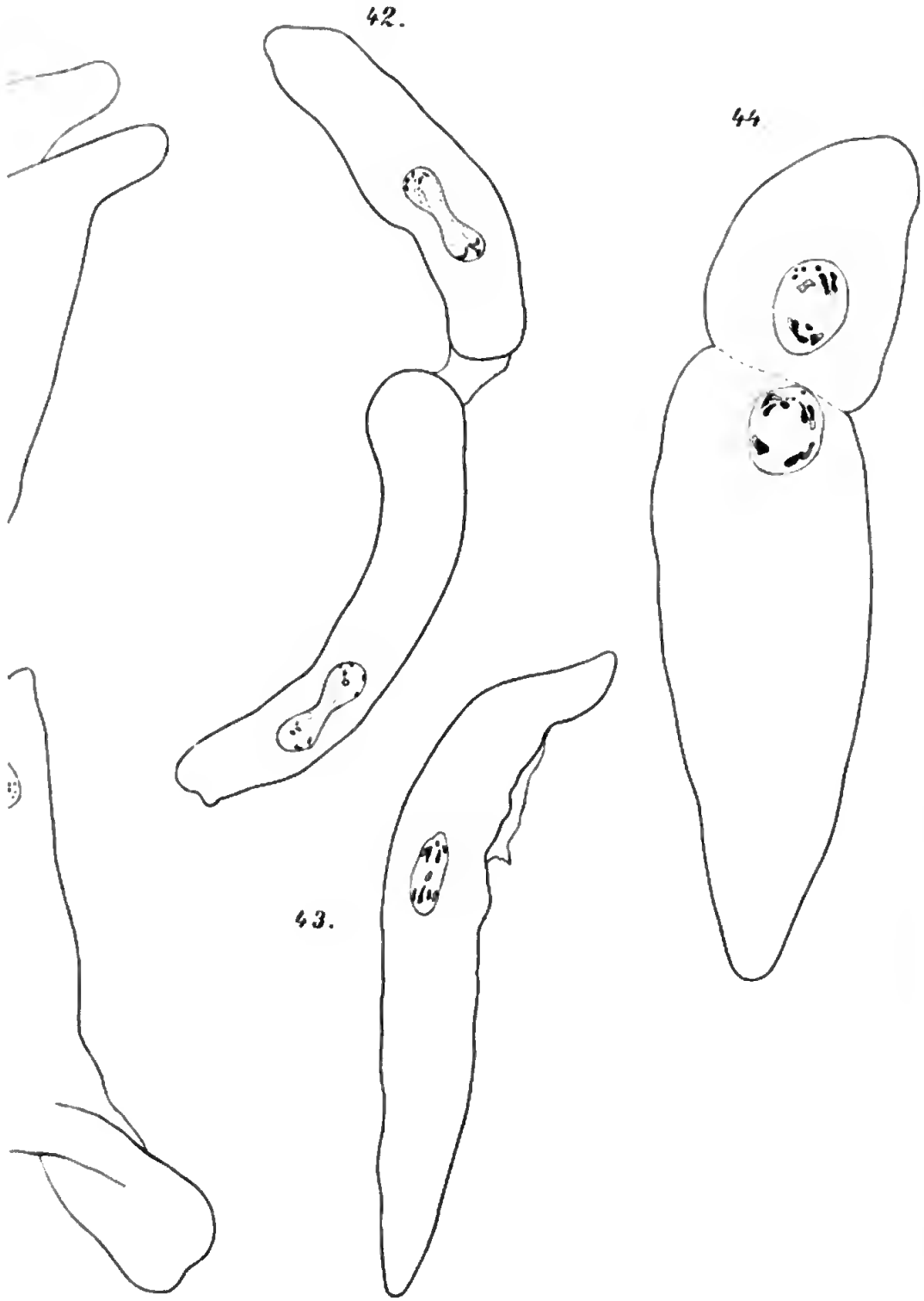

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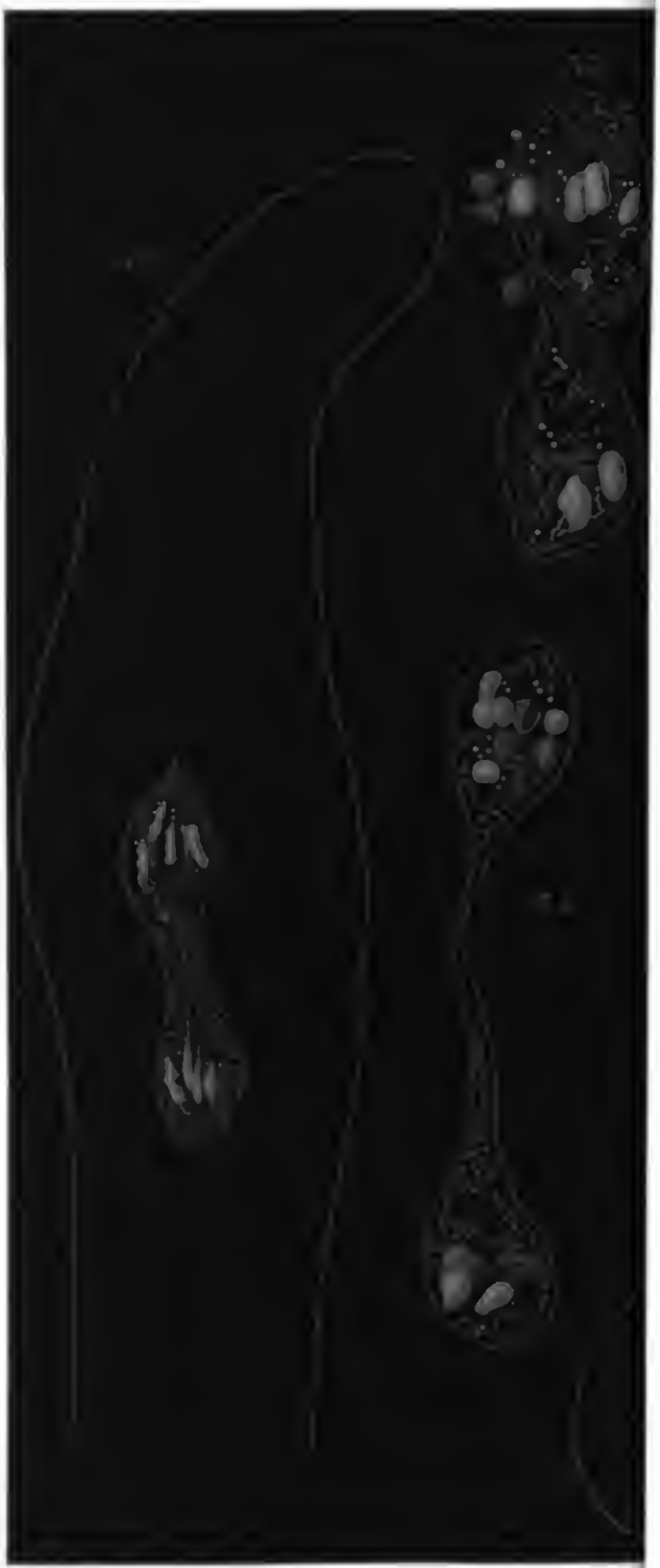

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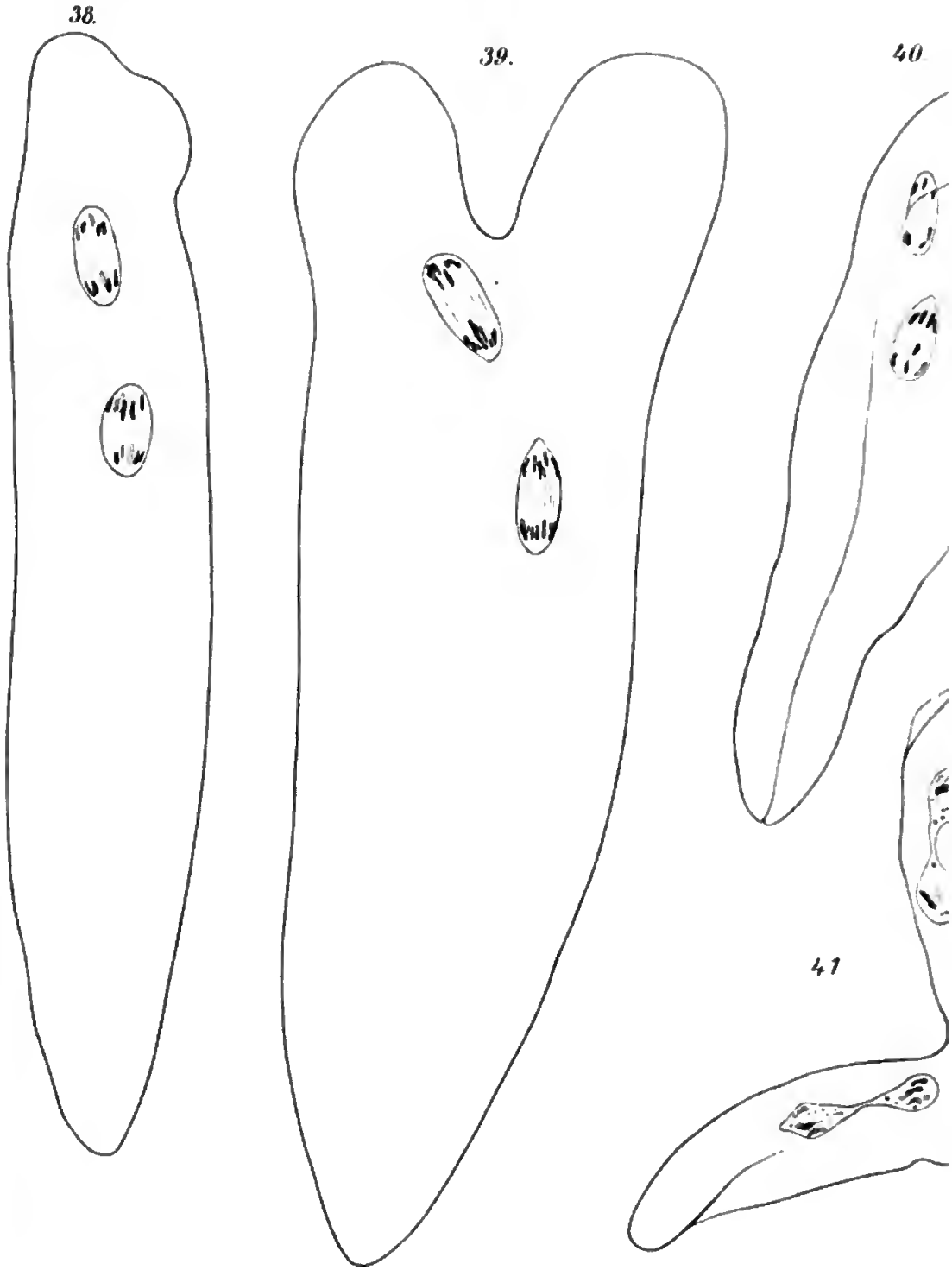

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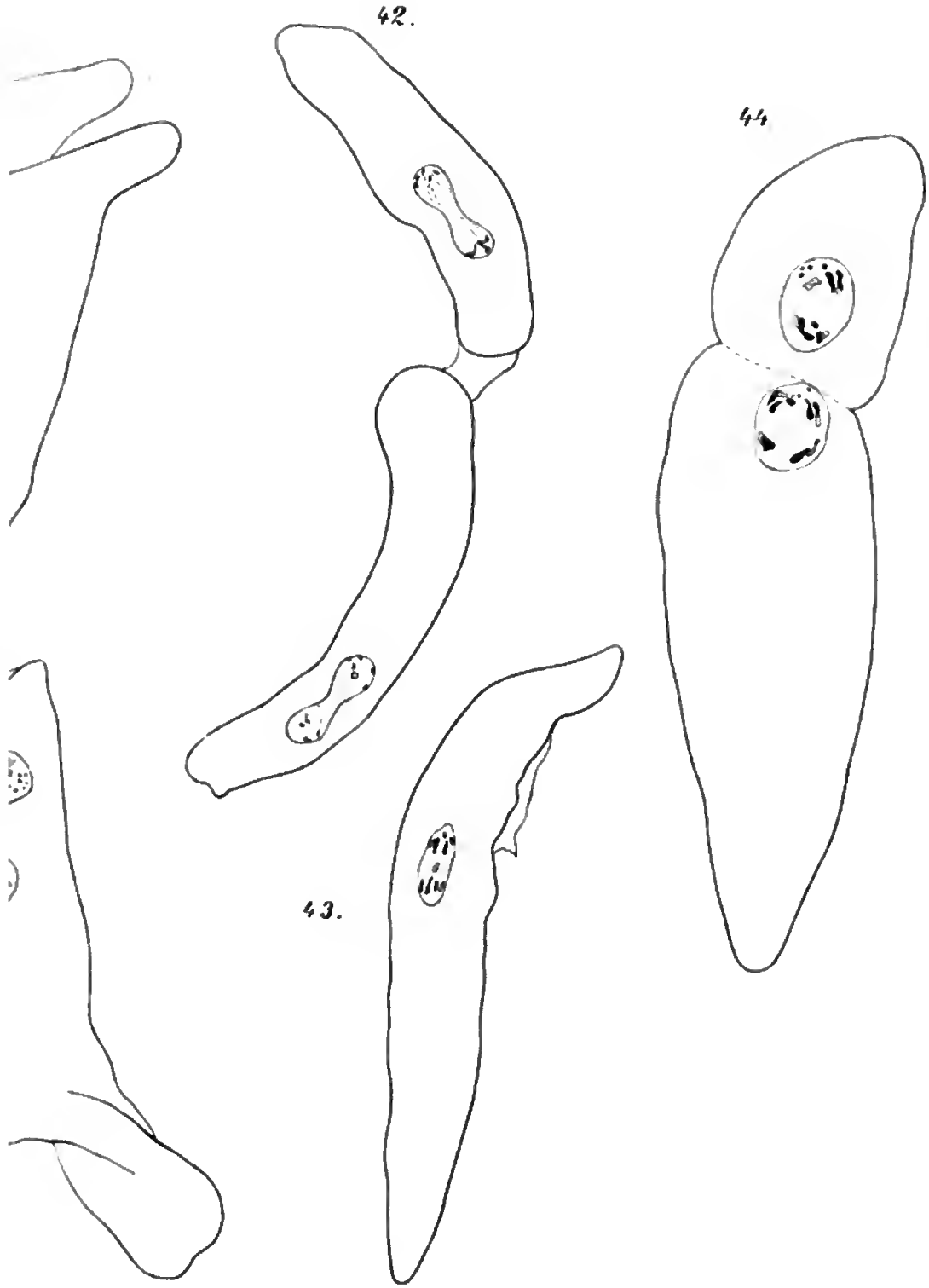

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