

## Some Observations on the Infusoria Parasitic in Cephalopoda.

By

**C. Clifford Dobell,**

Fellow of Trinity College, Cambridge; Balfour Student in the University.

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With Plate 1.

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### INTRODUCTION.

The infusorian parasites of cuttlefish are already well known from the excellent descriptions of their discoverer, Føettinger (5, 6), and the admirable figures of Gonder (7). From the work of the latter it would appear that the last word had been said about their morphology. But, as they are of particular interest on account of the peculiarities of their nuclear apparatus, I took the opportunity afforded by a recent stay in Naples (March to June, 1908) of re-examining these organisms. The results were somewhat unexpected, and are embodied in the following pages.

### OCCURRENCE OF THE PARASITES.

As is well known, three different Infusoria occur in cephalopods—*Opalinopsis sepiolæ*, *Chromidina* (*Benedenia*) *elegans*, and *C. (B.) coronata*.<sup>1</sup> The first—*O. sepiolæ*—has been recorded from the liver of *Sepiola rondeletii* (Føettinger, Gonder) and from the liver of *Octopus tetracirrhus* (Føettinger). Although I have examined fifty-five individuals of *Sepiola rondeletii*, I have never once met with the parasite. But I have encountered it in a hitherto unrecorded host,—*Sepia officinalis*,—and not only in the liver, but also in the kidneys.

<sup>1</sup> Following Gonder's nomenclature. *O. octopi*, Føett., is, as Gonder says, almost certainly identical with *O. sepiolæ*. Bütschli (2) united *Opalinopsis*, Føett., and *Benedenia*, Føett. (= *Chromidina*, Gonder) into one genus—*Opalinopsis*.

*C. elegans* was found by Fœttinger and Gonder in the kidneys of *Sepia elegans*, and by Gonder in the kidneys of *Illex coindetii* also. I have met with it in both these hosts—though rarely in *S. elegans*. I have also found it in *Sepia orbignyana*.

*C. coronata* was found by Fœttinger in the kidneys of *Octopus vulgaris*, and by Gonder in *Eledone aldrovandi*. I have found it only in *Illex coindetii*. I examined seven other species of cuttlefish in addition to those already mentioned, but with negative results. The results of the examination of all the cuttlefish is shown in the accompanying table.

TABLE.

CEPHALOPOD.	Number of individuals examined.	Number infected with		
		<i>C. elegans</i> .	<i>C. coronata</i> .	<i>O. sepiolæ</i> .
1. <i>Sepia officinalis</i> , L. .	73	0	0	1
2. <i>Sepia orbignyana</i> , Fér.	8	1	0	0
3. <i>Sepia elegans</i> , d'Orb.	82	4	0	0
4. <i>Sepiola rondeletii</i> (Gesn.), Leach.	55	0	0	0
5. <i>Illex coindetii</i> , Ver. .	9	5	2	0
6. <i>Octopus di filippi</i> , Ver.	7	0	0	0
7. <i>Octopus vulgaris</i> , Lam.	8	0	0	0
8. <i>Octopus macropus</i> , Risso	2	0	0	0
9. <i>Loligo vulgaris</i> , Lam.	13	0	0	0
10. <i>Loligo marmoræ</i> , Ver.	16	0	0	0
11. <i>Eledone moschata</i> , Lam.	24	0	0	0
12. <i>Eledone aldrovandi</i> , Raf.	2	0	0	0
13. <i>Ocythoe tuberculata</i> , Fér.	3	0	0	0
14. <i>Rossia macrosoma</i> (D. Ch.), d'Orb.	7	0	0	0

The great scarcity of the parasites is remarkable. Out of 309 cephalopods examined only eleven were infected—i. e. about 3·5 per cent.

It is possible that the organisms occurring in different hosts are different species, but it seems to me unlikely. Assuming that there are but three species of Infusoria, their occurrence may be briefly summed up as follows :

Parasites.	Hosts.
1. <i>Opalinopsis sepiolæ</i> .	<i>Sepiola rondeletii</i> (liver). <i>Octopus tetracirrhus</i> (liver). <i>Sepia officinalis</i> (liver and kidneys).
2. <i>Chromidina elegans</i> .	<i>Sepia elegans</i> (kidneys). <i>Illex coindetii</i> (kidneys). <i>Sepia orbignyana</i> (kidneys).
3. <i>Chromidina coronata</i> .	<i>Octopus vulgaris</i> (kidneys). <i>Eledone aldrovandi</i> (kidneys). <i>Illex coindetii</i> (kidneys).

This table combines all the results of the work of Fœttinger, Gonder, and myself as regards hosts. It may be noted that all the work on these organisms has been done upon material obtained from the Gulf of Naples.

*Chromidina elegans*, Fœtt. emend. Gonder.

The general morphology of this infusorian has been accurately described by Fœttinger and Gonder. I will here record only those points in which my observations are in disagreement with those of these two investigators.

A point which does not seem to have been noticed previously is that the body is not uniform in shape throughout its whole length. Immediately behind the head there is a

very well-marked flattening, so that in this region a transverse section would be elliptical—not circular. This feature is so distinctly seen in the living animal, and so characteristic, that it is really surprising that it should have escaped notice. (Cf. fig. 3.) The animal swims with great rapidity, and invariably with the head in advance.

Gonder was the first to find that a cytostome is sometimes present in Chromidina. "For the most part one finds these Infusoria . . . without any trace at all of a cytostome. Only by more exact observation does one notice, in a small number, a cleft at the extremity, or at another spot on the anterior end." He "also found Chromidinæ with a completely developed cytostome—though these were, of course, less common." (7, pp. 246—7.) Now I believe that this statement results from the circumstance that Gonder was dealing largely with young forms of Chromidina. The "rudimentary cytostomes" are truly rudimentary in the individual, though not in the species. For I have found that every large animal possesses a cytostome—and a well developed one. As is well known, a Chromidina reproduces by constricting off small portions of itself at the posterior end, which then become free, and develop into new individuals. Usually these portions are described as "buds"; but they are more correctly termed segments—being formed, not by budding, but by a process of segmentation, like the proglottids of a tape worm. It follows that a young Chromidina, just freed from its parent, begins life without a mouth. Hence we find all stages in the development of this organella if we examine individuals at different periods of growth. (Cf. figs. 4, 5, 6.) The constant presence of a mouth in the organism is of importance for understanding the nuclear apparatus.

The vacuoles of Chromidina are non-contractile.

Nuclear Apparatus.—This is the most important feature, regarding which I differ from the other observers. I will first briefly summarise what has already been said about it.

According to Föttinger, there exists "at times but a single nucleus . . . When there are several nuclear bodies these are merely fragments of the single nucleus. The latter, being capable of amœboid movements, may assume the most varied forms—push out extensions, become segmented, etc." To this account Gonder added that the nuclear substance undergoes a series of vegetative changes, so far as he was able to follow them, like those of *Opalinopsis* (see *infra*). He believes, further, "that those stages which we find in the posterior part of the cell or in the buds are the younger, those which take place in the anterior part of the cell the older." A cycle of nuclear changes is thus to be observed in one and the same animal at one and the same time. At first there is an arrangement of the chromatin in irregular fragments of different sizes. These then become converted into a network, which may then undergo a resolution into a system of strands, and finally give rise to a condition in which we see, once more, a number of irregular fragments. The net may also, it would seem, give rise to coarsely granular chromatin masses. Still another stage is described, but its relation to the others is not quite clear. It is a stage in which the chromatin and plastin re-arrange themselves in the form of a number of perfect nuclei—each with its membrane, network, etc. The nuclei appear to be of very variable size.

Now I am convinced that no series of vegetative changes in the nucleus such as Gonder describes really occurs. The appearances described—and very beautifully figured by Gonder—have, I believe, been wrongly interpreted.

In the living animal it is almost impossible to make out anything of the nuclear apparatus with certainty. It is, therefore, necessary to work chiefly on fixed and stained material. Unfortunately, the animals survive but a short time after removal from their host, no matter what precautions one takes. It is also a necessity, therefore, to fix the creatures immediately after removal. Moreover, if the host be allowed to die the parasites very quickly begin to

degenerate. In order to obtain satisfactory material I accordingly made preparations from the kidneys of the cuttlefish while still alive, fixing the smears, etc., as quickly as possible. When this is done the results are practically always the same after a reliable method of treatment. Excellent fixation can be obtained with any of the good fixatives in ordinary use—sublimate-alcohol (hot), picro-acetic (hot), and Hermann's solution being particularly good, especially the two former. The usual stains all give excellent results—even the very simplest giving quite exceptionally good pictures. I have found Delafield's hæmatoxylin and borax carmine (Grenacher) as good as anything one could desire. I used both moist film preparations—made by smearing the kidneys on a coverslip—sections, and the following method:—A small piece of the kidneys, containing many parasites, was fixed, stained entire, and finally teased up in clove oil. Isolated individuals could be examined in this way with great ease, though moist film preparations are perhaps the best. And the results at which I arrived were these. There is a nucleus constantly present in the form of a delicate network of chromatin and plastin. At no period in the living animal does it undergo a cycle of changes as described by Gonder. In addition to the network there are also to be seen in the cytoplasm—in greater or less numbers—particles which stain strongly with chromatin stains. (Cf. fig. 2.) From observations on a large number of organisms I am now convinced that this represents the normal condition of the nuclear material.

It now remains to answer the questions, "What are the chromatin particles in the cytoplasm?" and "What are the curious chromidial stages described by Gonder?"

Regarding the former, I think it may be regarded as certain that the chromatin particles are—in part, at any rate—ingested food material. As I have already shown, the majority of individuals—all those, in fact, which have attained any size—possess a mouth. And this very obviously serves for the ingestion of food, which appears to be largely com-

posed of the epithelial cells of the kidneys of the host. We do, indeed, see remains of cells in all stages of digestion (cf. fig. 8), and a careful examination of many different individuals has brought me to the conclusion that the majority of the chromatin particles in Chromidina are the remains of the nuclei of renal cells.

These ingested particles may be very strikingly demonstrated by staining the animal with neutral red intravital (fig. 3). The nuclear net remains unstained.

It is possible that the chromatin particles also constitute, in part, the micronucleus of the infusorian—the network representing the meganucleus. Multiple micronuclei are known in other Infusoria—e.g. in *Loxodes*, (cf. Joseph, 11).

Regarding the second question, I think there can be but little doubt that all the animals which show irregular lumps or granules of chromatin, in place of the delicate nuclear network, are abnormal. The appearances are caused by imperfect fixation. Almost immediately the animal dies, or is allowed to dry ever so little, the network breaks up, and its parts run together to form irregular chromatin masses. This can be easily proved by merely letting a smear preparation dry slightly in the air before fixation. The granular masses of chromatin then appear in nearly every individual in the preparation, after fixing and staining (cf. fig. 7).

Even in a well-preserved specimen it is often impossible to find the chromatin of the nuclear net continuous—because the distribution of the chromatin in the plastin network, which forms the basis of the nuclear apparatus, is not uniform. This is especially obvious in specimens which have been treated by a method involving differentiation after staining—e. g. iron-hæmatoxylin or borax carmine. The smaller masses of chromatin become decolorised before the larger—which apparently lie freely in the cytoplasm, though really imbedded in the plastin network (see fig. 1).

It is surprising that the nuclear apparatus in the head of the organism—when of large size—should have passed unnoticed. It is a most striking structure in the form of a

huge sling (fig. 6). Its gradual development from the simple network in a young "bud" can easily be traced (figs. 4, 5, 2, 6). The sling is seen to be composed of a number of parallel fibrils of plastin with chromatin granules imbedded in them (fig. 6).

In the process of segmentation ("budding") the nuclear net remains unaltered—from beginning to end of the process. This is well seen in fig. 1, where every stage in segmentation can be seen. Large segments are at first constricted off, and these subsequently divide in two.

I have never found individuals with the perfect "bladder" nuclei described by Gonder. Perhaps they are really the nuclei of the renal epithelium cells, either lying on the organism or after being ingested. The great size variation represented in Gonder's figure (Pl. 11, fig. 58) is worthy of note. I am inclined to think—after examining a large number of individuals—that they are not of normal occurrence during the vegetative life of the organism. But it is impossible to judge on negative evidence alone.

#### *Chromidina coronata*, Føtt. emend. Gonder.

This infusorian differs from the preceding in the single character already observed by Føttinger and Gonder—the possession of a ring of long cilia surrounding the head, crownwise (fig. 8). The nuclear apparatus is exactly like that of *C. elegans* in every particular. The remarkable sling in the network in the head is just the same, and is found strongly developed in large individuals only (fig. 8).

Reproduction takes place in a manner exactly like that seen in *C. elegans*.

#### *Opalinopsis sepiolæ*, Føtt.

*O. sepiolæ* differs considerably from the two infusorian parasites already considered. It has been described in some detail already, but the following points may be added to these descriptions (6, 7).



The vacuole, which is situated at the posterior end of the animal (fig. 9) is contractile. It pulsates at an average rate of about once a minute. It is one of the most characteristic features of the organism, and it is surprising that its contractions have not been remarked before. Most of the individuals which I observed contained crystalline bodies in their cytoplasm (fig. 9). There is no cystostome.

Although I succeeded in discovering but a single cuttlefish infected with this parasite, I was able to make a considerable number of observations upon it. For *Opalinopsis* survives, in carefully made preparations, for several hours after removal from its host, and continues to divide actively, thereby presenting a great contrast to *Chromidina*. The liver and kidneys of the infected *Sepia* were literally swarming with the parasites.

Very little regarding the nuclear apparatus can be made out in the living animal. My description is therefore based upon permanent preparations, made with the same precautions as those of *Chromidina*. And here again, I cannot agree with Gonder's interpretation of the appearances presented.

Föttinger found that "the nuclei . . . sometimes assume the form of a network, and all stages are to be found intermediate between these networks and scattered nuclei—spherical or rod-like" (6, p. 373).

Gonder believed that the changes seen in the nuclear apparatus were intimately connected with the division of the organism. The cycle of changes is as follows:—"1. A complete resolution and fragmentation of the lumps and particles into fine granules . . . 2. Division of the Infusoria; the animals attain their greatest size at the stage of complete resolution of the nuclear substances, whereupon they divide. 3. A reconstitution of the nuclear masses, i. e. the plastin collects itself at certain places in the walls of the alveoli, together with the granules—so that fragments arise which branch out into large bands and slings, out of which the nuclear masses—with which we started—are formed" (7, p. 254). All these stages are very accurately figured, and it

is from Gonder's interpretation of them only that I am compelled to differ.

I have found that when the animal is properly fixed and stained, the nucleus invariably has the appearance shown in figs. 10 and 11. That is to say, it forms a complete network of chromatin and plastin, lying in the cell—just like the nuclear net of *Chromidina*. The net is not always quite easy to make out in its entirety, owing to the manner in which the chromatin may be distributed in the plastin framework. Hence, when only a chromatin stain is employed, parts of the nucleus may appear detached (fig. 10). The size and complexity of the net vary a good deal. It often has a quite simple structure—especially in small individuals (figs. 12, 13).

Just as in *Chromidina* the network remains as such during division. All stages, from the very beginning (fig. 14) right up to the completion of the process of transverse division (fig. 15) are to be found. Division takes place rather rapidly—the organisms which I saw dividing taking about twenty minutes for the whole act.

Here again, as in *Chromidina*, the organisms which contain lumps and scattered fragments of chromatin are produced by imperfect fixation. The lumps appear as soon as the animals begin to die (figs. 17—19), and may take very different forms. The degeneration is also seen, as a rule, in the cytoplasm, which becomes more coarsely alveolar. This was noticed by Gonder, though he failed to realise its meaning. "The alveolar system changes its character with the nuclear changes. If the nucleus is broken up or completely fragmented—forming a chromidial apparatus—then the protoplasm has a coarsely alveolar structure" (p. 246). Gonder's figures show this very accurately (e. g. figs. 19, 20, 26, etc.). A condition in which the chromatin is completely dissolved in the cytoplasm (Gonder's fig. 19) has never come under my observation. It appears to me to be highly abnormal.

Although I have examined a large number of individuals of all sizes, and at all different stages of division, I have

never found any which contained a single nucleus, as figured by Gonder. I thought at one time that I had done so, but later I was able to prove that the large uninucleate cells (fig. 16) which I mistook for Infusoria in the preparation were really giant amœboid cells from the cuttlefish's kidney. Some of these cells attain a length of nearly 50  $\mu$ .

Neither in Chromidina nor Opalinopsis has any sign of conjugation been observed.<sup>1</sup> No sexual process of any sort is known.

Equally unknown is the method of dissemination in nature. No cysts or resting stages have ever been seen. It is a curious fact that—like their frequent companions, the dicyemids—the Infusoria are unable to live for more than a few minutes in sea water. How they reach their host is still a mystery.

I should like to correct here the statement made by Gonder (7, p. 246) that the colour of the liver is an index of infection. As a matter of fact, the liver in a perfectly fresh uninfected cuttlefish varies in colour from dark red-brown up to creamy white, apparently according to the relative amount of cellular and non-cellular substance which it contains. In livers of very pale colour, only a few shreds of living tissue are to be found. Colour seems dependent mainly upon metabolism, not parasites, though these might, of course, affect it occasionally to some extent.

#### The significance of the nuclear apparatus.

A comparative study of the nuclear apparatus of Chromidina and Opalinopsis brings some interesting points to light. I will here indicate a few of these.

As I have already shown, in both Chromidina and Opalinopsis the nuclear apparatus consists of a delicate network, composed of chromatin granules imbedded in a

<sup>1</sup> The figure given by Føttinger (8) showing "conjugation" in Opalinopsis is, as Gonder justly remarks, nothing more than a stage in division.

plastin matrix, which extends through the cell. This network represents the compact nucleus which we are accustomed to see in other organisms. To speak of it as a "chromidial net," as does Gonder, is, to my mind, misleading. For there is absolutely no indication that it is in any way comparable to the structure known as a chromidial net in Thalamophora, etc. It is merely a modification of the branched form of nucleus.

The branching type of nucleus has been long familiar to cytologists. It is well seen in the cells of certain insects, as we know from the work of the Hertwigs, Brant, Eimer, Balbiani, etc. (Cf. R. Hertwig's description (10) of the "amœboid" nuclei in the Malpighian tubule cells of *Pieris brassicæ*.) But the most instructive comparisons are to be made with the nuclear apparatus of other Infusoria.

Maupas (12), Gruber (8, 9), and others have described various forms of diffuse nucleus in the Infusoria. One of the most careful descriptions is that by Gruber (9) of the hypotrichous ciliate *Holosticha* (*Oxytricha*) *scutellum*, Cohn. In this organism both meganucleus and micronucleus lie scattered in fragments through the cytoplasm during vegetative existence. Before division, however, the fragments come together, forming a single mega- and micronucleus, both of which then divide, subsequently fragmenting once more in the daughter individuals. This formation of a compact nucleus before division does not appear to take place in all "multiucleate" forms, e.g. *Loxodes*. In *Trachelocerca*, *Uroleptus*, and *Epiclinites* also the nucleus is diffuse (Gruber, 9).

It is in the parasitic Infusoria, however, that the most interesting forms for comparison with Chromidina and Opalinopsis are to be found. In *Fœttingeria actiniarum*, Clap.,<sup>1</sup> a nuclear apparatus very like that of *Opalinopsis* has been described by Caullery and Mesnil (3). In

<sup>1</sup> = *Fœttingeria* (*Plagiotoma*, *Conchophthirius*) *actiniarum*, Claparède emend. Caullery et Mesnil. The animal lives in the coelenteron of various sea-anemones.

the youngest animals, the nucleus is roughly horse-shoe shaped, but in large individuals it takes the form of a mesh-work of chromatin containing nucleoli at certain points. The network, which varies in its form, is described as consisting of a system of "tubes," and as being "amœboid." It bears, as Caullery and Mesnil have pointed out, a very striking resemblance to the nuclear apparatus, as I have seen it, in *Opalinopsis*.

But the most interesting comparisons are to be made with various *Anoplophryinæ*. Recent research has brought to light many interesting facts regarding this group. As is well known, in *Anoplophrya* the nucleus is band-like, running down the middle of the body of the elongate organism. The animals possess a series of vacuoles and a method of segmentation which resemble the conditions seen in *Chromidina* to a remarkable degree. But at first sight the nucleus appears totally unlike. The means of comparison have been given us by Caullery and Mesnil (4), who have discovered a remarkable new member of the group—*Rhizokaryum concavum*, C. et M. In this animal—a parasite of certain species of *Polydora*—there is a nucleus consisting of a thick axis, from which numerous branching processes are given off ("like a root with numerous rootlets"). According to these observers, in *Anoplophrya* also the central nuclear cylinder sometimes shows little pointed appendages, thus presenting an appearance intermediate between a simple band and a branching stem like that of *Rhizokaryum*. From the latter condition it is not difficult to imagine how a reticular nucleus like that of *Chromidina* might have arisen from an originally compact nucleus. The last barrier between the infusorian parasites of cuttlefish and the *Anoplophryinæ* has now been broken. And it is certain, as Neresheimer (13) hinted from his study of *Opalina*, that the parasites from cephalopods are not related to *Opalina* but to *Anoplophrya*.

One or two other points of interest may be briefly touched upon. The most interesting is the apparent absence of a

micronucleus in the parasites of cephalopods. Nor is a micronucleus described in *Fœttingeria*. In *Rhizokaryum* the micronucleus is spindle shaped. Some very interesting observations have recently been made upon a form very closely allied to *Anoplophrya* by Awerinzew (1). He names this animal (a parasite of the marine worm, *Ophelia limacina*) *Bütschliella opheliæ*; and he finds that the micronucleus becomes visible only when the animal is about to divide. In *Chromidina*, however, a micronucleus is never visible at any stage during segmentation (cf. fig. 1).

As I have already pointed out, the chromatin particles, which are normally present in the cytoplasm, may in part represent the micronucleus. A curious formation, apparently from the nucleus, of similar particles occurs at a certain stage in *Bütschliella*. Another interesting feature of this organism is that it may undergo a simple transverse fission, thus combining both the method of reproduction seen in *Chromidina* and that of *Opalinopsis*. *Bütschliella* also possesses contractile vacuoles.

Of the deeper significance of the net-like nucleus we know nothing. It is as yet quite impossible to say why one organism should possess a single compact nucleus, whilst others of similar size and apparently performing similar functions should have nuclei in the form of a net or scattered fragments. It looks at present as though it were immaterial how the nuclear substances are disposed in the cell so long as they are present. However, the matter can be elucidated by further research alone.

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The foregoing pages embody a small part of the results of the work which I did whilst occupying the British Association Table at Naples from March to June of the present year. I desire to thank the British Association Committee for their kindness in assigning me the Table. I wish also to tender

my warmest thanks to the Goldsmiths' Company for their grant, without which I should not have been able to carry out my work in Naples. I trust the remaining results will be ready for publication before long.

CAMBRIDGE;  
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EXPLANATION OF PLATE 1,

Illustrating Mr. C. Clifford Dobell's paper on “Some Observations on the Infusoria Parasitic in Cephalopoda.”

FIG. 1.—*Chromidina elegans*, posterior end, showing various stages in the formation of buds. The chromatin is alike at all stages. (Hot picro-acetic, borax-carmin, differentiated acid-alcohol. Leitz  $\frac{1}{2}$  in.  $\times$  1.)

FIG. 2.—*C. elegans*. Medium sized individual, entire. Note the nuclear network, chromatin granules, mouth, etc. (Sublimate-alcohol (hot), Delafield's hæmatox.  $\frac{1}{2}$  in.  $\times$  1.)

FIG. 3.—*C. elegans*. Living animal: stained *intravivam* with neutral red. ( $\frac{1}{8}$  in.  $\times$  5.) The food particles, vacuoles, and mouth are well seen.

FIGS. 4, 5, 6.—Three stages in the development of the head of *C. elegans*. 4. A small individual, without a mouth. 5. A larger animal, with a small mouth and feebly developed chromatin sling. 6. Very large individual, with well developed mouth and strongly developed sling. ( $\frac{1}{2}$  in.  $\times$  1 (enlarged to scale). Hot sublimate alcohol, Delafield.)

FIG. 7.—Posterior end of *C. elegans*, forming segments. The animal had died before fixation, thus giving rise to nucleus in the form of chromidia. (Sublimate alcohol, Delafield,  $\frac{1}{2}$  in.  $\times$  1.)

FIG. 8.—Head of *Chromidina coronata*, large individual. The chromatin sling is well seen (cf. fig. 6). *m* = position of mouth. *c* = an ingested cell from the kidneys. Various other cell remains are also to be seen. (Hot picro-acetic, borax-carmin.  $\frac{1}{2}$  in.  $\times$  1, enlarged.)

FIG. 9.—*Opalinopsis sepiolæ*. Ordinary individual, showing contractile vacuole (*c.v.*), crystalline bodies, cuticular striation, etc. Living animal. ( $\frac{1}{2}$  in.  $\times$  1.)

FIGS. 10, 11.—*O. sepiolæ*, stained, showing nuclear network. Large individuals. (Sublimate alcohol, Delafield.  $\frac{1}{2}$  in.  $\times$  1.)

FIGS. 12, 13.—Two small *O. sepiolæ*. (Sublimate alcohol, Delafield.  $\frac{1}{2}$  in.  $\times$  1.)



FIG. 14.—*O. sepiolæ*. A large individual beginning to divide. Note persistence of nuclear net. (Sublimate alcohol, Mayer's paracarmin.  $\frac{1}{12}$  in.  $\times$  l.)

FIG. 15.—*O. sepiolæ*, at end of division. The nuclei are still in the form of a net. (Sublimate alcohol, Delafield.  $\frac{1}{12}$  in.  $\times$  l.)

FIG. 16.—Giant amoeboid cell from liver of *Sepia officinalis* infected with *Opalinopsis*. Length  $43 \mu$ . (Sublimate alcohol, Delafield.  $\frac{1}{12}$  in.  $\times$  l.)

FIGS. 17, 18, 19.—Degenerate forms of *O. sepiolæ*, with fragmented nuclei. (Sublimate alcohol. 17, Delafield; 18, 19, Grenacher's alum-carmin.  $\frac{1}{12}$  in.  $\times$  l.)

[With the exception of figs. 3, 8 (in part), and 9, the cilia are not shown.]



