

also, that this last amoeba, because of plenty of room, remained within the parent wall for some time after all the others had crept out.

It is evident from this observation that excystment depends upon a number of things. First of all, cysts must be ripe, that is, just ready to bud when put into a medium suited for excystment, or a medium is required which will stimulate a cyst to reach maturity and then to bud. External factors, as well as internal conditions of the cyst, must be *considered* since they are of equal importance. Chemical stimuli, temperature, and osmotic pressure all have their part to perform in this as well as in other culture experiments. The small percentage of amoebae which were known to excyst was not only due to proper conditions but also to the fact that allowance was made for immature and for moribund cysts.

SUMMARY

Excystment of *Councilmaniana laffeurii* took place in Ringer's solution plus .01 per cent of dextrine only, and not in any of the other media used. Of the sixteen tubes of Ringer's solution and dextrine only three (a little more than 20 per cent) were found to contain excysting amoebae.

Cysts must reach the proper phase in their life-history in order that excystment can take place and the surrounding medium must have within it the necessary chemical and physical conditions to make possible this excystment.

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THE CYTOLOGY OF TINTINNOPSIS NUCCULA (FOL) LAACKMANN

WITH AN ACCOUNT OF ITS NEURONOTOR
APPARATUS, DIVISION, AND A NEW
INTRANUCLEAR PARASITE

BY

ARTHUR SHACKLETON CAMPBELL

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INTRODUCTION

Cytological evidences presented by a study of *Tintinnopsis* offered in this paper may be of advantage to further investigations in revealing in a striking manner great structural differentiation and integrated behavior in this interesting, neglected, and large group of primarily marine, house-building ciliates. Particular attention is devoted to the neuromotor system, described for the first time for any of the tintinnids, and to the nuclear and cytoplasmic phenomena at the critical period of division. The relation of the house or shell to the neuromotor system and the method of its origin is discussed.

ACKNOWLEDGMENTS

The writer takes this occasion to express his indebtedness to Dr. C. A. Kofoed, at whose instance the investigation was undertaken, for kindly and suggestive aid. The Scripps Institution of Oceanography, of La Jolla, through its Director, Dr. T. Wayland Vaughan, has rendered invaluable aid, not only in allowing the writer the generous use of ample facilities during the several weeks of the summer of 1925 which he spent there, but also in supplying an abundance of well prepared material during the greater part of the year.

TECHNIQUE

Collections with a number 25 silk bolting cloth net were made by the writer at various periods throughout the year, depending upon tides and bright days. Most of the material used was collected at the Key System Railway pier, Oakland, California. The incoming tide early in the morning of a bright sunny spring day proved to be the most productive of abundant material. During the summer, the writer collected material at the Scripps Institution of La Jolla, California, under somewhat similar conditions.

As soon as containers were brought into the laboratory the contents were filtered quickly so that the excess amount of sea water might be eliminated. The precipitate, consisting of the organisms of the catch remaining upon the filter paper, was washed quickly and carefully into a large, shallow crystallization dish and allowed to remain at least one-half hour in a little sea water before being killed.

It was found after considerable experimentation that by this simple method the majority of the tintinnids, as well as other plankton, could be killed in an expanded state. Contracted animals are useless. The killing agent was always used hot, and if poured in suddenly with a rapid rotary motion nearly every animal in the crystallization dish was killed while excellently expanded.

Various fixing reagents were used. None proved satisfactory but Schaudinn's alcohol-mercuric chloride-acetic acid mixture, and this only when heated to boiling. Animals treated in Schaudinn's fluid heated to 90° C. were nicely expanded with open peristomes and membranelles spread out.

Material, collected and prepared as described, was stained in a number of ways. The best staining was obtained by using Heidenhain's iron-alum haematoxylin very carefully differentiated in one-half per cent iron-alum. Both aqueous and alcoholic solutions of this stain were used. In general, the aqueous solution gave the more uniform results. It was used as described by Sharp (1914).

All material intended for permanent slides was treated by the centrifuge method. By this method, hydration, staining, differentiation, and dehydration may be rapidly and efficiently accomplished.

Material for sectioning was carried through as described by Sharp (1914). Sections were cut in series at five and ten microns. Sections were stained either on the slide or before imbedding.

Maximum optical equipment, which is essential to the best observations, was used throughout this investigation.

SYSTEMATIC POSITION

O. F. Müller (1786) recognized and described, under the name of *Trichoda inguinus*, a marine tintinnid. Since that time numerous investigators, especially Brandt (1906, 1907), have revealed the extent and variety of these interesting forms. Kofoed (MS) regards the group as Tintinnoina, a subdivision of the heterotrichous ciliates.

GENERIC RELATIONS

The genus *Tintinnopsis* was founded by Stein (1867) for *Tintinnopsis beringiana*. The genus has been enlarged and emended by Daday (1887), Biedermann (1893), and especially Brandt (1907). More recently Jörgensen (1924) has further emended the genus *Tintinn-*

nopsis. This author considers *Tintinnopsis nucula* and *T. ventricosa* as members of the newly proposed genus *Stenosemella* which he separates from other members of the old genus *Tintinnopsis*. This separation is made upon the firmer lorica and narrow, thin-walled collar about the rather constricted mouth in these two species. In his key to the genera he separates these two species from the remainder on the basis of their double lorical wall.

It seems best to retain the older and wider application of the name *Tintinnopsis* as defined until other evidences than these slight differences are available.

Systematic distinctions in this and in other genera of tintinnids are confined almost altogether to the structure of the lorica. In large measure this is justifiable since the lorica expresses in its structure the fundamental, peculiar constitution and behavior based on structure of the animal which resides within it.

SPECIFIC RELATIONS

Although a long series of observations have been made upon *Tintinnopsis* since the time of Fol (1884) it seems unwise to offer a complete list of synonyms under which *Tintinnopsis nucula* may have been known. An extensive series from widely separated regions is essential to a more complete understanding of the exact limitations of this form. For this reason and to avoid confusion, it seems best to always write *Tintinnopsis nucula* as attributed to (Fol) Laackmann. A list of the most significant synonyms includes:

Codonella nucula Fol (1884)
Tintinnopsis nucula Laackmann (1907), Brandt (1907), Wailes (1925)
Stenosemella nucula Jørgensen (1924).

Fol compares this species closely with *Codonella* (*Tintinnopsis*) *ventricosa* Fol, from which it may be distinguished by smaller size and by being less incrustated with extraneous matter. This species was first placed in the genus *Tintinnopsis* by Laackmann (1907).

Considerable variation in size is indicated by investigators of this species. The question of geographic races or local forms may enter into the final allocation of *Tintinnopsis nucula* and the related *T. ventricosa* (Fauré-Fremiet, 1924). These geographical races may integrate the two species completely. The variations due to the disturbing factors of growth and reproduction may enter into a con-

sideration of these differences. At present, the greater size of *T. ventricosa* chiefly differentiates it from *T. nucula*. Extensive collection may either unite these two species or else indicate that *T. ventricosa* is rare; it may be absent from the California coast, although this is doubtful. For the present we must still consider these two species distinct.

The number of membranelles of the adoral zone in both *Tintinnopsis ventricosa* and *T. nucula* is twenty-two. This condition sets off *T. nucula* and *T. ventricosa* distinctly from other members of the genus *Tintinnopsis*, in which the number of membranelles is twenty or less.

HABITAT AND BIOLOGY

In common with most of the tintinnids, *Tintinnopsis nucula* is wholly marine in its distribution. Many species of tintinnids are found upon the high seas and some are found only under those conditions. Other species (particularly members of the genus *Tintinnopsis*) although fewer in number, are found near, or are limited to coastal waters. Among such neritic planktons is *Tintinnopsis nucula*. This species has been recorded from nearly all seas. The writer has been able to obtain specimens at almost any time of the year in San Francisco Bay. Although no accurate quantitative counts have been made, it was noted that the animals were more abundant in the late spring months, especially after a great increase of diatoms or dinoflagellates. This condition was noted by Entz, Jr. (1909), for various tintinnids and has been accurately reported upon by Fish (1925) for *T. beroides* in the restricted area of Woods Hole. In the bay waters few empty lorices were found, nearly every lorica containing live, normal animals. In the summer collections at La Jolla, however, where oceanic conditions prevail, there was a preponderance of empty shells. *T. nucula* is much less abundant at La Jolla than at San Francisco. It is a neritic species and is less delicate both in texture and viability than the genera and species of the high seas. Although *T. nucula* is a coastal form, in common with other members of the genus, transitions to brackish water have been reported (Steuer, 1910). This is easily understood if we consider the varied environment and salinity of harbor waters.

The writer was unable to cultivate or culture these animals in any way. They do not thrive in the laboratory under any conditions and

frequently die within an hour of collection. A few will live in well aired, large culture dishes for about twenty-four hours at low temperatures.

Tintinnopsis nucula feeds principally upon diatoms, dinoflagellates, radiolarians, small chrysomonads, and bacteria. Sponge spicules are sometimes found within the cytoplasm. The remains of food organisms are commonly found within the cytosome in the posterior part, or their shells are found to be attached to the lorica as part of its structure. In numerous instances the silicious skeletons of radiolarians, spicules of sponges, and valves of diatoms are discharged by the anus and stored within the fundus of the lorica until the time of fission (pl. 15, fig. 38). Usually the smaller specimens of these organisms are used as food, although in some cases observed large diatoms and dinoflagellates were ingested (fig. B, *fd.b.*).

The type of food ingested indicates that *Tintinnopsis* is largely omnivorous in feeding habit. Both vegetable and animal foods are taken indifferently and both may be found in food vacuoles in the same animal (fig. B, *fd.b.*). After a diatom or dinoflagellate pulse these may predominate as the source of food.

GENERAL MORPHOLOGY

LORICA

The shell or lorica of *Tintinnopsis nucula* is elaborate. Brandt (1907) gives, in his monograph of the tintinnids, what is probably the best account of this complex lorica, but since he treats certain features incompletely I have given the description below.

The lorica of *Tintinnopsis* is usually yellow or brown in life. Part of this coloring matter is derived from incrusting, foreign bodies. The lorica is approximately olive-shaped, with the widest point within the upper third of the height and the truncate oral end (fig. A, *col.*). The lorica may also be described as vase-shaped, ovoid, beaker-shaped or cordiform, and it is thus described, particularly by Brandt (1907). It is closed aborally. Thus the lorica is rather short and wide. There is no caudal prolongation or pedicel as in *T. campanula* Daday (Faure-Fremiet, 1924, fig. 29). The lorica is of firm consistency. It usually tears when sectioned. The body (fig. A, *bod.*) of the lorica gives no evidence of any distinct helicoidal coiling such as is prominent in the collars of the genus *Codonella* or in the body of *Corlella annulata* (Entz, Jr., 1909, pl. 10, fig. 1).

The *nuchal band*, surrounding the base of the collar, is always distinct (fig. A, *nu.b.*). It separates the collar from the body of the lorica and is a thickened band belonging properly to the body of the shell rather than to the collar or its base. The lorica is about two microns in thickness throughout, without the foreign, irregular bodies. It may be slightly thicker near the *fundus* (fig. A, *fun.*).

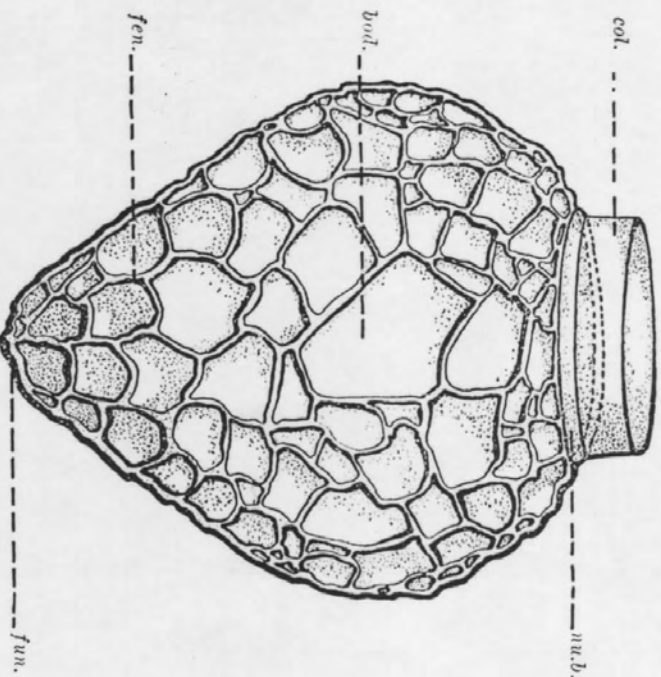


Fig. A. Semidiagrammatic figure of the lorica of *Tintinnopsis*. Abbreviations: *bod.*, body; *col.*, collar; *fen.*, fenestrations; *fun.*, fundus; *nu.b.*, nuchal band. $\times 1150$.

The *collar* (fig. A, *col.*) surrounds the oral aperture of the lorica and arises inside the nuchal band. It may consist of one, or at the most two, non-separable annuli. The disposition of these rings is probably correlated with the slight spiraling of the somatic ciliation and of the ciliary membrane (pl. 12, figs. 6, 8) which aids in the shaping up of the lorical components. The collar is frequently broken off, possibly during life. It is usually without reticulations or other structures and seldom has a great mass of agglutinated foreign bodies upon it (pl. 12, figs. 6, 8). Windows, characteristic of *Dictyocysta*, are never found. However, in certain loricas found in the open sea there is a marked tendency toward the formation of irregular, half-

moon-shaped openings in the collar. The collar is less firm in consistency than the remainder of the shell. The margins of the collar are smooth and without teeth or other ornamentation except as this appearance is given by added foreign matter. Both the collar and the body of the lorica are possibly colloidal (Entz, Jr., 1909).

There is no indication of a lorical diaphragm such as that found regularly in *Codonella* and similar forms. Entz, Jr. (1909, pl. 8, fig. 16), figures a lid-like detritus mass closing the oral aperture in *Codonella orthoceras* Haack. This massing of irregular materials I have found in *Tintinnopsis*. It represents an aggregation drawn into the aperture by the membranelles and may be protective in function or concerned in closing off the lorica preparatory to encystment.

The lorica has a reticulate structure (fig. A, *fen.*). The wall is thin, translucent, and apparently structureless between the elevated margins of the external reticulations. The inside of the shell is smooth and with but very slight indications of this external fenestration. The elevated ridges stain deeply with iron-haematoxylin.

Coating the exterior of the lorica there is a varying amount of agglutinated matter. Many of these particles are built into the intimate structure of the lorica. For the greater part this detritus is faecal, consisting of bits of radiolarians, diatoms, sponge spicules, or other bodies of organic origin; although other highly refractive bodies are met with which may be formed from the substance of the shell. This extraneous matter often conceals the real structure of the lorica (Calkins, 1902). The cavity of the lorica frequently contains a mass of stored faeces and secreted foamy matter (pl. 15, figs. 31, 38), which is used by the freed daughter animal at the time of binary fission to construct a new lorica. There is no groove or other special structural adaptation for the reception of this mass in *Tintinnopsis* (pl. 15, figs. 31, 38).

The size of the lorica is variable but within certain definite limits. In a measured series of loricas the average external dimensions were forty-two microns in length, from the extreme aboral apex or fundus (fig. A, *fun.*), to a line drawn at the center of the diameter of the oral aperture at the base of the nuchal band. At the oral end the collar, above the nuchal band, averages nearly six microns in height. The widest point of the body of the lorica is at a point about two-thirds above the fundus. At this position the lorica may measure about thirty microns across. The length-breadth relation is thus about 1:1.5. In diameter the collar averages twenty microns at the base.

The diameter of the aperture of the collar is slightly greater than at the base. There is considerably less variation in these measurements than those reported by others (Brandt, 1907; Entz, Jr., 1909; Jørgensen, 1924).

CYTOSOME

Although there is an extensive literature dealing with the general morphology of marine ciliates, little actual cytological work has been attempted. Studies upon the cytology of *Tintinnopsis* of various species have been made by Entz, Jr. (1909). Other papers of general nature include those of Schweyer (1909), Merkle (1909), and Fauré-Fremiet (1908, 1924). These papers deal with numerous genera and species of tintinnids.

GENERAL ACCOUNT

The highly contractile animal of *Tintinnopsis nucula* measures when fully expanded nearly seventy microns in length from the stalk at the base to the base of the reflexed peristome margin. The diameter of the cytosome is partly conditioned by its state of expansion. In a fully expanded animal it is slightly less than the circumference of the collar, or about twenty microns in diameter. Careful comparisons show these measurements to be closely corresponding to those of the living animal, the reagents used causing but slight shrinkage. In a fully extended swimming animal about one-half of the organism may be projecting freely above the mouth of the collar. A disturbed animal may, in *T. nucula*, completely withdraw into the lorica and fill the larger part of its cavity.

Tintinnopsis nucula is colorless and refractive. Within the cytoplasm, material derived from ingested diatoms and flagellates forms yellow vacuoles.

According to Fauré-Fremiet (1924, fig. 31), the body of some species of *Tintinnopsis* is attached to the bottom of the lorica without a distinct pedicel. *T. ventricosa* is figured with a terminal attachment by this author. The attachment of *T. nucula* is certainly not similar. Entz, Jr. (1909), failed to find any indication of the attachment but presumed it to be terminal. The attachment of *T. nucula*, as also in *T. ventricosa*, is without a distinctly differentiated pedicel and may best be described as characteristically subterminal

(pl. 12, figs. 3, 5) within the fundus of the lorica, as seen in both living and fixed animals. The region of attachment is always below the cytopyrge and it terminates the column (fig. B, *at.*). The attachment is derivable from a pseudopodial type characteristic in the Stentoridae and described by Faure-Fremiet (1908). It is somewhat comparable to that described by Schweyer (1909) in *Cyrtocapsis ehrenbergii* or to that in *T. lusus-undae* which I have repeatedly watched in the living plankton at La Jolla.

The column (fig. B, *clm.*) in the extended state is narrowly campanulate or pyriform, approaching gradually apically to the point of attachment (fig. B, *at.*), or sometimes rather abruptly (fig. C, *at.*), and increasing in diameter orally. In transverse section it is circular or subcircular. At the oral or distal end it broadens to a spreading, distinct, expanded, thickened peristome collar (fig. C). The column is cylindrical and its ventral and dorsal aspects may be distinguished by the position of the cytopyrge (fig. B, *cytop.*) and by the continuation of the broad ciliary membrane and lateral field (fig. B, *clm.*) slightly spiraling down part of its length, both on the ventral surface (pl. 12, fig. 5). The columnar surface is covered by a thin, unornamented pellicle. Body cilia follow the twenty-two longitudinal myonemes, the alternating spaces between them being hyaline bands. At a point slightly below the peristome collar the ventrosinistral side of the column is expanded into a *lateral lobe* (fig. B, *lobe*) which is highly plastic and capable of being thrust in and out. This lobe is frequently siderophile. In certain other members of the genus *Tintinopsis* it is a fixed, rigid structure. It was thus described by Faure-Fremiet (1924) in *Tintinopsis campanula* and I have been able to determine its permanence in *T. dadayi*. The functions and activities of this lobe I will describe in a later section. The column is unsupported by a cytoskeleton such as that found in the genus *Strombidium* (Amigstein, 1913). It depends upon the turgidity of the cytoplasm for support.

The *peristome collar* (fig. G, *cl.*), which is the thickened edge of the flaring and truncated oral end of the column of the animal, always lies nearly in the same plane, that is, its distal end is not noticeably displaced anteriorly or posteriorly. It is located peripherally to the membranelle zone. When seen face on it appears as a single, flat spiral enveloping the peristome field. The spiral condition obtains also in *Stenlor* and in other Heterotricha (Johnson, 1893), but is generally steeper. The direction of the spiral is left-handed, or

leiotropic, viewed from below. Between the distal and proximal ends of this spiral is a narrow, deep space or *cleft* (fig. E, *cleft*) and through it the ciliary membrane passes vertically down the surface of the column, on the inner side (figs. B, E, *clm.*) of the peristome collar. This ciliary membrane, which is a neuromotor structure and a direct outgrowth and transformation of the distal end of the adoral membranelle zone, arises from it in a manner to be described later.

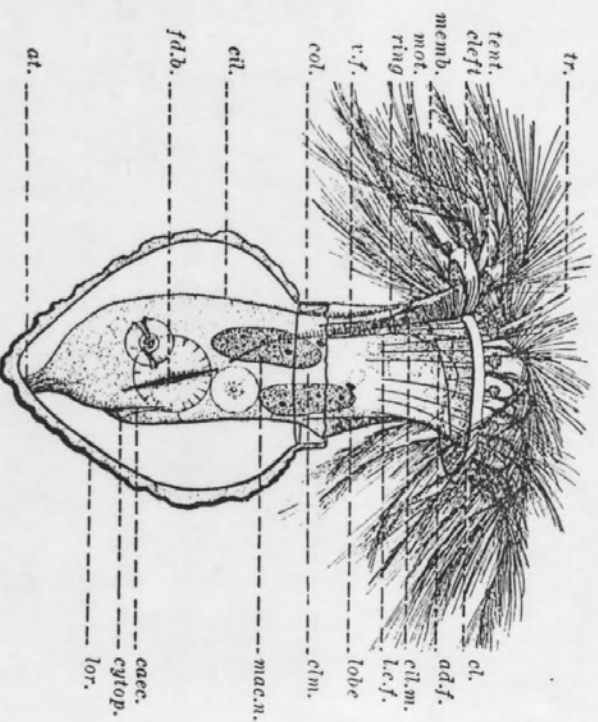


Fig. B. Semidiagrammatic figure of *Tintinopsis* showing the general morphology of the cytosome. Abbreviations: *ad.f.*, adoral fiber of the neuromotor apparatus; *at.*, attachment to the lorica; *caec.*, caecum; *cil.*, somatic cilia; *clm.*, ciliary membrane; *cl.*, peristomal collar; *clm.*, column; *cleft.*, cleft in the peristomal margin; *col.*, lorical collar; *cytop.*, cytopyrge; *fd.b.*, food body; *lob.*, lateral column lobe used in shell-building; *lor.*, outlines of the lorica; *lc.f.*, lateral ciliary field; *mac.n.*, macronucleus; *memb.*, membranelle; *mot.*, neuromotorium; *ring.*, circumoesophageal ring; *tent.*, tentaculoid; *tr.*, trichocysts; *v.f.*, ventral fiber of the neuromotor apparatus. X 850.

The plane of the peristome collar is either somewhat oblique (fig. G) or nearly perpendicular to the main axis (fig. B) of the column. The oral surface of the collar, which may be about five microns wide, carries on its oral face the crown of membranelles and tentaculoids (fig. E, *memb.*, *tent.*). The proximal end of the whorl ends somewhat abruptly and the point where it ends carries three membranelles which enter the infundibulum. The distal end tapers as it approaches the cleft (fig. E, *cleft*) and overlaps the proximal end for a short distance.

The *peristome field* (this term, as used in this paper, denotes the same series of structures described by the term *frontal field* in *Stentor* and related forms, and is more nearly descriptive of these organs in *Tintinnopsis*) is concave and somewhat disc-shaped (figs. D, E). It occupies nearly the whole of the anterior or oral end of the animal except the peristome collar, the inner margin of which limits it, and together with its own structure and associated structures forms one of the most complex parts of the whole organism. It is a deep, ~~laterally~~ spiraling plate which arises from the inner margin of the collar, as described, and passes downward, or adorally, into the eccentrically placed *infundibulum* (fig. G, *inf.*). Its surface is marked by striations, regularly placed with bands of clear substance between, spirally disposed, and entering the *infundibulum*. Dorsolateral of the *infundibulum* and within the peristome field there arises a highly mobile, prominent eminence or *oral plug* (fig. D, *o.p.*). The whole peristome field is covered with a thin pellicle which like the collar surface is never ciliated.

The structure which I have designated as the *oral plug* (fig. D, *o.p.*) is a most remarkable organ. Its physiology is described in a following section on the living animal. The oral plug forms one of the most characteristic organs of the tintinnids. On account of its extreme mobility and prominence, it was early noted and described by Stein (1867) and called by him the piston-organelle. This term is probably not descriptive of its function in the living animal, hence I have redesignated it. This oral plug may be homologous with the less conspicuous structure of the same name in *Balanidium* (McDonald, 1922), or in *Diplodinium* (Sharp, 1914). Its great mobility, situation, and sensitivity may be taken as evidences of this homology. Very delicate fibers may run between the surface markings of the peristome field (Entz, Jr., 1909). These are probably neuromotor fibers.

The organs of feeding may be considered as made up of the *infundibulum*, *cytostome*, and *gullet*. The pre-oral cavity or *infundibulum* (fig. D, *inf.*) descends from the peristome field, plunging into the slightly spiral gullet. No marked structural changes are associated with it. It forms a deep funnel-shaped depression in the peristome field which narrows as it progresses. It may be as much as three-eighths the breadth of the peristome field. Its wall is lined by the regularly placed membranelles and associated accessory combs. The mouth or *cytostome* is the entrance to the gullet. It is marked

by no special structural modifications and through it food pellets are passed into the gullet below.

The *gullet* (fig. D, *gul.*) leads directly from the mouth. It forms a curved tube, the convex side of which is directed toward the outer body wall and the concave side toward the oral plug. It is frequently as much as one-quarter or even one-half the length of the column. Its diameter at a point near the mouth may be about ten microns when fully extended. In section it is circular. There are no special internal myonemes, retractile strands, or other contractile elements surrounding or associated with these organs of food-taking, as in *Diplodinium* (Sharp, 1914). The gullet is covered by a thickened pellicle and extending down its length are several oral membranelles. These are made up of short cilia that arise from the adoral crown and course down the gullet in a lengthened spiral. Accompanying them are accessory combs. The *infundibulum* and gullet are very extensible and allow the passage of large objects such as whole diatoms more than twice the diameter of these organs when contracted.

A prominent *cytopyge* (fig. B, *cytop.*) marks the lower part of the column of the animal on the ventral surface at a point slightly above the attachment to the lorica. The *cytopyge* is a papilla or raised, clear, elongated point in the pellicle. When fully open it may extend inward as a tube and may be distinguished as being divided into two parts, a *caecum* (fig. B, *caec.*) and a *cytopyge* (fig. B, *cytop.*). The *caecum* is a tubular structure while the *cytopyge* is slot-like. The *caecum* is anterior to the *cytopyge* and extends upward and behind it. There are no supporting structures in connection with these organs as are seen in the Ophryoscolecidae (Sharp, 1914).

These organs of defecation serve the organism in the function of the discharge of the solid remains of food. Through the *cytopyge* the indigestible valves of diatoms and the plates composing the cuirass of dinoflagellates are passed, as well as other faecal material. Fluid excretory products are probably discharged through the *cytopyge* in part, and in part by the general surface of the body, there being no contractile vacuole. Although I have examined many animals, both living and preserved, I have at no time been able to distinguish any evidence of a contractile vacuole. Entz, Jr. (1909), lists a series of both marine and fresh-water forms for which he claims at least one of these vacuoles, opening generally into the *infundibulum*. In one species, *Tintinnidium pusillum*, he reports a non-contractile vacuole. In no case have I been able to confirm either this observer or others

(Entz, Sr., 1884) regarding these asserted vacuoles. It is entirely possible that they may be simply food vacuoles which may be readily found in this region of the body.

ECTOPLASMIC STRUCTURES

The whole surface of the body is coated with a thin, delicate, scarcely distinguishable *pellicle* which is slightly less than one micron in thickness. That part which covers the peristome surface and the peristome collar and related structures which extend into the gullet, is somewhat thicker and stains intensively with iron-haematoxylin. The *pellicle* about the cytopyrge is also somewhat thicker. I have been unable to distinguish in the *pellicle* any evidences of striations or other markings. The *pellicle* is highly flexible, allowing the very mobile cytoplasm within to throw it freely into folds. It is retentive and protective as well as supporting in function.

A highly differentiated and separated *ectoplasm* is not clearly evident in *Tintinnopsis nucula*. There is no boundary layer (Sharp, 1914; McDonald, 1922) or other definite, distinguishable line of demarcation between the ectoplasm and endoplasm in this form. The large alveoles of the endoplasm grade into the smaller, delicate ones of the periphery which compose the ectoplasm. Deeply staining granular bodies of the endoplasm may extend well toward the ectoplasm. At the extreme periphery the protoplasm becomes more dense and very finely granular.

Extending inward, and equidistantly placed in transection, are narrow (less than two microns across) hyaline bands or "*Zwischenstreifen*" which run vertically. Beside these clear areas the cilia rise from basal granules which are somewhat deeply set into the outermost region of the granular portion of the ectoplasm. This structural differentiation of the ectoplasm into clear areas and finely granular *myonemes* (fig. C, *my.*) recalls the structure of the parasitic *Balanidium* (McDonald, 1922). So far as I have been able to determine, in both living and stained organisms these granular and hyaline bands do not branch. My interpretation of these structures confirms closely that of McDonald (1922) and need not be further dwelt upon here. It corresponds closely to that of others (Maier, 1903) and is probably general in the Heterotricha.

The *myonemes* fade posteriorly and are difficult to follow. They usually meet at the stalk. Anteriorly, they extend into the reflexed

margin of the collar and possibly connect with the basal granules of the adoral membranelles. There is a little evidence that these granular *myonemes* are made up of alternating clear and denser matter, although the small size of *Tintinnopsis* makes it difficult to determine this fact satisfactorily. The *myonemes* constitute one of the most powerful effector systems in the organism.

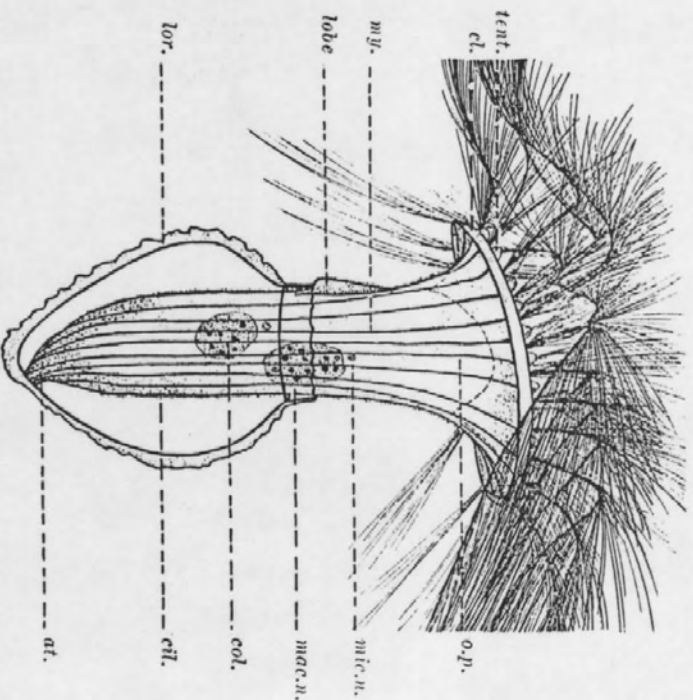


Fig. C. Semidiagrammatic figure of *Tintinnopsis* showing especially the *myonemes* and other external features. Abbreviations: *at.*, attachment to the lorica; *cl.*, somatic cilia; *cl.*, peristome collar; *col.*, lorical collar; *lobc.*, lateral column lobe; *lor.*, lorica; *mac.n.*, macronucleus; *mic.n.*, micronucleus; *my.*, *myonemes*; *o.p.*, oral plug; *tent.*, tentaculoid. X 850.

A peculiar system of intensively staining, elongate *trichocysts* covers the collar and tentaculoids and follows the protoplasmic accessory combs into the peristome in many tintinnids (figs. D, E, *tr.*). Some of these bodies may even enter into the wall of the infundibulum. Entz, Jr. (1909), by whom they were especially noted, homologizes them with the trichocysts covering the surface of many ciliates. This author states that Stein (1867) may have had them in mind, in describing "*Trichocysten*" in the fresh-water tintinnid, *T. fluviatile*.

In *Tintinnopsis nucula* these trichocysts are clustered especially about the tentaculoids (fig. B, D, *tr.*). A few were found scattered in a random manner in the peristome or upon the collar. In size they vary from one and one-half to two microns long by about one-half micron in breadth. No indication of their function has been determined by a study of the living animal. The presumption that they represent trichocysts is a very strong one although there is no indication of trichocyst structure. Their clustering about the tentaculoids may indicate that these organs are concerned with the capture and paralysis of small flagellates and other organisms used as food, or that they have a protective action as in *Paramecium* (Jennings, 1906). No indication of expelled threads have been noted in my preparations. No regularity of arrangement can be determined for them. There is no evidence that these structures are taste organs or tactile organs. If such were the case it might be expected that there would be demonstrable connections with the neuromotor system. Such is not the case. In *Paramecium*, the trichocysts are connected with, and are part of, the neuromotor system (Rees, 1922). In *Tintinnopsis*, on the other hand, there is no clear evidence that this is the case.

Fauré-Fremiet (1924) describes, in *Tintinnopsis ventricosa* and in *T. campanula*, a single series of stiff, elongate cirri surrounding the collar and extending from the column. These cirri are asserted to act as supporting rodlets which aid in keeping the animal extended when swimming. I have been unable to find any evidence of these cirri in either the living animal or in permanent preparations. They are certainly absent in *T. nucula* and it is suggested that they may constitute a specific distinction between these closely related forms. I have been unable to find them in *T. campanula* among any of my own preparations.

The *tentaculoids* make up a distinct ectoplasmic modification peculiar to the tintinnids. They were early noted by Haeckel (1873). They are especially prominent in the genus *Tintinnopsis* (Fauré-Fremiet, 1924; Jörgensen, 1924). Although I feel that these organs, from their position, shape, association with trichocysts, and connections with the gullet may be neuromotor structures, demonstrable connections with the neuromotor system have not been shown by any of my preparations. The small size of these organisms has so far prevented the determination of these exceedingly fine elements. It is highly probable that they may be connected with a peripheral sensory network not unlike that of the lattice structure in *Euploides* (Yocum, 1918).

The *tentaculoids* (fig. B, *tent.*), when expanded, are ball-shaped or claviform structures which rest upon a short pedicel and are arranged between the adoral membranelles upon the peristome collar. There is one of these tentaculoids beside each membranelle (fig. C, *tent.*); they are thus the same in number as the membranelles. If examined in the living animal, it may easily be seen that the tentaculoids are retractile and may be freely withdrawn almost completely into the substance of the accessory combs themselves, so that they are lost to view. This observation I have confirmed upon fixed and stained animals (figs. B, C, D, E, *tent.*) in which the tentaculoids are found in all degrees of expansion and retraction.

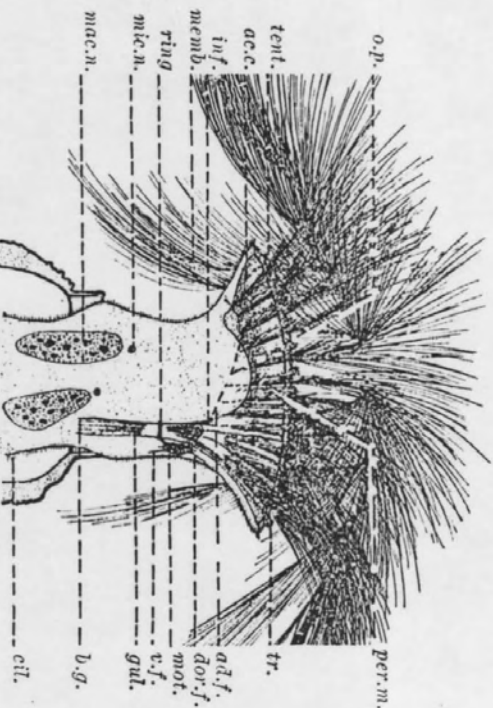


Fig. D. Semidiagrammatic figure of median sagittal section through the anterior portion of *Tintinnopsis*. Abbreviations: *acc.*, accessory comb; *ad.f.*, adoral fiber; *b.g.*, basal granules; *cil.*, somatic cilia; *dor.f.*, dorsal fiber; *gul.*, gullet; *inf.*, infundibulum; *mac.n.*, macronucleus; *memb.*, membranelle; *mic.n.*, micronucleus; *mot.*, neuromotorium; *o.p.*, oral plug; *per.m.*, peristome margin; *ring*, circumoesophageal ring; *tent.*, tentaculoid; *tr.*, trichocyst; *v.f.*, ventral fiber. X 850.

Extending from the proximal end or base of each tentaculoid is an *accessory comb* (fig. E, *acc.*). These accessory combs are narrow and conical, being widest at their distal part, and they extend to the floor of the peristome field in a slightly spiral course following the membranelles into the infundibulum. The accessory combs are hyaline, ectoplasmic structures and seem to bear no direct relation to the neuromotor system (figs. D, E, *acc.*). Trichocysts may be scattered over the surface. Under certain conditions the tentaculoids stain intensely with iron-haematoxylin and the accessory combs are also equally stained

(pl. 12, figs. 5, 9); both structures then appear homogeneous and dense. At other times they seem to take less stain and are clear and without structure (pl. 12, figs. 1, 7). The tentaculoids and accessory combs in the peristome field constitute the "*Begleitkämme*" and "*Deckplättchen*" described by Entz, Jr. (1909), or "intercalary cirri of the adoral ciliary plates" (Kofoid, 1905).

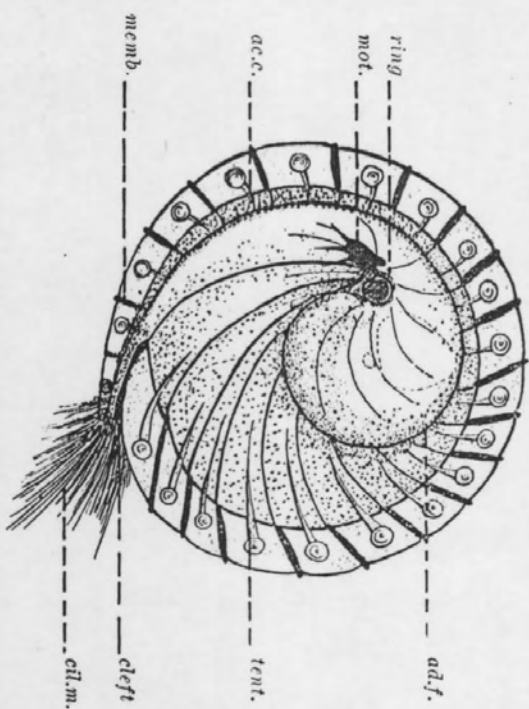


Fig. E. Semidiagrammatic figure of *Tintinnopsis* from above downward showing the peristome field. Abbreviations: acc., accessory comb; ad.f., adoral fiber; cl.m., ciliary membrane; cleft, cleft in peristome margin; memb., membrane; mot., motorium; ring, circumoesophageal ring; tent., tentaculoid. X 2150.

ENDOPLASMIC STRUCTURES

The endoplasm includes the great trophic mass of the cytosome together with its inclusions. It is surrounded by the thin and slightly differentiated ectoplasm. The endoplasm is highly fluid, especially during the active periods of feeding and division. The mobile oral plug is largely made up of endoplasm. Posteriorly the stalk may change rapidly from a stiff gel to a fluid sol and become detached and then reattached in a short time.

I have observed few clear indications of flowing currents within the endoplasm. Tracing the food vacuoles in fixed specimens has enabled me to determine that the course of these vacuoles is almost directly anterior-posterior in direction. Often they remain in the aboral part of the organism, which serves as a region for the storage

of undigested food (fig. B, *fd.b.*). No one who has watched the living animal can fail to be impressed with the great mobility of the organism and the fluidity of its contents.

The endoplasm of fixed and stained specimens is highly alveolar; more rarely is it granular. The collar and peristomal regions are made up of a denser, less alveolar cytoplasm while the column is alveolar to a greater extent. The living animal appears to possess clear and very finely alveolar protoplasm.

That the mitochondria or chondriosomes are cell organs is a well established fact (Cowdry, 1924). In *Tintinnidium inguiculatum*, Faure-Fremiet (1908) established the presence of phospholipin spherules about one micron in diameter which he claims are organized into a mitochondrial apparatus visible in the living animal. In living specimens of *Tintinnopsis nucula* treated by a special technique (Causey, 1925), I have been able to demonstrate rodlets staining deeply with Janus green B. No regularity of arrangement or number can be made out, nor are there any evidences of division at the time of binary fission. These results agree with those of Causey (1925) upon *Paramecium caudatum*. From their relationship to food vacuoles it seems clear that these rodlets are connected with metabolism. Spherical mitochondria associated with katabolism are rare or absent. Vacuoles constitute a prominent feature of the endoplasm. These are largely filled with the remnants of diatoms, dinoflagellates, or other articles of diet, and some of which lend their coloring matter to *Tintinnopsis*. These food vacuoles occupy, mainly, the posterior parts of the organism. Here they may await complete digestion and persist for a long time.

Within the endoplasm of some individuals (especially during the period of fission) may be found a loose massing or cloud of chromidial substance (Cowdry, 1924) near the region of the origin of the newly forming peristome. These chromidial granules stain deeply with iron-haematoxylin, borax-carmin, or with eosin. The question of their origin and fate will be dealt with in a later section.

NUCLEI

The nuclear system of *Tintinnopsis nucula* is made up of two macro-nuclei and two micronuclei, constant in form and size in the normal interkinetic condition. They form the most striking landmarks in a stained preparation, lying slightly below the middle of the upper half

of the organism (fig. C, *mac.n.*). In the living animal they are scarcely visible but may be brought out with dyes, especially aceto-carmin or methyl-green. It is evident from fixed and stained material (especially at the time of mitosis and binary fission) that the nuclei, due to active changes in the endoplasm, may shift their position within the animal. They usually retain their position in the middle region at a point near the outer wall of the column near the ventral side (pl. 12, fig. 5). This condition is especially notable at the time of binary fission (pl. 14, fig. 16). Each micronucleus is associated with one macronucleus.

The two *macronuclei* lie separated and equidistant from the right and left sides of the animal, one somewhat below the other (fig. B). They are entirely distinct and without any indications of commissures or connections of any kind. In no wise can they be said to be associated segments of a single nucleus nor are they comparable to the nuclear condition of *Stylonychia* (Lang, 1901). They form two distinct somatic, cell nuclei.

In form and size the two macronuclei are alike. They are usually bean- or kidney-shaped (pl. 12, fig. 9) or more rarely assume an ovoid, round, or even irregular form (pl. 12, fig. 3), possibly due to disturbing features in reorganization. In these latter cases there is frequently a vacuole surrounding the nucleus with its wall at some distance. Each nucleus is rarely more than twenty microns in length and about eight microns in diameter, with slight variations in proportion.

The *finer structure* of the macronuclei does not seem to have been particularly determined in *Tintinnopsis nuda*. Entz, Sr. (1884), determined that in a dying tintinnid the macronucleus, upon disintegration, discharged granules of various sizes. Entz, Jr. (1909, pl. 20, fig. 5), figures a macronucleus containing granules differing in size, some with vacuoles around them. To these vacuoles I shall refer later in my consideration of nuclear division and parasites.

Each macronucleus has a distinctly staining, rather heavy nuclear wall (pl. 15, fig. 22) toward the ends of which the chromatin granules which compose the mass of the nucleus are less evident (fig. C, *mac.n.*). In optical section this area is clear. In a favorably stained nucleus the chromatin substance is found to be aggregated into dense, black masses or granules of different sizes. Some of these are about one or two microns, or even three microns, in their greatest diameter. Other granules are scattered between these greater masses which stain

intensely with borax-carmin. No regularity of arrangement of the larger granules through the nucleus can be made out. Usually there are about twenty of these larger masses, although the number may be less. I have been unable to determine the cytological significance of these granules. They may represent the so-called macrosomes and microsomes (Anigstein, 1913; Bishop, 1923). As in *Balanitium* the larger granules may be trophic chromosomes (Hegner and Holmes, 1923). In a deeply stained nucleus the smaller masses as well as the larger are stained equally with iron-haematoxylin. The artificial division of chromatin granules into the so-called macrosomes and microsomes is probably a matter of physical chemistry and not of a fundamental division of chromatin into two contrasting nuclear substances. Wilson (1925) has used the term *microsome* in an entirely different sense to refer to the smaller cytoplasmic granules, hence the term should not be applied to nuclear granules. The macronucleus stains a uniform deep orange with Mallory's connective tissue stain.

The two *micronuclei* (fig. F, *mic.n.*) are alike in form and structure. A description of one will fit the other. Each is exceedingly small. In the resting condition or interkinetic period, the micronucleus may be about three microns in diameter. It is ovoid or globular. It may rest in a dorsolateral position, toward the upper or oral end of the animal, in a depression in the macronucleus. More often the micronucleus is separated at some distance from the macronucleus, but it may come to lie at almost any position within the organism not more than ten microns away from the macronucleus. The interkinetic micronucleus is spherical or subspherical with the flattened side at right angles to the longitudinal axis of the macronucleus. It is surrounded by a nuclear wall. Within the micronucleus there are numerous small granules. Usually the micronucleus is only slightly stained so that no structure can be determined within it. Often it appears as a single large homogeneous mass (fig. C, *mic.n.*). Each micronucleus always retains its identity and generally maintains itself in rather intimate association with one of the macronuclei.

NEUROMOTOR APPARATUS

The term *neuromotor apparatus* denotes a system of integrated fibers associated with the motor organs and connected with a central, massive body or motorium. Because this system of fibers connects with the motor organs and is integrated to the motorium, it is credited

with the function of coordinated conductivity of neuroid excitomotor impulses (Sharp, 1914; Kofoid, 1916; Taylor, 1920; Herrick, 1924).

Since the neuromotor system of *Tintinnopsis nucula* is intimately associated with feeding, locomotor, and other reactions of the organism, I will treat in this section not only the fibers and motorium but will also include the ciliary membrane, somatic cilia, and membranelles as part of that system. The myonemes, tentaculoids, and trichocysts have already been dealt with above, and while they constitute motor or effector organs, the lack of clearly determined fibrillar connections of these organs and the others treated in this section has led me to consider them separately. It must be understood that by so doing I do not consider the myonemes, tentaculoids, and trichocysts necessarily independent effectors. A more refined technique used upon these small ciliates may reveal evidences of intimate connections between all these organ systems.

To Entz, Jr. (1909), credit must be given for having recognized and described fibrillar elements in the collar which descend into the region of the gullet. He failed to comprehend their significance and did not connect them in any way with a motorium. He concluded that the motor functions of contraction reside in the myonemes (myophanes), or within the plasma itself.

The neuromotor system of *Tintinnopsis* is composed of seven distinct parts. These are (1) the membranelles, (2) the ciliary membrane, (3) oral membranelles, (4) the somatic ciliation, (5) the fibrillar connecting system, (6) the circumoesophageal ring, and (7) the central neuromotorium. These parts are intimately connected in a unified system. I shall treat them in order.

The adoral membranelles are twenty-two in number. No differences in number have been observed. Each is about twenty-five microns in length, measuring from the extreme distal tip to the margin of the thickened collar. They form a leiotropic, spiral crown about the collar, with which they are connected, and pass into the peristome where at the base they are united to the adoral fiber from the motorium. Beside each membranelle is a tentaculoid. Three of the adoral membranelles descend into the infundibulum where they are gradually modified in structure to become the oral membranelles and plunge into the gullet.

The adoral membranelles are blade-like (fig. B, *membr.*). They compare closely with the membranelles of *Diplodinium*, but are flat, blade-like ciliary brushes. The outermost cilia of each membranelle

are especially thickened and rather rigid, forming primary cilia. These cilia afford a rigid support against which the remainder of the components may beat since they are held out rather stiffly at right angles from the body. Their distal ends are held less rigidly and beat more freely. At the base of these membranelles are three basal granules which are about two microns in diameter. They are connected by fibrils in such a manner that each one forms the apex of an equilateral triangle with the base uppermost. Through the base passes the adoral motor fiber.

At the base of the flat, blade-like membranelles the innermost cilia are frequently distinctly separated, forming a row which has been taken to be a distinct ciliary line (Entz, Sr., 1884). In prepared specimens or in diving animals, this appearance is common. If a fresh specimen is examined, it is found that this row is actually only a part of the adoral series of the innermost row of the cilia which make up the adoral membranelle. This may be confirmed upon prepared material (pl. 12, fig. 9).

The cilia which compose the adoral membranelles are somewhat loosely held together by a thin, delicate coating which is dissolved away by reagents or at the disintegration of the living animal, thus allowing the component cilia to become separated.

The adoral membranelles do not seem to possess the long ciliary rootlets described by Sharp (1914) in *Diplodinium*.

The ciliary membrane descends on the ventral surface of the column (fig. B, *cil.m.*) from the distal end of the adoral zone. This membrane was first noted by Faure-Fremiet (1924) and described by him as a "frange bordante." The author failed to find any connection between this membrane and the adoral membranelles.

Before proceeding with the description of this organ, I will call attention to the fact that this membrane is not a true undulating membrane (Mlnechin, 1922). The term undulating membrane is best reserved for the structure found in flagellates only, particularly the trypanosomes and intestinal *Polymastigina*. In the flagellates of these types, the undulating membrane is a projecting, folded structure built of pellicle or cuticle which carries with it an internal marginal fiber connecting with the blepharoplast, and usually with a parabasal body. In the ciliates, on the other hand, the organs described as undulating membranes are never of this structure (Chambers and Dawson, 1924); instead they are made up of fused rows of cilia, each cilium arising from a basal granule. It seems best to differentiate these

analogous structures by a term which will not convey the idea of homology. Hence I propose the name "ciliary membrane" to describe, and at the same time convey the idea of possible functional identity for, these structures in ciliates.

The ciliary membrane of *Tintinnopsis* is a structure which finds no parallel in other ciliates. It is a system of elongate, highly flexible cilia, arising from rather large, globular, basal bodies (fig. G, *cil.m.*). The cilia which arise from these basal granules extend in a single line down the ventral surface of the organism rather more than half the length of a fully extended animal (pl. 12, fig. 5). They form a continuation of the adoral membranelle system and arise by a metamorphosis of the cilia composing that series. There is a gradual change in their form, as they pass over the peristome collar cleft, comparable to the changes in the adoral and oral membranes in *Diplodinium* (Sharp, 1914) but opposite in its direction. The longest cilia which compose the ciliary membrane are those nearest the adoral membranelle series from which they can be distinguished because they are single cilia only. They are about the same length as the adoral series at their adoral origin. As the membrane proceeds down the length of the body the cilia grow progressively shorter until they reach a point where they are indistinguishable from the body cilia in length.

This ciliary membrane functions in house-building and repair. It is the *house-builder* and aids in the removal of faecal wastes by wafting detritus outward and away from the lorica. The possibility that the ciliary membrane may store up detritus from the outside by bringing materials into the lorica is expressed in another section. The powerful current set up by the membrane sweeps these masses from their storage place within the lorica and carries them forward until they are out of the collar. At this point they are picked up, partly by the membrane and partly by the active adoral membranelles, and plastered over the lorica (pl. 15, figs. 31, 38). This ciliary membrane is connected through its basal granules by fine fibrils to the adoral fiber from the motorium. *The importance of this matter is that lorica-building, which was suggested especially by Kofoed (1905) to take place through the activities of the animal, is actually a function of the neuromotor system, acting particularly through the ciliary membrane and membranelles.* The lorica is then, in part at least, a construction; it is a result of coordinated activities, dependent for its being upon the resultant activities of a central, dominating neuromotor system (pl. 15, figs. 31, 38). I shall return to this matter later.

The oral membranelles (fig. D, *membr.*) are three in number. They arise by a sudden differentiation and change from the blade-like adoral membranelles to flat plates composed of fine cilia which continue their course in a slight spiral down the length of the gullet. I can add very little to the excellent descriptions of these organs given by Johnson (1893) in *Stentor*, and McDonald (1922) in *Balanidium*, with which they compare closely. Each membranelle ends at the base in the gullet in a distinct globular basal body (fig. D, *b.g.*).

The somatic ciliation of *Tintinnopsis* is confined to the column. The cilia which make up this series are short, less than two microns in length, and are very difficult to see in the living animal. They arise in longitudinal rows in the hyaline bands which follow the myoneme system closely (fig. C, *cil.*). Each cilium arises from a single basal granule which is located in the alveolar layer just below the myonemes.

Toward the posterior region the cilia and basal granules cannot be traced (fig. B). Anteriorly, the cilia are slightly longer than those of the middle region. The somatic cilia on the left side of the ventral surface form a special field in the region of the ciliary membrane and are distinguishable by their length only. They are not connected to that organ and constitute a separate, although functionally related, ciliary field which may be called the *lateral ciliary field* (fig. B, *l.c.f.*), described by Faure-Fremiet (1924). These cilia function as subsidiary organs to the ciliary membrane.

I have been unable to relate the somatic cilia either directly or indirectly to the motorium. They follow the myonemes closely and are parallel to them. Whether or not they form connections anteriorly with the adoral neuromotor fiber I am not certain.

The fibrillar system is composed chiefly of the adoral, dorsal, and ventral fibers, and the circumoesophageal ring. The adoral fiber is a large granular fiber extending from the motorium around the peristome at the inner base of the collar (fig. D, *ad.f.*). It connects with the adoral membranelles, the oral membranelles, and the ciliary membrane. It is the principal fiber trunk of the neuromotor system. That the myonemes and somatic cilia connect with it is not clear from any of my preparations. The presence of twenty-two ciliary lines and twenty-two membranelles is suggestive of such connection. The small dimensions of *Tintinnopsis* prevent the determination of these connections. At the point of junction between the adoral fiber and each membranelle there are three basal bodies, described above in

connection with the adoral membranelles. There is considerable evidence that the adoral fiber is not homogeneous in structure; its granular structure and its probable differential activity may be taken as evidences of this heterogeneity. Besides the adoral fiber, there are two *dorsal fibers* (fig. D, *dor.f.*) extending in the ectoplasm adorally. They end freely and fade out as they progress. A *ventral fiber* (fig. B, D, *v.f.*) extends downward from the motorium ending freely in the superficial ectoplasm. It may be homologous with the fibers passing toward the *microneurons* in *Diplodinium* (Sharp, 1914).

The most interesting of the fibers is the *circumoesophageal fiber* or *ring* (figs. B, D, *ring.*). It connects with the motorium by a fiber. The circumoesophageal ring surrounds the gullet completely, exactly as analogous rings extend about the gullet in lower worms, and compares with the same structure in *Diplodinium* (Sharp, 1914). Short fibers from the circumoesophageal ring project below, surrounding the gullet.

The *neuromotorium* (fig. B, *mot.*) is the central, massive body from which the fiber system described in the previous paragraph arises. It is located in the ectoplasm of the ventral wall of the column (fig. D, *mot.*) at a point rather above the middle of the length of the gullet. In form it is cigar-shaped and dorsoventrally flattened, the ventral fiber springing from its lower end and the two dorsal fibers from the upper end. The adoral fiber arises below the dorsal fibers.

The motorium, circumoesophageal ring, and adoral fiber are differentiated with iron-haematoxylin in favorable whole-mounts. With Mallory's connective tissue stain the motorium and ring are stained red. The ring has not been recorded or figured by any previous investigator of the *Tintinnina*.

The evidence that the system of fibers and the central body really constitute a neuromotor system rests upon the staining capacity, integration, and finally and most conclusively, upon experimental grounds that do not need to be reviewed again here (Sharp, 1914; Yocum, 1918; Taylor, 1920; Rees, 1922; McDonald, 1922). To the evidences already recited by these investigators, I can add little. The ciliary membrane, which is attached to the upper part of the length of the ventral body wall, is an organ the activity of which is displayed in the building and repair of the lorica. This organ is a part of the neuromotor apparatus as I have shown. Its actions are only explainable upon the assumption of intimate, functional, integrated union with the central apparatus. This connection I take to mean that the

neuromotor system acts toward the accomplishment of specific ends. Feeding, locomotor, and building operations are carried out by neuromotor organs.

LIVING ANIMAL

Observations upon the living animal are difficult. Its small size, opaque house, and dense agglomeration of foreign bodies prevent the larger part of the organism from being clearly seen. At the most only the upper half or less, about the adoral region, is all that can be made out. The rapid movements of the animals are a distinct hindrance to observation. It is very difficult to keep them alive for any length of time in the laboratory. Most of my observations were carried out at La Jolla, during the summer of 1925, where conditions for the capture and observation of living material were more satisfactory than in Berkeley.

Locomotion is effected either forward or backward something after the fashion of a cephalopod mollusk. The usual direction of progression is backward, the fundus of the lorica leading and the membranelles acting as propellers. Locomotion is usually jerky or darting; sudden starts alternating with short periods of rest. If observed under a binocular dissecting microscope, in a shallow dish of water, the animals will be seen to swim about in an agitated manner, striking first the surface film and then, with a sudden motor-reaction, reversing the direction of locomotion and descending rapidly to the bottom. This reaction is often carried out regularly and rapidly until the disintegration of the animal. If animals are resting quietly upon a frond of alga or upon the bottom of the dish, they may be observed to travel with the long axis parallel with the direction of locomotion, rotating in a manner comparable to the normal locomotion of other heterotrichous ciliates (as *Stentor*), the adoral membranelles acting by a lash-like stroke. In this case the oral end leads the body. A zigzagging course is common in a free-swimming animal.

The animal may freely rotate about within the lorica. In a resting animal, the anterior end is pushed in and out of the lorica frequently, the whole membranelle system being easily and completely withdrawn. The living animal may break away completely from the lorica and swim free. Whether such animals are ever able to form a new shell or to enter a deserted one, has not been determined. The number of empty loricas found in any plankton catch is usually greater than the number containing active animals. This condition

probably arises from the readiness of the animal to react to an unfavorable condition by freeing itself from its shell. These empty loricas may represent the reaction of the animal to the crowded and unusual conditions of the plankton-bucket, teeming with living matter. The rigid loricas persist for a long time.

Behind a free-swimming animal a trail of slime is usually evident. Food may become entangled in it and may be ingested when the animal rests upon a substratum.

There is a marked tendency toward gregariousness in *Tintinnopsis*. If a number are present in a dish they will be seen to gather together in a short time. In collecting, I have frequently noted that they occur almost in swarms, each individual dancing in the swarm as midges dance about flowers on a warm summer day. This phenomenon is possibly a chemotropism. It appears to be independent of light.

I have been unable to follow the behavior of living, dividing animals. In numerous instances the new adoral zone could be determined and its actions observed.

The somatic cilia beat toward the oral end of the animal in a uniform manner. These short cilia are difficult to see, but it may be determined, by a careful examination of animals which have been slowed down, that they carry detritus and faecal pellets out of the lorica and discharge them through the collar. These cilia may reverse their motions and beat backward, that is, from the oral end toward the stalk. This may be seen by watching bits of carmine passing along the column. This unusual manner of carrying in bits of matter may account, in part, for the storage of detritus in the fundus of the lorica.

The work of the ciliary membrane is important. These long cilia are united in the living animal to form a sheet in such a fashion that the membrane acts as a unit. Its motions are undulating and seem usually to have their origin as a tremor posteriorly which slowly, and then more rapidly, travels toward the peristome collar. If a bit of carmine becomes attached to it, it may be seen to be picked up and passed from cilium to cilium inward toward the peristome, where the inner row of cilia may pick it up and pass it toward the infundibulum. Its further fate cannot be traced. In locomotion, the ciliary membrane may be either held out rather rigidly, acting something like a fin disposed ventrally, or it may be in active motion and serve as an accessory locomotor organ. With its aid the animal may not only rotate itself, but the shell also. The usual function of the ciliary membrane is, however, neither locomotor nor food-gathering. It is the

chief neuromotor organ, aside from the membranelles of the adoral zone, connected with the repair and building of the lorica. Bits of faeces from the fundus of the lorica are picked up and passed outward, or secreted material and parts of diatoms are brought into the lorica through its agency. Sometimes one finds the remains of diatoms or dinoflagellates (pl. 15, fig. 31) on the lorica, probably secured in this manner. Bits of matter gathered by the ciliary membrane are held within the lorica.

The active twisting and turning of the animal within the lorica probably indicates that it may free itself from its posterior attachment and become reattached (Fauré-Fremiet, 1908). A local, specialized, mucus-secreting area occupies the extreme tip of the attachment and serves to aid in holding the organism to the lorica. This area is sometimes darkly stained.

Turning to the peristome region, a striking activity seen there in the living organism is that of the oral plug. In an animal which is dying, this organ pumps rapidly up and down with a piston action. In the normal, free-swimming animal, on the contrary, the plug is rigidly held expanded. Its normal function is difficult to determine. It may serve as a closing organ as the oral plug does in *Balanidium* (McDonald, 1922), or it may aid in feeding; by pushing action it may serve to crowd large dinoflagellates and diatoms down the rather narrow, but widely extensible, gullet.

The tentaculoids are extensible, claviform, or stalked, globular organs surrounding the peristome collar. They may be seen to expand and contract readily. Trichocysts are present on their globular, distal surfaces. It is very difficult to witness the action of the accessory combs in a living animal. They may be accessory feeding organs. The contractile tentaculoids may be freely withdrawn into the substance of the combs since these two organs are structurally related (fig. D, *acc., tent.*).

The adoral ciliary crown of membranelles waves back and forth with a slow motion. Waves of contraction are initiated at the base and pass outward along the outer edges, the inner cilia moving secondarily. At a point about two-thirds above their origin on the collar, the inner cilia seem to break free from the sheathing and beat freely. They then act semi-independently. The membranelles may act together in a coordinated manner or they may act independently of each other, especially when in contact with a substratum or in feeding. The motion of the free membranelle is so directed that the

effective stroke is from inward outward. When the direction of locomotion is reversed, the animal may reverse the direction of the effective stroke. Usually the avoiding of an obstacle in the pathway of a moving *Tintinnopsis* is accomplished by a reversal of the direction of locomotion and by a rotation of the body; the animal bores its way through the medium.

In the oral membranelles, undulating waves of beating pass from a point of origin toward the mouth in such a manner that the effective stroke is directed inward. The oral membranelles may beat constantly, even when the animal is contracted within the lorica. Their power of organizing small particles into food boluses is obvious.

DIVISION

The division of *Tintinnopsis nuda* has not been the subject of previous special study. Entz, Jr. (1909) and Schweyer (1909) have discussed the results of their observations upon various species of *Tintinnopsis*. My account differs so radically from that of these writers that I shall treat it in special detail.

Division stages are obtainable in animals collected at almost any time during the day. Most division phases seem, however, to occur in the early hours of the morning. In plankton catches made during the early morning, from two to nine o'clock, almost all phases are present. These results are comparable to those obtained in the dinoflagellates (Hall, 1925 a, b). They demonstrate that the rhythm of fission is part of the cyclic ebb and flow in the diurnal pulse of living matter; this rhythm is not confined to this group of protozoan organisms.

CYTOSOME

The earliest steps in preparation for division are seen in the cytosome rather than in the nuclei. The membranelles are formed and the new cytosome is almost completely developed before the nuclei have undergone visible changes or have shifted their position. During the whole period of binary fission both nuclei and cytoplasm act together toward the accomplishment of the common end.

It is very noticeable that the early stages of fission are abundant, and frequently in a plankton catch almost every animal is in some early phase of division. The late stages are few in all cases. It is probable that these early stages require a long period for their development and the final stages are rapidly completed.

The beginnings of division in the cytosome are to be looked for on the ventral side in a position midway between the area marked by the macronuclei. The earliest stage that I have been able to see is indicated by a line in the pellicle (pl. 13, fig. 13). This line, which is short and just below the end of the ciliary membrane, is distinctly marked as a transverse clear area below which a row of basal granules soon appears. The line crosses the level of the midpoint of the macronuclei. Slightly later the right end of this line starts to curl inward. Its left end starts to grow upward. As the line curls upward its spiral course becomes rather tightly wound (pl. 13, figs. 14, 15). When this condition is reached short cilia arise from the basal granules. The cilia soon grow long and assume the appearance of short adult membranelles. Shortly before they have taken on this structure, the cytoplasm surrounding the newly differentiating cytosome becomes massed about it so that the posterior daughter forms a lateral bud on the cytosome (pl. 14, fig. 19). This bud grows rapidly until it takes over the whole mass of the posterior protoplasm of the organism (pl. 15, figs. 21, 25).

Entz, Jr. (1909), claims that the basal bodies of the adoral membranelles arise in connection with either the macronucleus or the micronucleus. They are said to arise as granules emitted from the nucleus, passing through the cytoplasm in a definite manner and finally becoming deposited about the newly forming peristome. Hartmann and Schilling (1917), apparently without actual observations, criticized this view, asserting that it remains an unconfirmed and unique case. My observations both upon whole mounts and sections sustain this criticism. In no case have I been able to trace granules from any nucleus to the outer wall in the asserted definite course; the granules described by Entz, Jr. (1909), are possibly parasites. Maier (1903) considers that the basal granules arise as local cytoplasmic bodies at the surface of the pellicle. No evidence regarding this view can be demonstrated at this time in my materials. It is probable that the basal bodies arise by outgrowth in connection with the newly forming neuromotor apparatus, although conclusive evidence is yet lacking.

When the posterior zooid has reached the stage described in the preceding paragraph, the anterior or apical zooid, together with all its parts which occupy the upper half, elongates and rapidly assumes the appearance of a typical *Tintinnopsis*. This elongation continues rapidly until the animal is composed of two zooids (pl. 15, fig. 25)

each functioning separately, but connected by a thin, short strand of protoplasm. I cannot come to any satisfactory conclusion regarding the nature of the fission line. It is produced as a white line not unlike that in *Stentor* (Johnson, 1893). The forces which operate to effect the separation of the two zooids are probably internal and involve changes in sol-gel reversible protoplasm. I am confident that the myonemes are not cut at first by the fission line but simply become bent sharply inward. Until a very late stage in division they are accurately matched ~~on~~ either side of the fission line, although the stripes are somewhat narrower at the posterior end of the distal zooid. Later changes finally cut the myonemes apart as the fission line widens and deepens. When finally freed the two daughters are each provided with a lorica.

The fission stages described above resemble closely those of *Stentor* (Johnson, 1893). They represent a mode of division probably common in the strongly polarized heterotrichous ciliates.

The external changes in *Tintinnopsis* described above are accompanied by internal changes and reorganization. Profound changes in viscosity are evident. The cytoplasm becomes extremely fluid. This is evinced by the swinging of the macronuclei into a characteristic position (pls. 13, 14). These currents may be compared with the cytoplasmic streaming of cleaving metazoan eggs (Chambers, 1924). The modifications in viscosity, depending upon a probable solution of the cytoplasm, appear to be regular. It is probable that they are evidences of a regular physiological and general reorganization of the whole organism at this critical period of binary fission.

Rapid changes in growth and in lengthening of the anterior zooid result in its physiological isolation from the newly formed one. This isolation probably results in the independent further differentiation of the new cytosome. Physiological isolation may stimulate the rise of parts in certain cases, especially in the lower worms and in some protozoans, notably *Stentor* (Child, 1915).

I have been unable to trace any dedifferentiation and redifferentiation in the ciliary zones of the anterior daughter zooid comparable to that in the *Hypotricha* and *Holotricha* (Yocum, 1918; Rees, 1922) during binary fission. The fission of *Tintinnopsis* resembles that of *Stentor* (Johnson, 1893).

Agamic reproduction or binary fission in the ciliates is a process closely resembling the fission of metazoans such as *Planaria* or *Vais*, the strobilization of Scyphozoa, or the formation of gemmules in

sponges and Bryozoa. The binary fission of *Tintinnopsis* is to be regarded as allied to this type of reproduction of functional individuals, each with a similar genetic pattern (Kofoid, 1923).

NUCLEI

Nuclear and cytoplasmic changes are nearly simultaneous. The micronuclei generally complete their division before the macronuclei have formed a reconstruction zone. The micronuclei behave alike and usually begin their divisions about the same time although they are neither synchronized nor mutually dependent throughout mitosis. A description of the behavior of one will apply equally to the other.

The interkinetic micronucleus is a spherical or subspherical, granular, and rather faintly staining body, already described. During the first part of the prophase it moves away from the interior polar macronuclear depression in which it is frequently lodged. It moves toward the level of the middle of the macronucleus and while so doing swells and increases greatly in size, from two or three microns to nearly five microns in diameter (pl. 15, fig. 33). It becomes more granular. The granules which compose this mass become aggregated into the central portion of the micronucleus, leaving the margins clear (pl. 15, fig. 34). It is very difficult to see in this small nucleus any organization into a spireme. Shortly after the granules of the micronucleus have become aggregated into this central mass the nuclear wall shows signs of a slight elongation (pl. 15, fig. 35). The polar caps form thickened crescents about the two ends of the forming spindle and the chromatin granules become distributed along the spindle fibers. These granules may now be called chromosomes (pl. 15, fig. 36). The course of events at this point is not clear but as far as can be made out from preparations it seems that these chromosome threads become massed into a metaphase clumping in the center of the spindle, leaving the area toward the ends of the polar caps clear and free (pl. 15, fig. 37). In the anaphase the chromosomes become separated into two groups, one at either end of the now elongate nucleus, massing densely about the polar cap (pl. 15, fig. 29). In the final phase the two halves of the nucleus pinch away from each other until they are connected only by a delicate strand of the nuclear membrane (pl. 15, fig. 27) which later breaks and frees the two daughters. The internal change which accompanies these telophase changes is a progressive break-up of the chromosomes into granules which are at

first crowded about the pole of the nucleus and which later form a loose cloud within the nucleus (pl. 15, fig. 27).

At no time during the course of this mitotic process is it possible to accurately estimate the number of chromosomes. Their number is certainly great, possibly more than a hundred, and their small size, combined with irregularity of linear structure, prevents an accurate counting. I am unable to determine the nature of the division of the chromosomes.

As is the case with the micronuclei, the macronuclei divide nearly simultaneously. A description of events in one macronucleus will apply also to the other. The structure of the resting nucleus has been already given in a former section and need not be repeated.

The first indication of division in the macronucleus is the formation of a clear zone transversely across the center (pl. 15, fig. 23). Usually this clear zone is exactly across the center but sometimes it separates the two parts of the nucleus into slightly unequal portions. No changes in shape are at first associated with these changes. When the clear zone is complete and there is no indication of cloudiness within it, the nuclear wall constricts in such a manner that the nucleus assumes a dumbbell shape. This constriction narrows and pinches in until the nucleus becomes finally divided in two (pl. 15, fig. 24).

The clear zone or reconstruction band is without structural differentiation. It is the same as the "*Kernspalt*" of other ciliates. It is formed in *Tintinnopsis* by a migration of the chromatin substance away from this region of the macronucleus. As division proceeds, longitudinal strands appear on either side (pl. 15, fig. 24). These may be somewhat similar to the strand-like organization of the chromatin in the macronucleus of a dividing *Cycloposthium* (Dogiel, 1925).

At the beginning of division, the larger macronuclei within the macronucleus are scattered irregularly throughout its substance. As the process proceeds they become aggregated at either end. No evidence of their division can be made out in my material although it appears that their number is nearly constant (Hegner and Holmes, 1923). They may represent trophic chromosomes. The smaller granules are aggregated simply at either end of the clearing nucleus. The nuclear wall does not break down at any time during the normal process of division. Vacuoles surrounding larger granules are sometimes evident. The newly formed macronucleus of *Tintinnopsis* is

about one-half the size of the one from which it came. It grows rapidly and in the period just before the separation of the daughter animals has assumed normal size.

A regular and constant series of changes in position is assumed by the macronuclei in division. These are probably conditioned by the streaming and changes in viscosity of the cytoplasm during this exceedingly plastic period. The two macronuclei occupy the middle region of the animal and are about equidistant during the trophic period. By the time the reconstruction band appears they have migrated in such a manner that their inner, concave sides form an almost complete circle about the ventral surface which is to be the site of the newly forming, daughter cytosome (pl. 12, figs. 7, 9). They approach gradually and by the time the daughter cytosome is almost fully formed they are in close approximation. They then divide; the adoral halves of the macronuclei are retained by the anterior animal and the posterior halves move into the cytoplasm surrounding the (posterior) daughter cytosome (pl. 15, fig. 21).

During the whole period of nuclear changes described above, there are no indications of nuclear conjugation (Laackmann, 1906) either of the micronuclei or of the macronuclei. The process of nuclear division in *Tintinnopsis* nucula is comparable to the nuclear behavior of other ciliates.

LORICA

The fate of the lorica during binary fission is interesting in that the parent lorica is inherited. In a dividing pair of zooids, the anterior (oral) animal first secretes a delicate, transparent, irregular, foam-like substance from its protoplasm, which is at first slightly viscous and easily picks up the stored faecal reserves from within the fundus of the parent lorica. This mass is then passed anteriorly by the action of the ciliary membrane of the posterior zooid (pl. 15, figs. 28, 31). This foreign matter, excreta and foamy secretion, is firmly cemented together and forms the new shell. The matrix rapidly and progressively hardens into the characteristic architecture of the lorica. Thus in large part the workmanship of the construction, especially of the posterior end, falls upon the posterior member which itself acquires the old lorica at the final separation of the two zooids. From time to time the animal within a lorica may add to its own lorica by its own neuromotor activities.

The sources of the matrix are to be found in the deposition of materials from the animal. This matter may be (1) a general secretion from the ectoplasm of the animal of the colloidal substance which hardens into the prismatic elements. For this there is no evidence in *Tintinnopsis nucula*. However, in *Tintinnidium primum* [nomen nudum] Busch (1925) reports this to be the case, the secretion arising from the peripheral vacuoles over the general surface. (2) Material poured out from the mouth or from the anus and stored as faecal pellets and as fecal matter within the lorica, often in sufficient amount to account for the material forming the shell, or (3) a local secretion from the labile and active lateral lobe. The evidences for the second source are the extraordinary metabolic activity of the animal, the large vacuoles which crowd the cytosome, and the well-developed cytophyge. The evidence which favors the third view is to be found in the staining of the lateral organ. It is sometimes deeply stained with haematoxylin, especially in a dividing pair, and this dark siderophile condition (fig. B, lobe) may be taken as evidence of secretory activity. The lateral lobe is in a position to shape up chiefly the smooth interior lamina of the lorica, and the ciliary membrane, the outer reticulated surface. Kofoed (1905) suggests: "The type of shell structure in *C. fasciata* suggests the slow rotation of the animal in a constant direction during the deposition of the shell-forming substance and the location and limitation of the region of its extrusion to a single place upon the animal."

Not only secretion but also activities are principal agents in the assemblage of the house in these forms. The substrate arising from the lateral lobe and the stored reserve are profoundly affected by stereotyped neuromotor behavior. While secretion determines the material of the lorical pattern, behavior moulds its characteristic architecture, unlike the shells formed in larger part by secretion in some rhizopods, or possibly wholly by secretion in the internal skeleton of radiolarians. The ciliary membrane, lateral ciliary field, adoral membranelles, and the lateral lobe act together in a coordinated manner in the building of the shell, using the faecal matter stored within the parent lorica together with a secreted substratum and adding to these extraneous matter gathered from outside by the animal. Thus the construction of the lorica becomes a matter of neuromotor activity, through the cooperation of several organs toward the accomplishment of a common end.

Kofoed (1905) stressed the relationship between the number of adoral membranelles and the serrate margin of the lorica in *Tintinnus serratus*. In describing *Tintinnopsis reflexa* he remarks: "The significance of this limitation in proportions lies, it seems, in the dependence of this projecting portion of the shell upon the length of the cilia and intercalary cirri of the adoral plates." Kofoed (1905) was unacquainted with the ciliary membrane, which appears in *Tintinnopsis nucula* to be one of the principal neuromotor agents acting in this function of house-building. Some of the tintinnids unprovided with a ciliary membrane, examples of which are *Tintinnus lusitanae*, *Cyrtarocylis serratus*, and *C. ehrenbergii*, do not agglutinate foreign bodies nor do they build them into their permanent structure.

The slight spiral structure of the collar of the lorica is evidently the morphological expression of the leiotropic disposition of the shell-forming organs. While *Tintinnopsis nucula* does not clearly show evidence of spiral structure in the body of the lorica, that tendency is found to be present or even pronounced in other tintinnids, as in *Cyrtarocylis fasciata* (Kofoed, 1905).

In binary fission the posterior daughter zooid acquires the old lorica. The older peristome and its organs, which belong to the anterior daughter zooid, have had secreted and built up for them a new lorica by the progressive and joint action of the parent and the offspring. This condition is different from that of certain dinoflagellates (Kofoed, 1908) in which part of the skeletal plates are passed to each daughter, and from the ciliate *Coleps* in which conditions comparable to those in dinoflagellates occur. In the case of *Tintinnopsis*, however, the shell is passed *in toto* to one daughter and a wholly new one formed for the freed daughter.

PARASITES AND DEGENERATION

Parasites have been known for some time to inhabit various tintinnids. Most of these are described as voluminous cytoplasmic inclusions which by their growth ultimately disrupt the organism (Laackmann, 1906; Duboseq and Collin, 1910). These are supposed to be parasitic dinoflagellates and are comparable with the various other parasitic members of that group described by Chatton (1906). So far as I have been able to determine there are no other parasites described from tintinnids.

I have encountered such voluminous cell inclusions in only two or three cases. In all these cases the inclusion occupied the posterior part of the organism.

In a great many instances, especially in the plankton collections during the late summer and early fall months, dividing *Tintinnopsis* revealed, when stained and mounted, abnormal nuclear conditions which so resembled the stages of nuclear conjugation described by Laackmann (1906) that I was led to make a special study of them. In some collections nearly every animal was in some stage or other of division associated with disintegrating nuclei (pl. 13, figs. 10, 12). These disintegrating nuclei were in all cases distinctly filled with the large granules. Many of these were surrounded by distinct vacuoles. That these vacuoles indicate solution of the nuclear contents is evident.

Thin sections, carefully differentiated after staining in Heidenhain's iron-haematoxylin, revealed the structure of the granules surrounded by a vacuole. In each there is a clear, non-staining, central area in the midst of which a single intensely staining granule is distinctly visible (pl. 13, fig. 11 a). In certain cases examined carefully, it was found that the central granule had divided into two parts which were connected by a thin, deeply stained, connecting fiber (pl. 13, fig. 11 b). In other cases, still more rare, the central granule was divided into four parts in a cross (pl. 13, fig. 11 c).

Numerous instances of the escape of these bodies into the cytoplasm have been observed (fig. F, *pa.*). The nuclear wall in these cases is partially disintegrated and broken down. The parasites are discharged freely into the cytoplasm, and are carried about in such a manner that they form a loose cloud-like mass about the region of the newly forming cytosome (pl. 13, fig. 12). There is considerable evidence from the examination of many of these parasitized *Tintinnopsis* that the parasitic infection, possibly accompanied by the extrusion of chromatin into the cytoplasm, so stimulates the animal that plasmatomy is initiated. In no case, among hundreds of individuals, was division ever carried out beyond the spiral inrolling of the ciliated daughter peristome. The animals which reach this stage are usually, but not always, incapable of further development and degenerate and disintegrate. The parasites increase in size in the cytoplasm after being freed from the nucleus. I cannot connect them with the voluminous inclusions or dinoflagellates described above.

The loose cloud-like mass distributed about the daughter peristome is the chromidial mass or cloud which I described in a previous section.

Its appearance is comparable to that figured by Entz, Jr. (1909, pl. 19, figs. 1-9, and pl. 18, figs. 1-2), for *Cyrtarocylis*, and asserted to be present in other *Tintinnina*. In some cases, although rarely, it is present in an animal the nuclei of which are hardly abnormal. In these cases, the parasites may have been carried over in the cytoplasm by a recently divided infected animal and await there opportunity to infect the nuclei of the animal. Such instances might be compared with an incubation period. In a few instances, carefully differentiated,

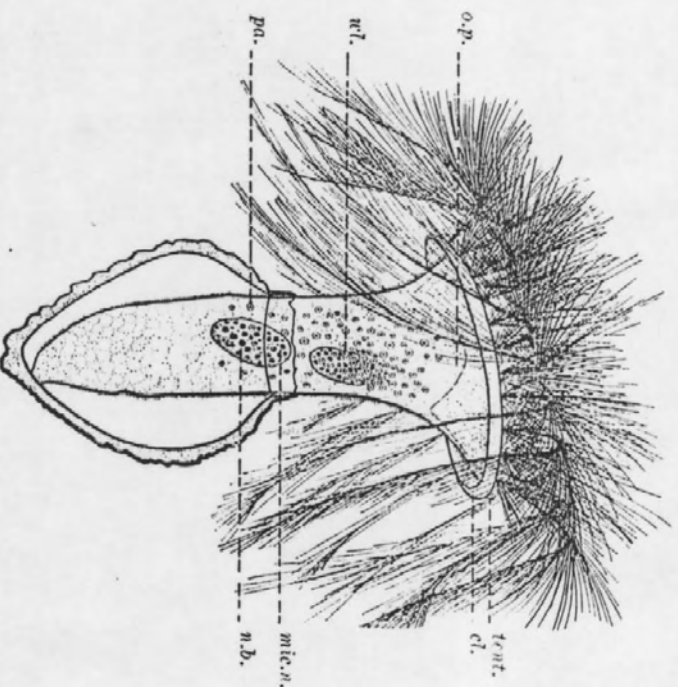


Fig. F. Semi-diagrammatic figure of *Tintinnopsis* showing cytoplasmic and nuclear phases of *Karyoclastis tintinnal* gen. nov., sp. nov., within the cytosome. Abbreviations: *cl.*, peristomal collar; *mic.n.*, micronucleus; *n.b.*, trophic chromosomes within the macronucleus; *o.p.*, oral plug; *pa.*, parasitic *Karyoclastis*; *tent.*, tentaculoids; *n.l.*, nuclear wall. X 850.

lightly stained whole mounts of some animals revealed the "nucleated" parasites exactly as they are found in sections (pl. 13, fig. 12). Usually they stain homogeneously and then their appearance, together with their regular distribution to the region of active cytoplasmic changes, has undoubtedly misled Laackmann (1906) and his follower (Merkle, 1909) to the erroneous conclusion that they were actually *chromidia* and essential to the normal reproduction of *Tintinnina*. I believe that this erroneous conclusion is a confusion of chromidia

with these parasitic organisms. That some chromidia, if properly and carefully stained, will reveal themselves as parasites in many Protozoa has been recently expressed by Kofoid (1921).

These parasitic bodies may possibly inhabit but one nucleus and cause its destruction only (fig. G) or they may attack both nuclei simultaneously (pl. 13, fig. 10).

These parasites are distinct in structure in many respects from *Nucleophaga* of fresh-water Protozoa (Dangeard, 1895) and from *Sphaeria* from the intestinal amoebas. For this reason I shall propose for their reception a new and provisional genus, *Karyoclastis*.

Karyoclastis gen. nov.

Intracellular parasite, primarily intranuclear but with a cytoplasmic phase. Ovoid, globular, or irregular in outline. Individuals separate. An outer mantle, possibly cytoplasm, staining gray in iron-haematoxylin, surrounds an inner clear area in the midst of which is a single black-staining granule. Undergoes binary and repeated fission by the division of the central grain. Division in nucleus of host causes a dissolution and vacuolization of nuclear substance so that the individuals are set free into the cytoplasm. Unknown relations and uncertain position.

Karyoclastis tintinni sp. nov.

Karyoclastis inhabiting various marine members of the Tintinnina. Having alternating cyclic phases within the nuclei and the cytoplasm. Spores set free by the disintegration of the host or carried along by binary fission from host to host, possibly stimulated by parasitism. Morphological characters identical with those of the genus. Host, *Tintinnopsis nucula*, from San Francisco Bay.

Among the *Tintinnopsis* collected are certain rare forms which may be degenerate forms (pl. 15, figs. 26, 30). The adoral zone is lost and the organism becomes contracted into the fundus of the lorica. In one or two cases (pl. 15, fig. 32) the animal was freed from the lorica. These forms may represent either of the following conditions, (1) degenerations due to parasitic infection, (2) normal phases in the life-cycle. The first seems probable since in these forms the nuclei are usually abnormal and do not present the condition of a normal process. Parasites are also usually present in them.

I have not seen any forms of *Tintinnopsis nucula* which might be designated as resting cysts (*Dauercysten*). In *Cyrtrocylis* such

cysts are reported (Busch, 1919). They are surrounded by a hard cyst wall and do not resemble the degenerate forms which I have described above.

Endogenous budding or sporulation does not occur in *Tintinnopsis* so far as I have been able to determine. Nearly all cases of reported endogenous budding (Walker, 1909) have proved to be either cannibalism (McDonald, 1922) or due to ingested foreign bodies. In a few cases I have seen within the cytoplasm small dinoflagellates which might have been mistaken for spores or internal buds. Wailes (1925, pl. 2, fig. 18) has reported spores in *Tintinnus subulatus* Ehr.

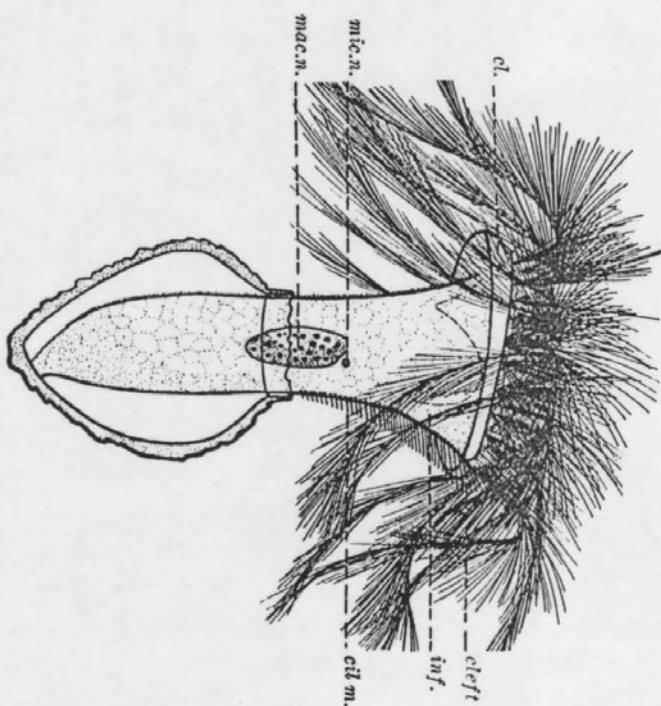


Fig. G. Semidiagrammatic sketch of *Tintinnopsis*. Form with a single macronucleus. Abbreviations: cleft, cleft in peristome; cl.m., ciliary membrane; cl., peristome collar; inf., infundibulum; mac.n., macronucleus; mic.n., micronucleus. X 850.

Forms of *Tintinnopsis campanula* with but a single round or ovoid macronucleus and a single micronucleus are described by Laackmann (1906). They were designated by him as "*Jungenformen*" in the belief that they represented the products of macrospores and microspores which had conjugated. The youngest of these were supposed to be devoid of the adoral membranelle zone.

I have occasionally seen forms of this nature (fig. 6, *mac.n.*) in *Tintinnopsis nucula*. They are not limited to the spring plankton but are scattered throughout the year. In all, there is no evidence of growth in the adoral zone. They are normal save in having a single macronucleus. In several cases there were a single macronucleus and two micronuclei. In some cases these forms may be the result of the parasitism described previously or in others they may represent ex-conjugants and the reorganization after the period of sexual reproduction. The lack of extensive evidence regarding the so-called conjugating macrospores and microspores and the hypothetical resting stage in the depths of the sea (Laackmann, 1906) make my interpretations of a parasitic origin of the irregularities more plausible. Parasites are frequent in the cytoplasm and nuclei of these forms. The irregularities probably represent the results of differential parasitism and partial recovery. Conjugation of individuals of the usual type is known to occur (Bresslau, 1906) in these forms and will account for the most of the biological phenomena which this hypothesis attempts to explain. No evidence regarding hypothetical macrospores and microspores has been noted in my preparations. Inclusions that might be so regarded have always proved upon careful treatment to be food bodies, usually ingested dinoflagellates.

DISCUSSION

No account has appeared describing a neuromotor system of any member of the genus *Tintinnopsis*, or indeed of any tintinnid. In this paper there are described for the first time, a complex neuromotor system, the labial lateral lobe, the cleft of the peristome collar relating to the spiral organization of the adoral zone, as well as other minor features. An extended account of the structure and physiology of the ciliary membrane is given and its relations to house-building shown. An account of binary fission is offered which places *Tintinnopsis*, and probably the whole group of tintinnids, within the bounds of what we know regarding other ciliates.

The only previous mention of the ciliary membrane is that of Faure-Fremiet (1924) who failed to follow the course of this organ into the peristome and was unable to describe its functions. The mobile lobe which plays an active part in the building of the lorica has not been previously noted in *Tintinnopsis nucula*. The ciliary membrane finds no exact parallel in other heterotrichous ciliates without a lorica.

Tintinnopsis nucula possesses a complex neuromotor apparatus. This organ system comprises every motile organ of the body. The coordinating center or neuromotorium constitutes the directing center. It is located in the ventral ectoplasm. In form it is spindle-shaped or cigar-shaped with a ventral fiber, two dorsal fibers possibly connecting with the striated surface of the peristome plug, and a powerful adoral fiber which connects with the basal granules of the adoral membranelles, oral membranelles, and the ciliary membrane. A special fiber connects the circumoesophageal ring. This neuromotor system compares favorably with similar systems in other ciliates. In many respects it is one of the most centralized and compact neuromotor systems yet described in any ciliate. In centralization it stands in close association with the neuromotor system of *Diplodinium*. Both *Diplodinium* and *Tintinnopsis* are alike in the possession of a central motorium connecting a circumoesophageal fiber and with adoral fibers connecting the adoral membranelle zones. The neuromotor system of *Paramecium* (Rees, 1922), *Balanitidium* (McDonald, 1922), and *Euplates* (Yocum, 1918) are comparable in that they consist of fibers which are distributed to the motor organs that have their origin in a common motorium-mass and, in some, the existence of a neuromotor fiber related to the adoral zone.

As far as the neuromotor system of the ciliates has been carefully examined, it is composed of a single central mass located near the gullet which sends out fibers to the motor and feeding organs integrating them structurally. Taylor's experimental studies upon *Euplates* (Taylor, 1920) have conclusively demonstrated the assumption of previous writers that this system of converging intracytoplasmic fibers really represents a system of organs integrative of the neuromotor activities of these ciliates. That the neuromotor system actually integrates the activities other than locomotor of ciliates was forecast in *Follucina* (Andrews, 1920) in the following words: "Were there a complex nervous system we might refer much of these activities of *house-building* (italics mine) to it." In *Tintinnopsis nucula* such a neuromotor system is actually described and its part as the active agent in house-building is shown.

Yocum (1918) aptly points out the inherent weakness in the idea of the neuromotor function of the neurophanes described in some ciliates, and Entz, Jr. (1909), was unable to discover any evidences of these structures in *Tintinnoina*, nor have I been able to do so.

The protozoan individual may be unicellular or it may be multicellular. In the hypermastigote *Trichonympha*, Kofoid and Swezy (1919) state that "the organism and not its cells is the fundamental basis of differentiation." *Trichonympha*, it must be confessed, is a unicellular protozoan. However, this organism displays a complexity and integration of organs almost as structurally diverse and functionally able as its metazoan termite host. In *Proboscidiella* (Kofoid and Swezy, 1925) the organism is controlled by a variable number of nuclei. These hypermastigote flagellates display within themselves all stages of transition from the unicellular individual to that of the multicellular. In *Tintinnopsis nucula* the individual is multicellular. Its four nuclei are not a pair of discrete amphinuclei. Each nucleus of *Tintinnopsis* has its own behavior, divides independently, and finally is passed on to the two new daughters at the time of binary fission as a distinct, separate, independent unit. Dobell (1911, 1925) affirms that protozoan individuals such as *Tintinnopsis* are non-cellular; this author would claim that the criterion of cellularity resides not within the cytoplasm and its organ the nucleus but rather in the cell wall. The noted English protozoologist focuses his attention upon the cell wall and not on the contents. A cell is a "mass of cytoplasm surrounding a nucleus" (Wilson, 1925). Cells are interdependent masses of the living substance, protoplasm, organized into contrasting, changing systems. We may recall in this connection that in the annelid *Chaetopterus* experimental results have been obtained which demonstrate that differentiation may occur without cytoplasmic cleavage (Lillie, 1902). In the tapeworm, to cite another metazoan, the greater mass of the adult is syncytial, yet it cannot be stated that the organism is undifferentiated in any degree, or that it lacks cells. *Tintinnopsis* is a four-celled somatella. It may, however, under unusual conditions, contain but two nuclei (fig. G) or it may contain sixteen in the period of conjugation (Bresslau, 1906). The whole complex of organization and its visible, morphological expression in the differentiated somatella is the fundamental basis of organization in *Tintinnopsis*. This ciliate is not only cellular but it is also equally multicellular. Hardly an hypothesis applied to the Protozoa has been more misleading than the concept of them as simple unicellular organisms which are not subject to the same essential laws of organization and behavior as the more prominent forms of life with which we commonly deal (Kofoid, 1923); the cell remains, however, as the arena of evolution.

The chromidial hypothesis (Hertwig, 1902) cannot apply to *Tintinnopsis*. No evidence is brought forward that the chromidia which are described by Entz, Jr. (1909), are probably other than nuclear and cytoplasmic parasites. They are not a part of the nuclear cycle. The doctrine of the individuality of nuclei expressed in the familiar aphorism *omnis nucleus e nucleo* is clearly demonstrated in the binary fission of *Tintinnopsis*. The chromidiogony of *Polystomella* and of the protozoans in general is probably also to be attributed, in part at least, to internal parasites (Kofoid, 1921).

CONCLUSIONS

1. *Tintinnopsis nucula* is a frequently occurring marine ciliate which maintains itself within coastal waters. It is cosmopolitan in distribution and omnivorous in feeding habits.
2. *Tintinnopsis nucula* is a member of the genus *Tintinnopsis* and can be definitely placed in Brandt's (1907) definition of this genus. It agrees with Fol's (1884) description which was emended by Laackmann (1907). My material was less variable than that previously described. Measurements of the lorica show that it does not deviate more than five microns from the mean length. Its other dimensions are proportional. It is probably not a small form of *T. ventricosa*, its nearest relative.
3. No previous detailed description of the morphology of this species has been made. In this study, the ciliary membrane and the lateral lobe are described for the first time and their functions indicated. They are the chief organs concerned with the building and repair of the lorica. The peristome collar is shown to be a reflexed, spiral thickening of the margin of the column wall. Through it the ciliary membrane passes to the column. Within the peristome field the oral plug arises. The adoral membranelles are twenty-two in number; three of them send proximally into the gullet membranes which become modified to form oral membranes. The somatic cilia form a lateral field and a general somatic field. The cilia are organized in longitudinally disposed rows following the highly contractile myonemes. A stalk attaches the animal to the lorica. There are twenty-two extensible tentaculoids. From the proximal end of each tentaculoid extends an accessory comb into the peristome. The tentaculoids are armed with trichocysts. There is no contractile or non-

contractile vacuole in this species. Mitochondria are present within the cytoplasm.

4. The nuclear system of *Tintinnopsis nucula* is composed of four discrete nuclei, two of which are macronuclei and two are micronuclei. They are normally constant in number and position. The macronuclei are made up of large granules tending to be constant in number in a given individual. Additional small granules are scattered irregularly throughout the substance of the nucleus. The micronuclei are about three microns in diameter and possess a granular structure.

5. There is a complex neuromotor apparatus. The central massive motorium located on the ventral side of the column beneath the gullet may be presumed to be the directing center since it is intimately connected with most and presumably all the motile organs of the body. It is spindle-shaped. Anteriorly it gives rise to two dorsal fibers ending in the peristome and probably connecting with the striations in the ectoplasm of the oral plug. Posteriorly there is a single ventral fiber ending freely in the ectoplasm and directed toward the nuclei. The circumoesophageal ring surrounds the gullet and connects with the motorium by a fiber from its proximal end on the ventral side. The circumoesophageal ring extends fine fibrils partly down the length of the gullet. The adoral fiber extends from the motorium about the circumference of the peristome, connecting each membranelle with the others. It is the most prominent and easily distinguishable member of the neuromotor system. The myonemes constitute the contractile elements of the neuromotor system. They are twenty-two in number. They extend from the base of the animal to the margin of the peristome collar in an unbroken series and are granular in structure. Between them are the clear hyaline bands from which the cilia arise from basal bodies. A lateral field ciliary area arises from these cilia at the right of the ciliary membrane. The ciliary membrane is a structure made up of powerful, elongate cilia in a single series following a spiral course down the length of the column. It arises by a gradual change in the structure of the distal end of the adoral membranelle series. In function it is the most important organ in the building and repair of the lorica. Oral membranes are found in the gullet. These are three in number and arise by a direct transformation of the adoral series. They end in large basal bodies at the base of the gullet.

6. *Tintinnopsis nucula* is a quadrinucleate somatella. It is not only cellular but is also equally multicellular.

7. In fission, each nucleus gives rise by transverse constriction to two daughter nuclei. The division of the micronuclei is mitotic. The macronuclei contain a reasonably constant number of formed bodies which may represent trophic chromosomes. These divide and become distributed to the daughter nuclei. Linear organization of the chromatin occurs within the nuclear walls of the macronuclei. The adoral membranelle zone of the newly forming daughter animal arises posteriorly and ventrally in a constant position and the nuclei are oriented about it in a definite manner beneath it. The first sign of division is cytoplasmic and may be seen as a clear line across the myonemes which gradually spirals upon itself forming the peristome. From this line, the basal granules arise and from them the membranelles arise. Other organs appear to be formed *de novo*.

8. The lorica is retained by the posterior daughter animal of a dividing pair and is thus passed on from one generation to the next. The anterior animal of the dividing pair forms a new lorica, partly with the aid of the posterior animal. The neuromotor activities of the animal play a fundamental part in the construction of the lorica. The ciliary membrane is found to be a principal organ concerned with this function. The ciliary membrane is a neuromotor organ.

9. Viscosity changes in the cytoplasm evinced by profound movements of the nuclei during fission are evidences of the sol-gel reversible nature of protoplasm. These movements are characteristic and take a definite path which is constant.

10. Parasites inhabiting the macronuclei and responsible for abnormal degenerating forms, are described. A new genus and species of these parasites of doubtful position is erected. *Karyoclastis tintinni* is a parasite with an intranuclear and intracytoplasmic phase and with constant morphology and behavior. It undergoes binary and repeated fission within the macronucleus and is discharged, upon the dissolution of nuclear wall, into the cytoplasm. Misinterpretation of the nature of these parasites has led to the application of the chromidial hypothesis to the reproduction of tintinnids (Entz, Jr., 1909). *Karyoclastis* cannot be confused with the dinoflagellate parasites described by Chatton (1906) or with *Sporocystis* (Duboscq and Collin, 1910). It is unlike *Nucleophaga* (Dangeard, 1895) or the related *Sphaeria* from the nuclei of the intestinal amoebas. These parasites probably account for the irregular distribution of most of the forms with a single macronucleus in the plankton, and for other abnormalities.

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EXPLANATION OF PLATES

All figures drawn with camera lucida from whole mounts of preparations fixed in hot Schaudinn's fluid and stained with aqueous iron-haematoxylin. X 525 unless otherwise indicated.

PLATE 12

Fig. 1. Showing the myonemes with narrow bands between, the claviform tentaculoids, and ciliary membrane. The cytophyge is large.

Fig. 2. *Tintinnopsis* viewed from above downward. Showing the oral plug, peristome collar, gullet, and ciliary membrane. The trough through which the ciliary membrane passes is shown below. The position of the tentaculoids and membranelles is diagrammatically indicated.

Fig. 3. Shows especially the mode of attachment at the base of the lorica. Basal granules of the ciliary membrane are to be especially noted. The nuclei of this specimen are somewhat unusual in shape.

Fig. 4. Lorica from below showing the structure, particularly the elevated, irregular fenestrations.

Fig. 5. A fully expanded and attached specimen. The ciliary membrane and membranelles are clear. The nuclei are normal in shape and position. The tentaculoids are heavily stained.

Fig. 6. Lorica from above downward showing the nuchal band, collar, and fenestrations.

Fig. 7. The collar of this specimen is expanded but the tentaculoids are nearly contracted. The nuclei have begun to divide. The ciliary membrane and basal granules are clear. The circumoesophageal ring and motorium may be seen near the gullet, beneath the ciliary membrane.

Fig. 8. Lorica from the side showing general structure, especially of the collar, nuchal band, and fenestrations.

Fig. 9. A very clear view of *Tintinnopsis* showing the general structure. The tentaculoids, accessory membranelles, ciliary membrane, oral membranes, and column lobe are shown distinctly. The macronuclei are beginning to become oriented about the forming cytosome in preparation for division.

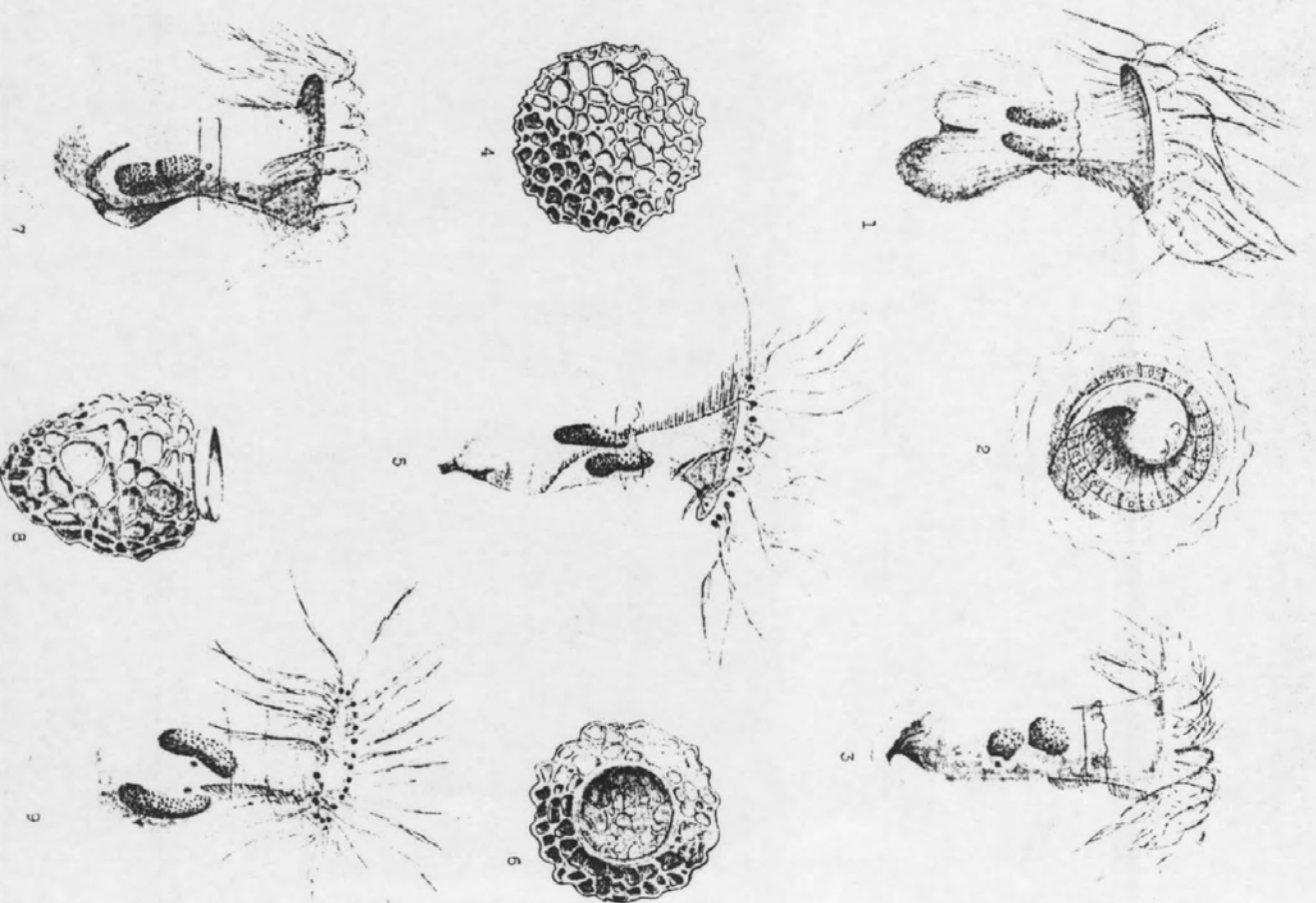


PLATE 13

Fig. 10. Showing an animal rather early in division. This specimen is heavily parasitized with *Karyoclastis tintinnii* gen. nov., sp. nov., in a characteristic manner.

Fig. 11. *Karyoclastis tintinnii* gen. nov., sp. nov., showing (a) an interkinetic individual, (b) one undergoing binary fission, and (c) one in multiple fission. Note the gray-staining mantle, clear central area, and central granule within which undergoes division. $\times 2000$.

Fig. 12. Cytoplasmic phases of *Karyoclastis*. The parasites are shown escaping from the macronucleus. Some parasites are still within the wall while the greater number have already passed out into the cytoplasm.

Fig. 13. The earliest stage in division. The line of basal granules is shown beneath and at right angles to the ciliary membrane, cutting across the myonemes.

Fig. 14. Early fission stage. The new peristome is already formed and the nuclei have swung into position. The behavior of parts is indicated.

Fig. 15. Rather the same stage as figure 14 but oriented differently to show structures not indicated in that figure. The macronuclei have begun to constrict.

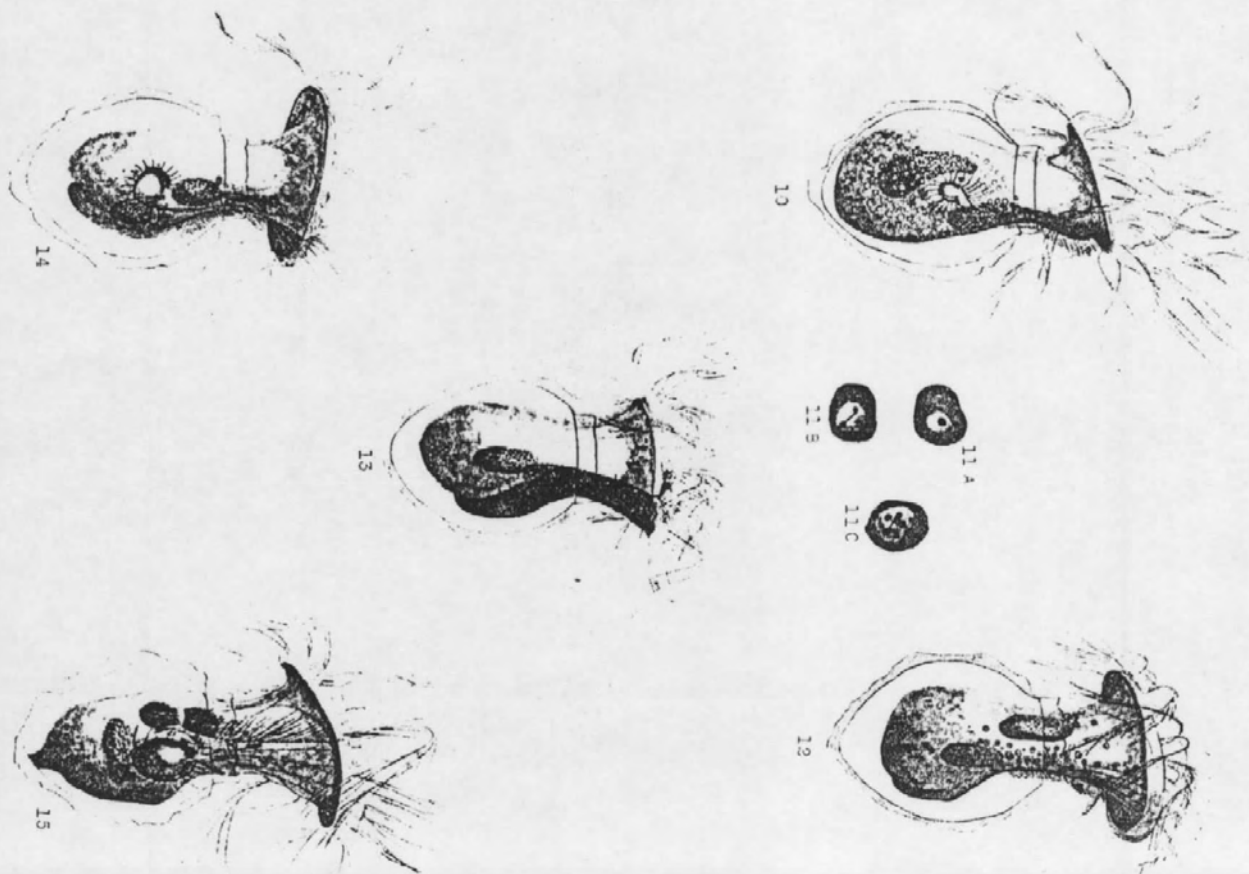


PLATE 14

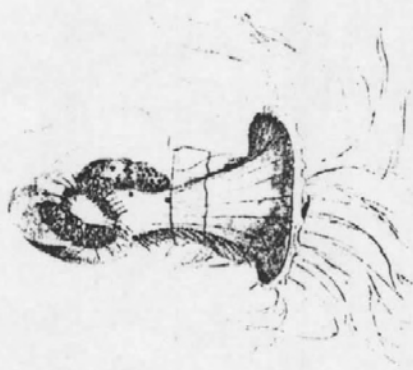
Fig. 16. Later than figure 15. The newly formed peristome has become independent. The micronuclei have divided and the macronuclei are not yet separated. The cytopyge is shown at one side.

Fig. 17. Division stage about the same time as figure 16 but showing the new membranelles growing out. The ring and motorium are distinct.

Fig. 18. A somewhat oblique view of a stage comparable to figures 15 and 16. The nuclei are in division.

Fig. 19. This specimen is somewhat later than previous figures. The nuclei somewhat obscure internal conditions. The motorium and ring are distinct. The tentaculoids are stained very pale but are fully expanded.

Fig. 20. A late fission stage showing the beginnings of separation of the two zooids. One nucleus has not yet divided, the others have separated and are passing into the two daughter individuals. The motorium is distinct.



15



17



18



19



20

PLATE 15

Fig. 21. A very late fission stage before the separation of the two daughters. The posterior animal is behind and will retain the lorica. All organ systems are formed and the animals are independent in every way except in the posterior connection.

Fig. 22. A single macronucleus and micronucleus especially showing the large trophic chromosomes. $\times 2000$.

Fig. 23. Showing the polar massing of the trophic chromosomes in the macronucleus during division. The reconstruction band is clear. $\times 2000$.

Fig. 24. A later stage of the fission of the macronucleus. Note the strand-like organization within the nucleus and the massing of the trophic chromosomes. $\times 2000$.

Fig. 25. Daughter animals almost ready to separate.

Fig. 26. Degeneration form with two macronuclei. Note that the organs are lost.

Fig. 27. Micronucleus in telophase, the chromatin is becoming granular. $\times 2000$.

Fig. 28. *Tintinnopsis* with stored faeces, some of which are being passed out by the ciliary membrane and attached to the lorica by the lateral column lobe. This animal is about to divide.

Fig. 29. Micronucleus in early telophase. The chromosomes are massed about the poles but have not yet lost their identity. $\times 2000$.

Fig. 30. Abnormal form with a single macronucleus.

Fig. 31. A *Tintinnopsis* somewhat like figure 28. Not dividing but showing the path of faecal masses from the fundus of the lorica to the outside.

Fig. 32. A degenerate form free from a lorica, with but a single parasitized macronucleus.

Fig. 33. Interkinetic micronucleus. Note the granular structure. $\times 2000$.

Fig. 34. Prophase micronucleus, enlarged, with the granules massed toward the center. $\times 2000$.

Fig. 35. Prophase micronucleus. The chromosomes have formed. Note the polar caps. $\times 2000$.

Fig. 36. Metaphase micronucleus. $\times 2000$.

Fig. 37. Anaphase. The chromosomes are massed about the polar caps. $\times 2000$.

Fig. 38. *Tintinnopsis* with faecal masses stored in the fundus of the lorica.

