Structural analysis of *Colpoda duodenaria* sp. nov.

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With plates 15—18.

The identification of unicellular forms of life has always been, for obvious reasons, exceptionally difficult and inconclusive as depending primarily upon the accuracy and completeness of their structural analysis. This in turn has frequently had to await the development of specially devised techniques which, along with hasty, uncritical publication, may largely account for the very retarded, unsatisfactory progress in systematic studies on various groups of the Protista. Moreover, the extensive literature comprising such studies is often scattered or unavailable and may be equally vague and confusing.

This rather intolerable situation, especially at a time when detailed, critical analysis of form and structure is no longer in vogue, is most unfortunate for present-day experimentalists some of whom, as a consequence perhaps, would hold inviolate their experimental methods yet as readily disregard the identity and standardization of their experimental material. The trend, then, is toward further confusion and can probably best be obviated by the experimentalists themselves who may not infrequently have to turn aside from their immediate experimental tasks and thoroughly analyse, describe and identify their experimental material. Otherwise they can scarcely hope to have their experimental results confirmed by other workers or help to reduce the taxonomic confusion now so common in experimental literature.

The present study was undertaken with reluctance and only after a fuller realization of the general predicament that now faces experimental protistologists. In this connection it should be noted that already six papers have been published [1]), the experimental material for which came from the original stock culture begun in 1930 at the University of Chicago, and titles of which gave the species as *Colpoda cucullus* or *Colpoda steinii*. Due to the uncertainty of these identifications a critical study of the structure of this ciliate was undertaken two years ago. This together with a careful survey of all the available literature on the genus *Colpoda* and closely related genera led finally to the conclusion that the structural parts of this ciliate differed so widely from those of any known descriptions as to require the status of a new species. We are confident that the diagnosis and illustrations given below will fully justify this conclusion. Supplementing the structural analysis of this new species there are now in progress detailed studies of its reproductive stages, the results of which will soon be published.

Brief historical account of the genus *Colpoda*.

1773. Genus established by O. F. Müller in his "Vermium terrestrium et fluviatilium", p. 56, with the following characterization:

> "VII *Kolpoda*. Vermis inconspicuus, simplicissimus, pellucidus, complanatus, inuatus."

Five species were described: *K. lamella*, *K. rostrum*, *K. Ren*, *K. cucullus*, and *K. meleagris*, giving figures which, so far as diagnostic details are concerned, are so incomplete that actual identification is now quite impossible.

1786. O. F. Müller, in his "Animalcula Infusoria", repeated his description (1773) of the genus and added eleven new species (p. 93). The sixteen species names were listed in the following order:

> "98. *K. lamella*, 99. *K. gallinula*, 100. *K. rostrum*, 101. *K. ochrea*, 102. *K. mucronata*, 103. *K. triquetra*, 104. *K. striata*, 105. *K. nucleus*, 106. *K. meleagris*, 107. *K. assimilia*, 108. *K. cucullus*, 109. *K. cucullulus*, 110. *K. cucullio*, 111. *K. Ren*, 112. *K. pirum*, 113. *K. cuneus*."

This enlarged genus shows that Müller's concept of it had become greatly broadened to include a wide variety of forms. Of his drawings, Figures 7—11 on Tab. XV, *K. cucullus*, depict the type which was later generally accepted for the genus. A list of synonyms was given for this *K. cucullus* which had appeared in publications up to that time.

1838. Up to the publication of C. G. Ehrenberg's "Infusionsthierchen als vollkommene Organismen" the genus *Colpoda* had become so extensive that 101 species names had appeared in print. This confusion was clarified by Ehrenberg who selected from Müller's first publication (1773) *Colpoda cucullus* as the type

[1]) Barker and Taylor (1931 and 1933), Thimann and Barker (1934), Taylor and Strickland (1935 and 1936) and Taylor, Brown and Strickland (1936).

species of the genus, and added more structural details for this species. While Ehrenberg's interpretation of the "life-history" was not valid, his figures (Taf. XXXIX Fig. V) make it certain that he was dealing with the genus *Colpoda* as we understand it today.

1859. F. Stein gave an analysis of the cystment process in a *Colpoda* which he wrongly identified as "*C. cucullus*". This was later designated by E. Maupas (1883) as a new species, *C. steinii.*

1883. Maupas, in his "Etudes sur les Infusoires Ciliés", gave a clear analysis of *C. cucullus* and established its identity. Maupas interpreted descriptions and figures of early workers (Ehrenberg, Dujardin, Perty) as representative of a species he was culturing. He also described a new species, "*C. steinii*" (wrongly identified as "*C. cucullus*" by Stein whose figures were copied by Saville-Kent, 1881—82). He cultured four types of organisms which he considered variants of "*C. steinii*".

1888. Fabre-Domergue reviewed the early history of encystment in *Colpoda* and reported chemical differences in the two types of cysts. His figures (Pl. 4 Figs. 56—57 and Pl. 5 Figs. 63—64) leave identification of the species uncertain.

1888. Rhumbler also studied cyst formation in *Colpoda.* Identification of the species is doubtful though Enriques (1908) considered it to be "*C. maupasi*".

1887—89. Bütschli reviewed the taxonomic history of the genus giving the early synonymies which have been generally accepted. Maupas' description of *C. cucullus* was quoted and his figures copied. The oral organelles of *C. cucullus* were differently interpreted and some details on the mouthparts of "*C. steinii*" were added. Figures of "*C. steinii*" (Taf. LXII, Fig. 8a—d) which are copies of Stein's figures (1859), show only its cyst formation. Enriques (1908) considered Bütschli's "*C. steinii*" as really "*C. maupasi*".

1908, a and b. Enriques studied conjugation in "*Colpoda steinii*", but discovered two forms in his culture. From isolated individuals two strains were obtained. The smaller was identified as "*C. steinii*" [1]) and the larger form given a new species name: "*C. maupasi*". The latter was identified with the species studied and figured by Rhumbler and by Bütschli as "*C. steinii*" and by Stein as "*C. cucullus*". *C. steinii* and *C. maupasi* were compared, significant nuclear details were added and complicated mouthparts analysed. The "labial appendage" and the "dorsal vibrating membrane" were found composed of closely set cilia, membrane-like. Bütschli considered descriptions of other species of this genus inadequate and stressed the importance of pure cultures for valid identifications.

1921. Bresslau gave photomicrographs of "*Colpoda steini*" showing both right and left sides from which can clearly be determined the number and direction of the 15 ciliary stripes and the size, shape and position of the cytostome (cf. our Pl. VII Figs. 29a and 29b).

1921. Yakimoff and Kolpakoff figure a "*C. steini*" which they found in faeces from a patient having colitis, but its species identity seems questionable.

1926. Klein presented the first drawing of the silverline system of "*Colpoda steinii*" (Fig. 13, p. 262) but details for certain identification are incomplete.

[1]) Enriques seems to have been the first to drop the "ii" ending of the original spelling of this species name. However, in accordance with Opinion No. 8 (1910) of the International Commission on Zoological Nomenclature: "Specific patronymics originally published as ending in ii . . . are . . . to be retained in the original form."

1926. KAHL added two new species, *C. aspera* and *C. Rouxi*. The latter became a synonym for *C. inflata* in this author's paper of 1931.

1928. REICHENOW figured nuclei of "*C. cucullus*" and "*C. steinii*". Identity is uncertain.

1929. KLEIN gave drawings and photomicrographs of the silverline system of "*C. cucullus*" and of "*C. steinii*" and drawings of the silverline system in division cysts of "*C. steinii*" (Fig. 1, p. 286). Apparently he had several species in his cultures and the various figures of the free swimming forms of his "*C. steinii*" (Figs. 4, 5, and 6, and 15 and 16) are not only inconsistent with each other but with the figures of division cysts (Fig. 1) which seem to represent three different species.

1930. KLEIN gave drawings of "*C. steinii*" in conjugation (Figs. 22 and 23) but his papers, being concerned with the nature of the silverline pattern, lead to confusion in the taxonomy of these *Colpoda*.

1931. KAHL in his extensive work revised the Family *Colpodidae* POCHE 1913 and re-diagnosed the genus *Colpoda*, adding considerable new details to the morphological analysis of this genus. Nine new species were added to the genus, a total of sixteen being recognized by this author: *C. praestans* PENARD, 1922, *C. irregularis* sp. nov., *C. tripartita* sp. nov., *C. patella* sp. nov., *C. Henneguyi* FABRE-DOMERGUE, 1888, *C. reniformis* sp. nov., *C. californica* sp. nov., *C. fastigata* sp. nov., *C. colpodiopsis* sp. nov., *C. penardi* sp. nov., *C. aspera* KAHL, 1926, *C. cucullus* O. F. MÜLLER, 1786 [1]), *C. steinii* MAUPAS, 1883, *C. maupasi* ENRIQUES, 1908, *C. inflata* (*Tillina inflata* STOKES, 1885, *Colpoda Rouxi* KAHL, 1926, *C. steinii* ROUX, 1901). Despite the additional information on the morphology of this genus such as the course of the ectoplasmic markings (interpreted by him as being "protrichocysts"), the shape and position of the cytostome with its complex ciliary organelles, the position of the contractile vacuole and the excretory pore, there remain various structural details to be clarified in all of the species of *Colpoda* so far described. KAHL emphasizes the difficulties and uncertainties in the systematics of this genus:

> "Die Systematik ist sehr schwierig, da manche Arten je nach Ernährung ihre Gestalt und Größe ziemlich ändern; die hier gebotene Übersicht, die zwar auf überaus zahlreiche eigene Beobachtungen zurückgeht, wird daher noch mancher Korrektur bedürfen; sicher fehlen noch mehrere Arten und wahrscheinlich wird man die eine oder die andere Art wieder aufgeben können (p. 273)."

1935. KAHL added another new species to this genus, *C. cavicola*, bringing the total number of species recognized by this author to seventeen.

Material and method.

The *Colpoda* used for this investigation were originally recovered six years ago from bank soil of an artificial lagoon on the campus of the University of Chicago. A pure strain of that culture has since been continously maintained on a selected species of *Pseudomonas* and used for experimental purposes in our Laboratories of Chemophysical Biology at Stanford University. This pure strain of

[1]) This date should be 1773.

Colpoda has remained vigorous during the six years of laboratory culture, with no apparent change in their rate of fission or in their experimentally induced excystment time (Taylor, Brown and Strickland, 1936). We have never observed any conjugation in this strain and, so far as studies of the nuclear behavior have been made, there has been no indication of endomyxis or similar phenomena.

For studies of fixed and stained preparations, several methods have been tried, of which the following were found to be the most satisfactory, according to the particular structure or structure-complex for which a given method was employed: (a) for studies of ciliation, especially of the body cilia, Breslau's method using China Blue suspension was excellent; (b) for critical analysis of the silverline system, including the mouth parts, and for identifying the cytopore and the cytoproct, a modification of Klein's silver nitrate technique has been invaluable; (c) for details of the nuclear complex, a modification of Heidenhain's haematoxylin stain, and Borrell's and Feulgen's nuclear techniques, following various fixatives, gave the best results, particularly for comparative studies which are as yet incomplete.

A modification of the dry method of Klein, rather than the wet method of von Gelei and Horváth (von Gelei, 1934), has afforded several advantages. The detailed structures of the mouth-parts which in the normally shaped animals are "hidden" down in the cytopharynx are sometimes revealed when the cytopharynx flattens out in the drying process. Moreover, the characteristic silverline pattern of this species and the convergence of ciliary meridians at its anterior and posterior ends are more clearly seen by this method than by the wet method.

Living *Colpoda Duodenaria* sp. nov.[1])

Size and body form of the various species of *Colpoda*, and of holotrichous ciliates generally, are so modified by food, temperature and other environmental factors that their frequent use as reliable criteria in the taxonomy of these organisms is largely invalidated, especially when the environment is unknown and uncontrolled. While this species is apparently one of the smallest in the genus *Colpoda*, we have observed in our controlled laboratory cultures of *C. duo-*

[1]) *Duodenaria*, "denoting twelve", refers to the 12 body meridians of cilia characteristic for this species. We are indebted to Dr. R. D. Harriman, Professor of Classics at Stanford University, for assistance in selecting this species name.

denaria striking variations, particularly in size, which well illustrate the unreliability of such criteria. Under exceptional conditions of our culture media, this ciliate has been found to vary in length from about 9 μ to as much as 60 μ. In cultures maintained for our experimental purposes, this length variation ranges from about 20 μ to 40 μ throughout the reproductive cycle. For a given length, the width may vary greatly according to the amount of recently ingested food.

Descriptions of vaious species of *Colpoda* commonly give their body form as "kidney-shaped" or "bean-shaped" with the mouth located in the "hilum" or notch on one side of the body. WENYON (1926) and KAHL (1931) describe the body of *C. steinii* as "flattened dorsoventrally" with its right side half-moon shaped and its left side usually quite straight; the mouth lies "near the left margin of the flattened ventral surface close to the median transverse plane" (KAHL), or "... at the end of a grove on the right hand side of the ventral surface in front of the middle of the body" (WENYON, p. 1178). The description of *C. steinii* given by YAKIMOFF and KOLPAKOFF (1921) considers the dorsal side convex and the cytostome on the ventral concave side (p. 553).

While we would interpret our ciliate as flattened laterally and its cytostome ventrally located and invaginated to the right, it is obvious that such disparities of interpretation may be rectified only by means of fundamental criteria derived from characters that are common to holotrichous ciliates generally. Sufficient amphasis upon structural similarities is quite as indispensable for proper taxonomic interpretations as is due recognition of essential differences. Similarities or common characters come to be discerned, however, especially for the holotrichous ciliates whose structural differentiations are proverbially inconspicuous, chiefly upon recourse to a plausible hypothesis of their genetic history.

Accordingly, if we consider the genus *Colpoda* as a modification of a more primitive, ellipsoidal or pear-shaped holotrich, such as delineated by BÜTSCHLI (1887, p. 1353), we may better interpret the body form and essential characters of *C. duodenaria*. According to BÜTSCHLI's hypothesis, the ciliary meridians of the primitive holotrich converge in the anterior and the posterior pole of the original polar, or body, axis. But since the circular mouth of this primitive ciliate (cf. *Holophrya*) marks precisely the anterior pole, it interrupts all the ciliary meridians which thereby impinge symmetrically upon it. This primary relationship of mouth and ciliary meridians remains essen-

tially unimpaired in any and all the phylogenic modifications of holotrichous forms known today. These philogenic changes, in this connection, consist mainly in a posterior elongation of the mouth, which tends in later phylogeny to foreshorten toward its posterior limits. This tendency has given rise to holotrichs whose ovoid or nearly circular mouth is now anterolateral rather than polar in position and whose ciliary meridians are correspondingly displaced and distorted.

According to our own interpretation, such distortions are conspicuously evident anterior to the mouth of various holotrichs through the formation there of a "preoral keel" or apical ring or suture. And while more primitively this apical ring and the posterior convergence of the meridians mark approximately the anterior and posterior poles of the primary body axis, in a holotrich such as *Colpoda duodenaria*, the elongated apical ring no longer represents the geometric anterior pole but lies on the oral (ventral) side of the body. This ring is, however, still the morphological apex and the meridians of cilia still converge in it. Moreover, this region of the body has become less regular and the meridians of cilia have become curved to meet this new configuration (Pl. 15 Fig. 1—3).

In *C. duodenaria* the apical ring, so evident in the silverline preparations, is elongated and roughly conforms in extent to the "preoral keel" (Pl. 15 Fig. 3; Pl. 18 Fig. 25, 27). The various ciliary meridians converge in the preoral keel and so indicate a distortion in a more advanced body form.

The periphery of the fairly large, C-shaped cytostome is vaguely marked on its left margin as it slopes into the cytopharynx which, in turn, is invaginated sharply to the right (Pl. 15 Fig. 1—3). This in-pushing of the cytostome toward the right deflects the adjacent meridians on the right side of the cytostome from their otherwise straight course between the apical ring and the posterior convergence. As a result, the region of the body immediately posterior to the cytostome gives the impression of being slightly twisted to the left (Pl. 15 Fig. 3).

In this holotrichous ciliate we have a convenient mark of reference in one of these meridians of the body cilia. Redifferentiation of the cytostomal ciliary field of a new daughter begins in a single meridian of the body. This stomatogenous meridian, "Hauptmeridiane" or "Richtungsmeridiane" of Klein (1927) and of von Gelei (1935) can be readily identified and used for reference

in describing the positions of other body parts. Designating this "main" meridian (SM) as No. 1 and counting (from an anterior view) clockwise around the body, we can number each meridian correspondingly and thereby locate any differentiated structures with reference to these meridians. We thus avoid the confusion due to diverse interpretations as to what constitutes a "ventral" or "lateral" side. With these criteria, it becomes possible to describe more accurately the body form of this ciliate, and to designate precisely its various differentiated structures either as specific characters or as homologues that have their structural counterparts in other ciliates.

While the ciliation of *C. duodenaria* can best be studied with staining techniques, results with which are described below, the living organism shows a fairly typical, holotrichous ciliary pattern. The body cilia are always paired and are inserted along the floor of 12 longitudinal grooves (Pl. 15 Fig. 1, 2). A preoral concentration of cilia forms a conspicuous, brush-like "keel", and a pair of long caudal cilia extends directly back from the posterior end. The latter may be nearly twice as long as the other body cilia, are motile, and seem to function like a rudder when the organism changes its direction of locomotion. With the exception of the caudal cilia and the ciliary organelles of the mouth, there are no other distinguishing marks in the ciliation of *C. duodenaria.*

The cytostome with its ciliary organelles forms one of the most distinctive taxonomic characters. More complete details of the cytostomal structures are presented under the caption: "Fixed and Stained *Colpoda duodenaria*". In the living organism, one can observe along the right semicircular margin of the cytostome a row of cilia which, as evident in silverline preparations, mark the outher boundry of a ciliary "field". This field bounds, somewhat dorsoposteriorly, the right cytopharyngeal wall. Along the posterior margin of the cytopharynx and extending down the left-posterior wall of the cytopharyngeal funnel, there is a series of narrow membranelle-like structures arranged in a clearly defined series of parallel clefts. This series is considered homologous with the adoral zone of membranelles of other holotrichs and possibly comparable with the adoral zone of ciliates generally. The distal portion of this membranelle zone of *C. duodenaria* protrudes from the left posterior margin of the cytostome and constitutes the "beard" which is characteristic of this species (Pl. 15 Fig. 1, 3; Pl. 16 Fig. 8). The cytopharynx diverts dorso-posteriorly into a short, narrow cytoesophagus and ends slightly to the right of the main body axis.

The contractile vacuole lies at the extreme posterior end of the body somewhat dorsal to the insertion of the paired caudal cilia where it empties through a localized pore, herein designated the cytopore (Pl. 15 Fig. 4). As later noted, the single discharge pore of the contractile vacuole has a persistent locus and is clearly discernible, especially in China Blue and silverline preparations. Small secondary vacuoles can be seen coalescing to form the next contractile vacuole after systole.

The cytoproct is scarcely visible in the living organism. The discharge of solid wastes near the posterior end can be seen but the exact location of this organelle can be definitely determined only by means of the silver nitrate technique (Pl. 18 Fig. 26).

The hyaline cytoplasm contains varying amounts of granules, food vacuoles and other inclusions. The more conspicuous of these inclusions, when few in number, are usually confined toward the posterior end of the body. In well-fed individuals, the cytoplasm becomes packed with food vacuoles and various other inclusions so as to give the organism a swollen contour and grayish opacity (Pl. 15 Fig. 4). *C. duodenaria* is remarkably hyaline when it emerges from the resting cyst.

The macronucleus is distinguishable in the living organism as an ovoid or ellipsoidal body usually near the center of the cell. In life it appears slightly more opaque than the cytoplasm, and finely granular and colorless. Its central position shows a globular Binnenkörper which is somewat more coarsely granular than the nuclear cortex. No other endosomes have been observed in the living nucleus but one or more besides the Binnenkörper may be seen in fixed and stained preparations.

The micronucleus is a compact, crescent-shaped organelle which, in vegetative stages, lies always adjacent to the macronucleus. When seen from an end view (Pl. 16 Fig. 14), the micronucleus appears spherical but careful focussing shows it to be distinctly crescent-shaped. Enriques' (1908) description and illustration of this organelle as spherical for *C. steinii* reveals one of the characteristic differences between these two species. That the micronucleus is crescent-shaped and that it remains intimately associated with the macronucleus is clearly demonstrable in the living organism during rapid cycloses of the cytoplasm. Then the whole nuclear complex may be turned and carried about without any apparent disturbance of the relative positions of the two nuclei.

Reproductive cysts.

C. duodenaria reproduces by fission only within a well-defined cyst membrane. Sometimes only two daughter cells are formed (Pl. 15 Fig. 5), more commonly four daughter cells result (Pl. 15 Fig. 6), and occasionally eight daughters (three successive divisions) are formed before the young individuals emerge from the cyst. Penn (1937) reports that in *C. cucullus* division sometimes occurs without the formation of a cyst membrane. We have never observed such a cyst-less method of reproduction in *C. duodenaria.* Its division process is being studied in detail and the results are to be published shortly.

"Resting" cysts.

This species readily forms resting cysts (Pl. 15 Fig. 7). In this state they can be dried and are highly resistent to extreme environmental conditions (Taylor and Strickland, 1936). Further studies on these cysts are in progress.

Fixed and stained *Colpoda duodenaria.*

Several improved staining techniques of recent years have helped greatly to advance our knowledge of the structural differentiations of unicellular organisms. For ectoplasmic structures, the silver nitrate method is probably one of the most useful yet devised, by means of which the silverline system, a remarkable complex of specific pattern, has been disclosed in various ciliates (Klein, 1936 and later).

We owe chiefly to this method, along with Bresslau's China Blue technique, our present analysis of the ciliation and the fibrillar system of *C. duodenaria.* The body cilia of this species, as noted above, are arranged in pairs along the floor of 12 well-defined meridional grooves. These grooves run the full length of the body from the apical ring to the posterior convergence excepting three of them which are interrupted by the cytostome. Two of these end at the posterior border of the cytostome and one ends at its left margin.

Both the body cilia and those of the mouth parts are fairly discernible in the living, unstained organism, but their delineation is strikingly enhanced in preparations stained with the China Blue suspension. The adsorption of this dye on the ciliary and body surfaces intensifies their surface outlines so as to make quite con-

spicuous most or all of the various cilia. As a consequence, their number and distribution can be definitely ascertained. Those anterior to the mouth where the converging meridians effect their crowding and produce finally the preoral keel are, however, finer and somewhat shorter. This difference in relative lengths of the preoral and body cilia is just the reverse of that described for *C. steinii* by Maupas (1883) who considered the preoral cilia longer than the body cilia.

Associated with this ciliary sytem, and even more significant for present purposes, is the fibrillar or silverline system. As revealed by means of the silver nitrate technique, the general pattern of this system is so definite and characteristic and its principal parts are, for a given stage, so constant that it assumes a rank of first importance both for the analysis of any reorganization changes and for taxonomic determinations.

In a fully differentiated *C. duodenaria*, the 12 meridional fibers of this silverline system tend to be invariable not only in number but also in their relations to each other and to the ciliary apparatus. Moreover, they are apparently always single since no "meridians of the second order" have so far been observed. These meridians converge posteriorly in a fibrillar polygon which sends other fibrils to the pore of the contractile vacuole (Pl. 17 Fig. 20, Pl. 18 Fig. 26, 28). Anteriorly the meridians converge in the apical ring which is elongated the full length of the preoral keel. Cross fibrils, the inter-meridional connectives, join adjacent meridians usually, but not invariably, at the bases of the cilia. Within each "basal ring" which marks the insertion of a pair of cilia, two basal granules can be seen. In meridian No. 2, near the contractile vacuole pore, can be observed the basal granules of the two caudal cilia (Pl. 18 Figs. 25, 26). These two granules lie nearer this pore than do those of any other meridian. This constant and unique relationship of the cytopore and the bases of the caudal cilia is doubtless of taxonomic value.

The stomatogenous meridian (No. 1) is continuous with the basal granules of cilia that constitute the ciliary field along the right cytopharyngeal wall. The cytostome also interrupts meridian No. 12 (Pl. 18 Fig. 25). The convergence of the other 10 meridians, on opposite sides of the elongate apical ring, is characteristically asymmetric. Four of these (Nos. 2, 3, 4 and 5) join this ring on its right side and five (Nos. 6, 7, 8, 9 and 10), which are closer together, join the ring on its left side. This asymmetry accounts for an apparent

discrepancy in the number of grooves (and, therefore the number of "teeth") in the preoral keel when viewed from its right side or from its left side. Inasmuch as the number of these "teeth" ("dentlatures" of MAUPAS, 1883, and ENRIQUES, 1908) has been used for species determination, it becomes important to account for this discrepancy and so rightly interpret this meridional asymmetry of the preoral keel. Meridian No. 11 shows one pair of basal granules to the left of the cytostome but beyond this pair, up to the indirect union of its fibril with the apical ring, no other basal granules (hence no cilia) appear.

As formerly noted, the mouth parts of *C. duodenaria* comprise externally (1) a row of cilia which are inserted singly along the right semicircular margin of the cytostome which constitute the outer border of the right cytopharyngeal ciliary field, and (2) a series of membranelle-like structures which begin along the posterior margin of the cytostome and, in parallel rows, continue down the left-posterior wall of the cytopharynx. The more distal of the series project from the mouth to form the conspicuous "beard".

Subtending this ciliary pattern of the cytostome is a cytostomal fibrillar complex which is best revealed by means of the silver nitrate method. Corresponding to the semi-circular row of cilia, seen in the living animal, along the right margin of the cytostome is a single row of prominent granules which are integrated with the silverline meridian No. 1. Usually 12 basal granules can be counted, representing, therefore, as many cilia in this row. Below this row can be seen, especially in preparations where the cytopharynx is flattened out, a series of granules in parallel rows (Pl. 18 Figs. 25, 27) making up the right cytopharyngeal ciliary field.

And, likewise, corresponding to the cytostomal memranelles, there are 12 rows of basal granules arranged in parallel series from the left cytostomal margin near meridian No. 11 down the left-posterior wall of the cytopharynx (Pl. 17 Fig. 21, Pl. 18 Figs. 25, 27). Some preparations show these membranelle basal granules clearly defined and variable in number, from 7 in the upper and wider rows to 2 in the last row deep in the cytopharynx.

Numerous connecting fibrils from the right ciliary field, which is connected to meridian No. 1 on the one hand and from the series of membranelles on the other hand, converge, with interlacing fibrillae, to join a crescent-shaped fibrillar structure in the dorsal wall of the cytopharynx (Pl. 18 Figs. 25, 27). In turn, this entire fibrillar net-work of the cytostome is united by other fibrils to meridian

No. 11 and so eventually to the apical ring. Thus the entire silverline complex of *C. duodenaria* is, in the fully differentiated organism, a completely integrated system.

The locus of the contractile vacuole at the extreme posterior end of *C. duodenaria*, which is constant in the living organism, apparently owes this constancy to its persistent pore. This pore is readily discernible in silverline preparations and, as stated above, lies near the center of the fibrillar polygon which receives the meridional fibers and, in turn, sends other fibers to the cytopore (Pl. 17 Fig. 20, Pl. 18 Figs. 26, 28). The site of this discharge pore represents morphologically the posterior pole of the organism.

The cytoproct may be identified also in silverline preparations where it may vary greatly in size, perhaps due in part to the technique employed (Pl. 18 Figs. 26, 28). Figure 26 shows an expanded cytoproct which lies anterior to the pore of the contractile vacuole and is associated with meridian No. 10. The cytoproct may, however, appear only as a heavy line at this juncture.

The nuclear complex of *C. duodenaria* was studied comparatively in preparations which were fixed and stained by the various methods noted above. The data thus far, however, are incomplete so that only a brief preliminary report of them can be given here.

The general appearance and interrelations of the macronucleus and micronucleus, as already described for the interfission stage of living *C. duodenaria*, obtain conspicuously in fixed and stained material where further structural details are revealed in both nuclei. In preparations stained with iron-haematoxylin, the elliptical macronucleus shows a definite membrane which encloses a finely grained nucleoplasm that usually contains one or more endosomes and, at its center, a deeply staining, spheroidal Binnenkörper. One or two endosomes may appear on either side of the Binnenkörper or all on one side, but sometimes they are entirely wanting. Their absence may then be due, however, to their tendency to destain quite readily. The micronucleus, following this stain, shows the same crescentic contour in lateral view, or spherical contour in end view (Pl. 16 Fig. 14, Pl. 17 Fig. 22) as seen in the living organism and noted above. If the preparation is greatly destained, the micronucleus is the last to lose the stain, but it remains homogeneous (Pl. 16 Fig. 14).

After Borrel's stain, the micronucleus takes on an intense cherry-red color. The nucleoplasm of the macronucleus appears dull red

but the Binnenkörper and endosomes are colored green from the counterstain (Pl. 16 Fig. 16, Pl. 17 Fig. 24).

Further differences in the nuclear complex are brought out with FEULGEN'S nuclear stain, which is reported specific for thymonucleic acid (LISON, 1936). The Binnenkörper and endosomes of the macronucleus may at times show scarcely any affinity for this stain, appearing rather as vacuoles in a lightly stained nucleoplasm. This reaction, however, varies considerably, depending partly on the technique and on other factors now under investigation. The cresent-shaped micronucleus may appear intensely red with the tips of the crescent remaining unstained (Pl. 17 Fig. 23). By increasing the hydrolysis, this nucleus may show a lengthwise differentiation of chromatin or, with further hydrolysis, vacuolization or a peripheral clumping and interlacing pattern may appear, all of which indicate that the micronucleus may not be continuously homogeneous even during its interphase (Pl. 16 Fig. 17).

Species diagnosis of *C. duodenaria.*

1. Sizes: In our experimental cultures, length ranges from about 20 μ to 40 μ throughout reproductive cycle; under exceptional conditions, length varies from 9μ to 60 μ; width for a given length indeterminate, as affected by ingested food.

2. Shape: Varies considerably depending on amount of food and other culture conditions. Roughly bean-shaped, flattened laterally with deeply invaginated cytostome on ventral side distorting the regular shape of the otherwise ellipsoidal body. (See illustrations.)

3. Cytostome: Outer opening C-shaped with funnel-shaped cytopharynx indented dorsolaterally slightly to the right of the median dorsoventral body axis. Adoral zone of about 12 primitive membranelles extending from left posterior margin of cytostomal opening, along posterior margin and down along the left posterior wall of the cytopharynx. A row of prominent single cilia along right cytostomal margin marking the outer edge of a ciliary field covering a portion of the right cytopharyngeal wall. This ciliary field best revealed in silverline preparations.

4. Body cilia: Arranged in 12 longitudinal rows set in pellicular grooves extending from "preoral keel" to posterior end of body. One pair of motile caudal cilia about twice as long as regular body cilia in meridian No. 2 at posterior end of body, which act as rudders in the swimming movements of the animal. Cytostome interrupts 3

meridians of body cilia, No. 1 and No. 12 at posterior edge of cytostome and No. 11 ends at left margin of cytostome.

5. Contractile vacuole: Single with secondary vacuoles around it. One cytopore at extreme posterior end of body, best seen in China Blue and silverline preparations.

6. Cytoproct: Dorsoposterior, in meridian No. 10 anterior to cytopore.

7. Nuclear complex: Ovoid macronucleus with prominent spherical Binnenkörper and frequently one or more other endosomes. Single crescent-shaped macronucleus lying adjacent to macronuclear membrane.

8. Reproduction by division cysts into 2, 4 and sometimes 8 daughter cells. Division without cyst formation never observed.

9. Resting cysts: Readily formed. Can withstand extreme environments in "dried" state.

10. Conjugation: Never been observed even though attempts to induce it have been resorted to.

11. Silverline system: Very distinctive and uniform pattern. (See illustrations.)

12. Food: Bacteria feeder. Has been cultured for several years on *Pseudomonas*.

Discussion.

The structural analysis of this *Colpoda* which has long been cultured and utilized in our experimental laboratories, discloses several essential characters that are so definite and unique as to require for it the status of a new species. These characters include (1) the consistently 12 meridional rows of body cilia, for which it receives its specific name, *C. duodenaria*; (2) a pair of caudal cilia, approximately twice as long as the body cilia; (3) a distinctive nuclear complex, comprising a crescentic micronucleus and a vesicular macronucleus, and (4) well-defined cytostomal organelles, including a series of about 12 membranelles on the left and a ciliary field on the right wall.

In comparison with other species of this genus, *C. duodenaria* is appearently most similar to *C. steinii* Maupas, altough various discrepancies in the literature describing *C. steinii* make its identity and so any comparisons uncertain. Breslau's photomicrographs (1921, Pl. 20 Fig. 4 and 5) of a "*Colpoda steinii*", fortunately show both sides of the same individual. From these photomicrographs it was

possible to reconstruct the course and number of the ciliary meridians (see our Pl. 18 Fig. 29 b), which for that individual are definitely 15, Eleven of these 15 meridians converge in the apical ring (preoral keel), one at the left margin of the cytostome and three at its posterior margin.

The ciliary pattern in BRESLAUS *C. steinii* is, therefore, distinctly different from that of our *C. duodenaria* whose pattern of 12 meridians is constant, and only 9 meridians converge on the preoral keel (Pl. 18 Fig. 29 a, b). Moreover, the cilia on his *C. steinii* are conspicuously more numerous and its cytostome shows a deep notch on both right and left margins, whereas our species is indented markedly only on the right margin.

In view of MAUPAS' (1883) original description of *C. steinii* as having 5 or 6 "dentelures" and of ENRIQUE'S (1908) designation of "6—7 dentellature frontali", both indicating, therefore, the number of ciliary grooves that converge on the "frontal lobe" (preoral keel) to be comparable with those shown in BRESLAU'S photographs, it appears justifiable to regard the species studied by BRESLAU as *C. steinii.*

Assuming that the number of ciliary meridians in the genus *Colpoda* is as constant for any given species as it is for our species which has been reared in our laboratory in pure culture now for several years, it seems likely that KLEIN'S (1926 and 1929) published drawings of the silverline system of "*C. steinii*" actually have to do with more than one species. Fig, 13 (p. 262) of his paper of 1926 is not sufficiently distinct for certain identification, but his drawings of division cysts in his publication of 1929 show clearly a variable number of silverline meridians. Fig. 1—8 shows 22 such meridians, and in figures 1—4 and 1—7 there are 12 meridians (characteristic of our species) and 15 meridians (as in BRESLAU'S photomicrographs) respectively. Evidently KLEIN has depicted the division cysts of three different species of *Colpoda.* Further discrepancy is noted in his drawing (Fig. 4) showing a "*Colpoda steinii*" with 20 meridians and (Fig. 6) a diagram of the anterior end of a "*Colpoda steinii*" with 29 ciliary meridians.

KLEIN (1930) has also described and figured the silverline system of *C. steinii* in conjugation (Figs. 22 and 23). For our ciliate, however, during the six years of its pure culture, there has been no indication of conjugation. We have attemped to induce its conjugation by various methods, including that described for *C. steinii* by ENRIQUES (1908), but thus far without success.

The occurence of species of *Colpoda* as parasites in the human intestine has been reported for *C. cucullus* by Schulz (1899) and for *C. steinii* by Yakimoff and Kolpakoff (1921); also for *C. steinii* in the land slug *Agriolimax agrestris* by Reynolds (1936). In any of these publications, the only figure given is that by Yakimoff and Kolpakoff and its details are inadequate for species identification.

It is obvious that the genus *Colpoda* is greatly in need of more careful morphological study in order to establish with certainty the status of the various species given in the literature.

Summary.

1. A new species of holotrichous ciliate, *Colpoda duodenaria*, is described.

2. The confused taxonomy of the genus *Colpoda* is indicated and discrepancies in the structural analysis of members of this genus are pointed out.

3. Need for accurate species identification of Protista used in experimental studies is emphasized.

4. Especially for the relatively less differentiated holotrichous ciliates, adequate identification would seem to require at least: (1) the exact number and course of the ciliary meridians, (2) detailed analysis of the cytostomal organelles, (3) accurate position of the cytopore and of the cytoproct, (4) detailed analysis of the nuclear complex by means of standardized staining techniques and (5) analysis of changes and fate of cytoplasmic and nuclear structures during reorganization in fission, conjugation, cystment, etc.

5. Reproductive and resting cyst formation is noted for *C. duodenaria*, details of which will be given in a subsequent paper.

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Explanation of plates.

Plates 15—18.

All drawings were made with the aid of a camera lucida except Figs. 27 and 28, Pl. 18.

Plate 15.

Fig. 1. Drawing from life. Right side. × 1950.

Fig. 2. Drawing from life. Left side. × 1950.

Fig. 3. Drawing from life. Oral (ventral) side. × 1950.

Fig. 4. Drawing from life. Left side showing typical cytoplasmic granulation. × 1950.

Fig. 5. Drawing from life. Division cyst; 2 cells. × 2400.

Fig. 6. Drawing from life. Division cyst; 4 cells (three visible in drawing). × 2400.

Fig. 7. Drawing from life. Resting cyst. × 2400.

Plate 16.

Figs. 8—19. Nuclei of *Colpoda duodenaria*. Schaudinn's fixing fluid and haematoxylin stain used in preparation except when stated otherwise. × 2000.

Fig. 8. Position and relative size of macro- and micronucleus commonly found in organism containing a medium amount of food. Macronucleus with the two endosomes in addition to the Binnenkörper.

Fig. 9. Macronucleus showing a single, faintly staining endosome.

Fig. 10. Note halo around Binnenkörper.

Fig. 11. Unusual type of Binnenkörper.

Fig. 12. Endosome irregular in shape.

Fig. 13. Macronuclear granules arranged more or less in linear order.

Fig. 14. Greatly destained preparation; micronucleus retains stain longest.

Fig. 15. Large macronucleus without endosomes other than the Binnenkörper.

Fig. 16. Borrell's stain; Binnenkörper unstained.

Fig. 17. Feulgen's nuclear reaction with marked hydrolysis. Note vacuoles in macronucleus and non-homogeneous micronucleus.

Fig. 18. Feulgen's nuclear reaction. The Binnenkörper is negative for stain.

Plate 17 (Photomicrographs).

Fig. 19. Fully differentiated, young organism showing silverline system of left ventrolateral side. Irregular convergence of meridians at preoral keel can be seen above cytostome. v. GELEI's formol-sublimat silver nitrate method. × 1120.

Fig. 20. Stilverline system, posterior end of organism; contractile vacuole pore at center of polygon. Compare with labelled tracing of photomicrograph of another specimen, Fig. 28, Pl. 18. KLEIN's silver nitrate method. × 1360.

Fig. 21. Rounded up individual; cytostome flattened, thus showing (1) at right (left side of organism), membranelle basal granules in rows, and (2) the ciliary field at left (right side of organism). Note basal granules in latter region. KLEIN's silver nitrate method. × 1400.

Fig. 22. Nuclear complex as seen in organisms fixed in SCHAUDINN's fluid and stained with haematoxylin. Micronucleus clearly crescent-shaped. Section, 5 μ. × 1150.

Fig. 23. Nuclei after fixation in SCHAUDINN's fluid and followed by FEULGEN's nuclear stain. Binnenkörper fails to give color reaction; micronucleus strongly positive. Approx. × 2360.

Fig. 24. Nuclei of slender form as seen with BORRELL's differential stain after fixation in SCHAUDINN's fluid. Approx. × 1380.

Plate 18.

Fig. 25. Drawing of silverline system of right ventrolateral side showing relation of meridians to mouthparts and contractile vacuole pore. Note position of caudal cilia (basal granules) in meridian No. 2. KLEIN's silver nitrate method. × 1312,5.

Fig. 26. Drawing of left dorsolateral side showing meridians and their course. KLEIN's silver nitrate method. × 1312,5.

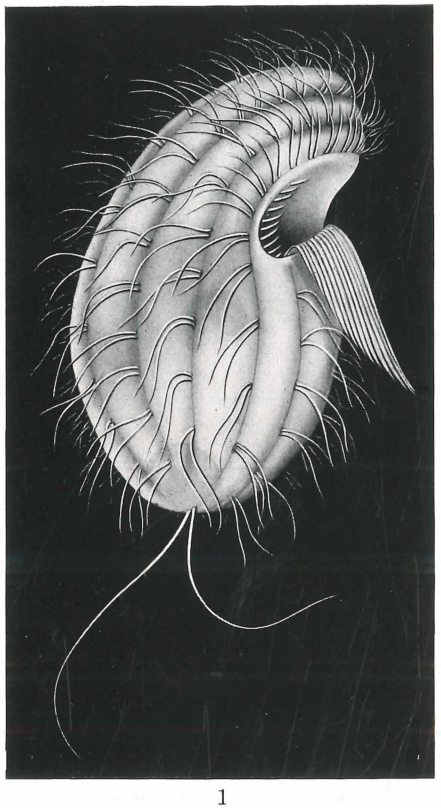
Fig. 27. Labelled tracing of a photomicrograph of silverline system of anterior end of organism. KLEIN's silver nitrate method. × 1312,5.

Fig. 28. Labelled tracing of a photomicrograph of silverline system of posterior end. Compare with Fig. 20, Pl. 17. × 1020.

Fig. 29a and 29b. Comparison of ciliary meridians of *C. duodenaria* and *C. steinii*: (a) *C. duodenaria*; (b) *C. steinii* as shown in BRESSLAU's (1921) Taf. 20, Figs. 4 and 5.

Legend.

AM = adoral membranelle zone.
AR = apical ring.
CC = caudal cilia.
Cpr = cytoproct.
Cst = cytostome.
CVP = contractile vacuole pore.
RCF = right cytostomal ciliary field.



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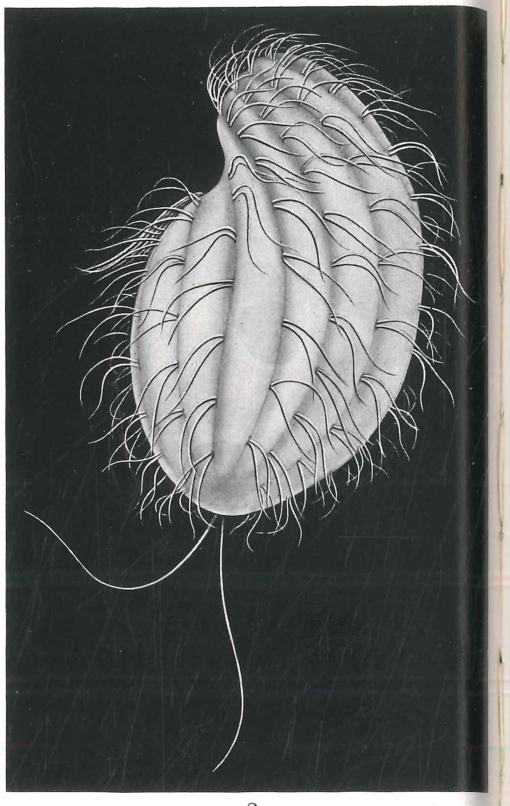

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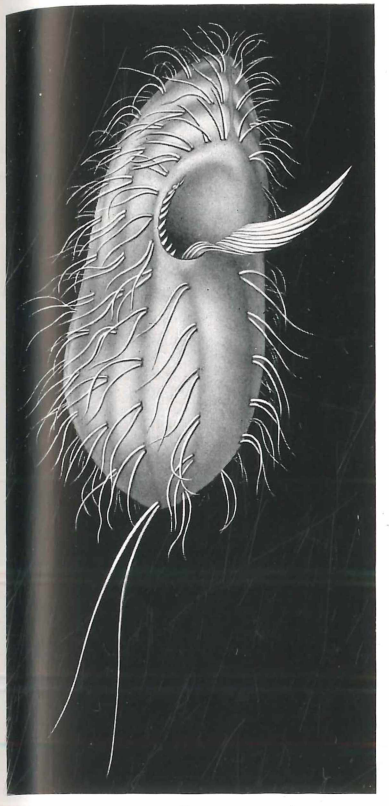

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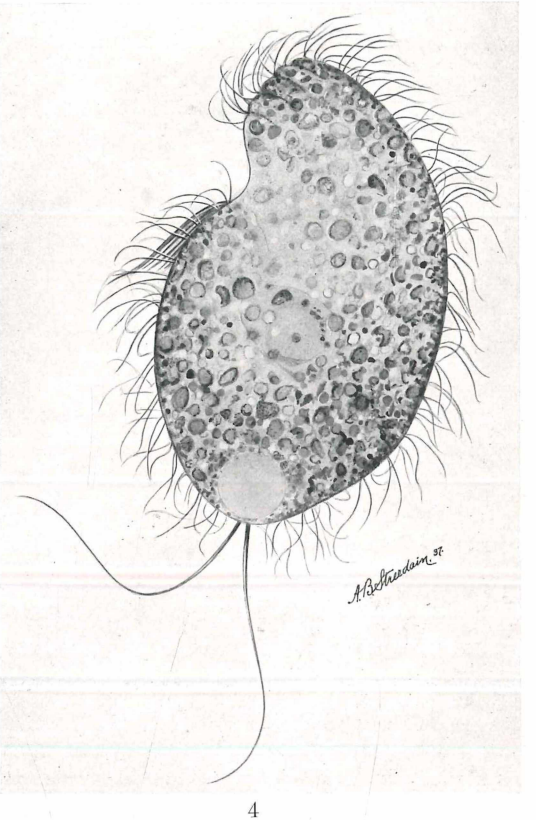

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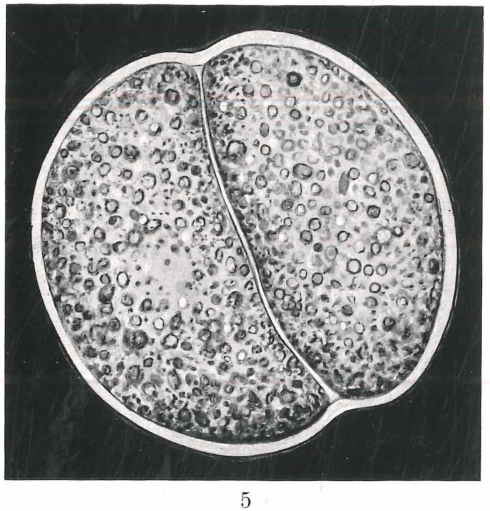

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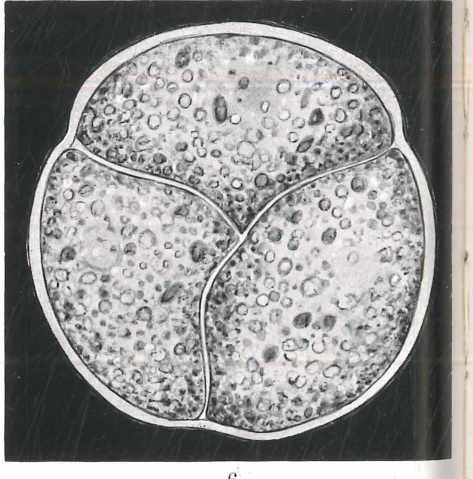

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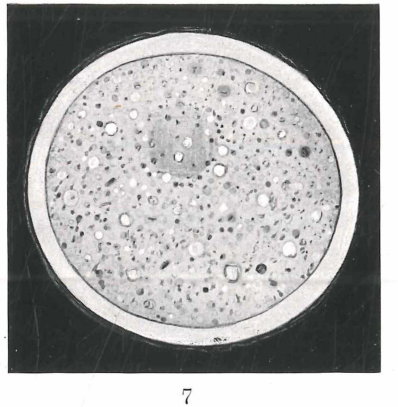

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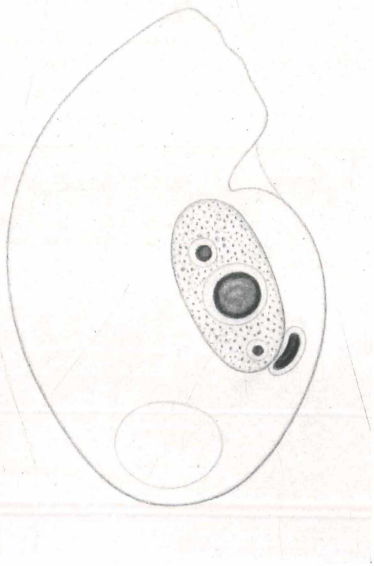

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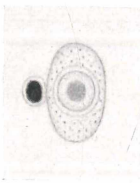

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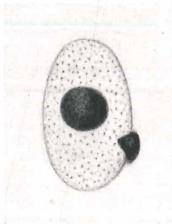

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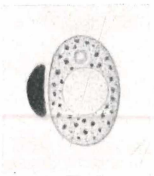

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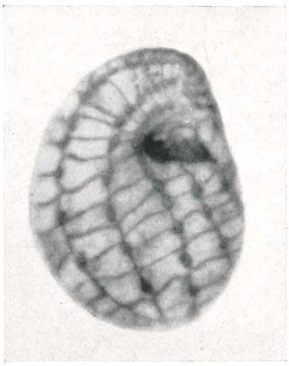
VERLAG VON GUSTAV FISCHER IN JENA.



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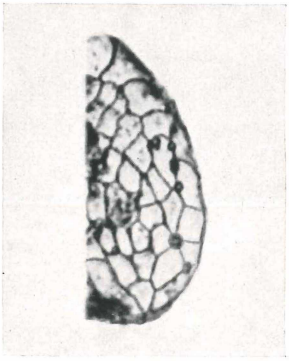

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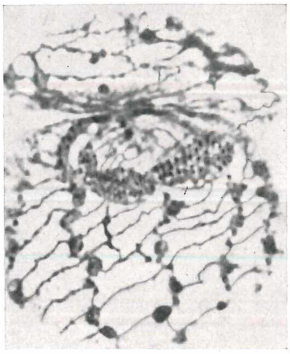

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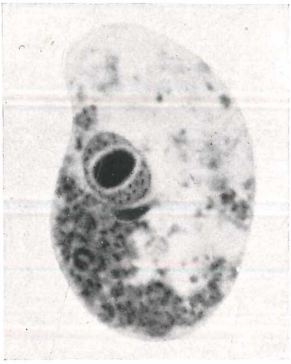

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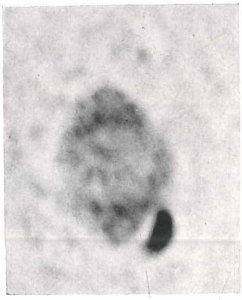

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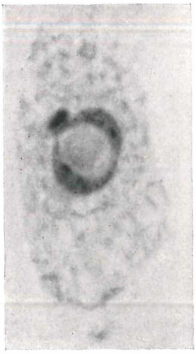

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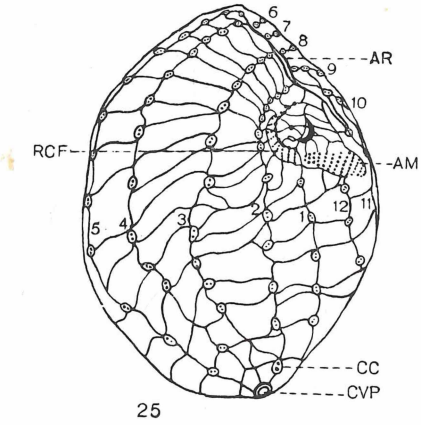

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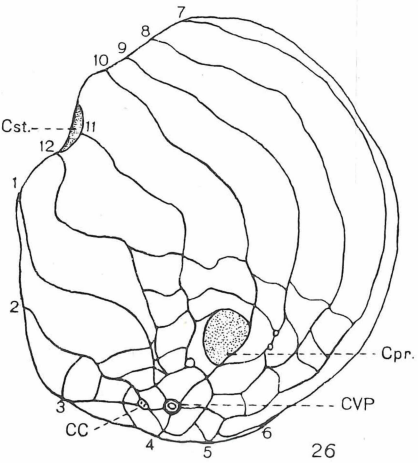

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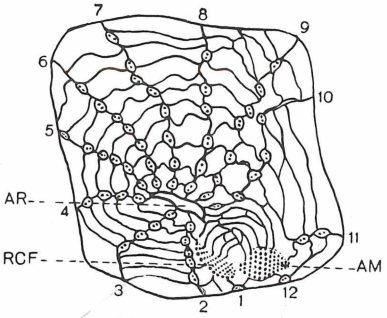

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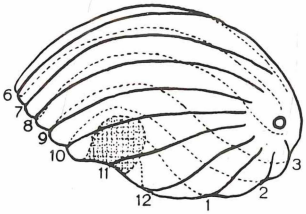

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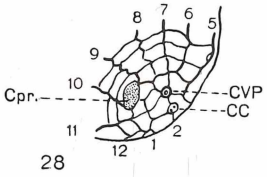

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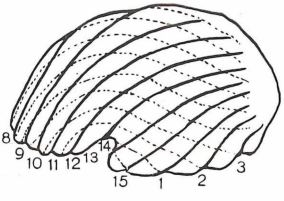

29b

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