Reorganization of the "Silverline System" in the reproductive cysts of *Colpoda duodenaria*.

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With plate 2—4.

Protoplasmic reorganization is now apparently characteristic for the protista during their fission, conjugation, regeneration and cystment. It has long been known that this reorganization involves the dedifferentiation and redifferentiation of the more conspicuous organelles, such as cirri, membranelles, cilia and flagella. But to what extent it may also involve the less conspicuous and often quite obscure structural differentiations has not thus far been adequately investigated.

The results of studies herein reported are concerned primarily with the reorganization of the silverline system of *Colpoda duodenaria* during its fission in quadrigenic cysts (reproductive cysts which yield four daughter cells). These results demonstrate not only the complete resorption and redifferentiation of the silverline network but also a remarkable reversion in cell symmetry, viz., from the asymmetry of the free-swimming *Colpoda* to a radial symmetry which becomes evident in the initial stages of the cell's redifferentiation.

This striking reversion to the more primitive type of symmetry and the complete resorption of all traces of the intricate silverline system, a detailed account of which is given below, indicate the profound nature of protoplasmic reorganization and that such phenomena may accordingly have their counterpart in other protista forms.

Technique.

The silver nitrate techniques of v. Gelei (1934) applied to the reproductive cysts of this ciliate provide a means of tracing the reorganization of certain structures denied by any similar methods so far tried out. Of these structures, the most significant are the intermeridional fibrils and the cytostome. This morphological study, therefore, is based almost entirely upon material prepared according to these methods.

The coverslip method of handling cysts was used throughout. Scrupulously clean coverslips were placed in Syracuse watchglasses over which was poured a culture of precystic organisms.[1]) When a sufficient number of cysts had been formed on a coverslip it was removed to another watchglass containing balanced medium. There the cysts were left until the greater number had reached the desired stage, when the coverslip was removed and promptly dropped into the fixing solution held in a Columbia coverslip staining jar. The fixatives selected were the "Formol-sublimat" and "Osmium-sublimat" mixtures (or a combination of these). Cysts were measured before and after a fixation period of thirty seconds and these measurements show that little if any shrinkage occurs. Furthermore, the shape of the cysts is beautifully retained, which is not altered by further treatment.

Both these methods (v. Gelei, 1934, pp. 109—110) gave good results although precipitates formed occasionally, particularly in early reorganization stages, even though careful washing, first in Osterhouts balanced medium and then in glass distilled water, preceded the addition of silver nitrate solution (2 %). Preparations with precipitates of course are misleading and therefore considered unsuited for critical study.

Sunlight or ultra violet light produced by a General Electric Type H-4 lamp[2]) was used to reduce the silver. In the following respects the artificial light is preferable to sunlight: (1) it is available at any time; (2) the light intensity is not variable and therefore the length of exposure (2—3 minutes) to obtain a desired result is always the same (the different stages of cystment require different time-lengths of exposure); and (3) clean, delicate unbroken

[1]) For method of obtaining numerous organisms of about the same age after fission, see Taylor, Brown and Strickland (1936).

[2]) This equipment was kindly lent by Dr. P. R. Needham of the United States Bureau of Fisheries.

lines are produced which may be made thicker before washing upon exposure to the light of a SPENCER (Model 370) microscope lamp. The ultra violet light was adjusted 11,5 inches above the preparation, which was laid in a Syracuse watchglass and covered with a minimum layer of distilled water. Heat (32 to 34° C.) from the lamp was reduced by placing the watchglass in a pan of water having a temperature of 20° C.

Preparations were studied in water or in glycerin, or they were first made more permanent by dehydrating in ethyl of tertiary-butyl alcohol, clearing in creosote or xylene, and mounting in neutral balsam. The temporary preparations are useful because there is no shrinkage and when the cysts become flattened by pressure of the coverslip, a greater area can be observed in a single focus of the microscope. They are, however, less transparent than "permanent" slides. The latter preparations were studied also immediately upon completion for experience has shown that most preparations begin to darken after a few days, particularly when they have been exposed to the microscope light, as they must be during study. Slides kept in the dark in a refrigerator remained unchanged for a longer time. A green cellophane screen was advantageous in the study of fine details.

The following description of reorganization refers chiefly to quadrigenic cysts because, in the first place, these cysts predominate in any flourishing culture, and secondly, with the methods of culture used in this laboratory, the extremes (di- and octogenic cysts) of reproduction can be almost entirely eliminated. Whenever a new supply of organisms was obtained from an old culture, or from protective cysts, at least two periods of division were allowed to occur before the cysts were used for this study. Pouring off the organisms which have not encysted at a given time brings about greater uniformity in the following encystment time, provided the amount of food is adequate for the number of organisms present.

Decription.

The first visible signs of reorganization in the "silverline system" of *Colpoda duodenaria* preceding fission do not appear until after encystment. Precystic organisms (Pl. 2 Fig. 3) were carefully scrutinized before and also after the general procedure was well known but no certain evidence was discovered. In the earliest stages of encystment the change is plastic in nature, as observed

by Klein (1929), and may be described as a mechanical adjustment to the spherical form. This alteration results in a phase wherein the organism's entire peripheral surface lies against the cyst membrane, except that the body grooves are not entirely lost. At first the peristomal cavity is still present. Later the membranelles and ciliary field are resorbed and then this region, too, lies flattened against the cyst membrane. The condition described is a decided advantage to the observer.

Since the earliest modifications of structure occur along the longitudinal meridians, these will be described first. Furthermore, the meridians share a similar history throughout the reorganization, except at certain points where they are intimately associated with the resorption and (or) development of more highly differentiated parts, particularly of the anterior end. For convenience of description, the latter will be considered separately.

Meridians.

Each meridian in the differentiated, free-swimming organism (Pl. 2 Fig. 1) is composed of a longitudinal fibril interrupted at intervals by rings within each of which a pair of cilia originates. Adjacent longitudinal meridians are united at irregular intervals by intermeridional fibrils. The pattern is distinctive but variable to a slight degree: (1) the intermeridional fibrils join the longitudinal meridian at irregular points with reference to the ciliary rings; (2) their number varies; and (3) occasionally they are atypical in form (Pl. 2 Figs. 2 and 6). Of the meridians which extend to the keel, both ciliary rings and interconnectives are more numerous at the anterior end of the body. The intermeridians are also closer to each other on the left side of the organism, just anterior to the polygon (Pl. 2 Fig. 3) where the ciliary rings are relatively scarce.

The present study has brought out certain new though minor details of structure. The number of meridians that each individual may possess has been found exceptionally to vary from eleven to fourteen, according to observations made to date. More than 95% of the organisms prepared for this investigation, however, had twelve. Furthermore, the oval ring, within which a pair of cilia arise, previously described (Klein, 1929; Taylor and Furgason, 1938) really consists of two rings (basal rings?). In all deeply stained preparations the outer border of the juxtaposed rings is more conspicuous, particularly, but not exclusively (Pl. 2 Fig. 5) when the dry method of Klein has been used. The cilia arise from a slight depression

or "dimple" in the body wall, and the slight amount of debris clinging to the base of the cilia, also adsorbing the silver nitrate, might cause this difference in appearance. A single granule (basal granule?) in each ring is present in some specimens but in silver nitrate preparations such granules are not so clearly defined as the basal granules of haematoxylin preparations.

Before the parental (old) cilia are resorbed, distinct ringlets make their appearance in the vicinity of the paired ciliary rings (Pl. 2 Fig. 9), and occasionally elsewhere along the longitudinal meridian. Their number increases, thereby producing a series of clustered ringlets (Pl. 2 Fig. 10), which later unite to form a longitudinal ribbon (Pl. 2 and 3 Figs. 11—14). The ribbon is not completed at the same time in all parts of the organism, a fact which may be related to the differences in the time of the first appearance of the ringlets at various points along the meridians. The old intermeridional connectives and the ciliary rings persist for a considerable time, the former being particularly conspicuous. The longitudinal fibers, on the other hand, gradually disappear during the formation of the ribbon, except near the polygon of the posterior end, where reorganization lags, and therefore resorption is delayed.

In the cysts examined in water immediately after reduction of the silver, the stages during which the old cilia are resorbed can be determined. The gradual evaporation of the water, when the edges of the coverslip have not been sealed, brings them into view when present. In digenic cysts the old cilia disappear soon after cleavage but in quadrigenic cysts at least some persist until the time of the second cleavage, when they are replaced by a completely new cover of small cilia. Both old and new cilia were not found in any individual but the earliest stages of the new, outgrowing cilia would be too small to determine their presence with certainty. It is doubtful that, during fission, a period exists which is devoid of either new or old cilia, as determined in silver nitrate preparations, but if it does, the period must be very short. These findings agree with observations made on cysts dehydrated in alcohol and preserved in balsam. In rather deeply stained individuals, the old rings are easily seen as a pair of white spots usually at the right edge of the cluster of small rings, and at a later stage at intervals along the ribbon network. They have been found also in lightly stained preparations (Pl. 2 Fig. 10), both temporary and permanent. During the stage described in the following paragraphs, the old ciliary rings disappear and the new become clearly defined.

The ribbon network is not homogeneous throughout: In many preparations structures can be distinguished which appear like chains of miniature rectangles, each with a distinct central granule. A slight modification of such structures is found in the remaining preparations, in which the granules are united by silverlines. In either case the chains are of unequal length and seem to be well distributed along the meridians. The right and left edges of the ribbon are dissimilar: Along the right edge looped fibrils make their appearance which proceed to grow toward the right adjacent meridian, and eventually replace entirely the old intermeridional fibrils. The network formed by the loops gradually become the intermeridians of the new daughters. Simultaneously with the growth of the "pro-intermeridians" the ribbon decreases in width and tiny, closely set ciliary rings in pairs can be distinguished therein. When these pairs come to lie farther apart, a longitudinal fibril is present.

The outgrowth of the looped threads can best be described by means of a series of figures drawn with the aid of a camera lucida (Pl. 2—4). It must be stressed that a detailed study of the earlier stages is a necessary preliminary to an interpretation of the more complicated pattern of the network of the later stages. Furthermore the regions of the organism should be taken into account, for at both the extreme anterior and the posterior ends, the outgrowth of the loops is delayed, as compared with the mid-region. When this study has been made it becomes clear that new loops are continuously arising over a period of time so that near the end of the period some intermeridians are fully differentiated, others are in earlier stages, and still others have apparently already disappeared. The length of the period probably depends in part upon the number of cleavages taking place (the greater the number of cleavages the greater the number of meridians to be formed), and new loops have been found in very large cysts after the second cleavage, a fact which suggests that a third fission might have eventually occurred in these cysts.

Each loop may form two meridians but it is not argued that they do so in all cases for contiguous loops appear to possess a common "fibril". Thus, for example, three contiguous loops lying side by side would form four instead of six intermeridional connectives, as would be the case were these three loops adjacent though not contiguous. The characteristic X-shaped fibril in the late stage of development (Pl. 3 Fig. 21) is a composite fibril formed by a small new loop appearing at the base of one side of an older loop.

This type of multiplication (Pl. 3 Fig. 18) might be interpreted as a splitting of a previous fibril but there is no visible difference in size of the fibrils and a small loop is not always found in the position just described but may occur without contact with an older, outgrowing one. It is fairly clear that some of the loops formed again disappear as they reach the meridians toward which they have been approaching, being replaced by others forming behind them. This re-dedifferentiation might be looked upon as evidence of an abbreviation, and not a total absence, of the period eliminated when cleavages follow one another without an intervening differentiated, free-swimming period.

Eventually this period of fibril formation slows down, no additional loops appear and those now present are fully differentiated or are in the form of Y's with longer or shorter stems. Y-fibrils are sometimes found even in the excysting, young organism. From evidence found in free-swimming forms (Pl. 2 Figs. 2 and 6), it seems entirely likely that a few new intermeridional fibrils are being formed throughout this growth period.

During early stages, as shown in Figures 15 and 16 (Pl. 3), the outgrowth of the looped fibrils are at irregular intervals along the meridians. The presence of groups or patches of looped fibrils is therefore characteristic of these stages. Later the irregularity is scarcely noticeable for new loops apparently may arise anywhere along the right side of the meridian.

The following differentiated parts arise along the old meridian: (1) paired ciliary rings, (2) basal granules (and therefore cilia), (3) intermeridians, and (4) longitudinal meridional lines.

It is possible that all these differentiated parts form within or from the ribbon network. This might explain why the ribbon decreases in width. A different interpretation might be given the ribbon network: It is known for other ciliates that before the appearance of new cilia, the pellicle is resorbed above each ciliary primordium, permitting the new organ to arise above the surface. If this resorption occurs in dividing *Colpoda*, the reticulum might be caused by the silver being deposited at points of discontinuity of the pellicle. The fact remains, however, that the cilia do arise along the ribbon network and the pro-intermeridians do so as well. A more detailed analysis of the ribbon network, including the chains of similar small structures, will receive further study in connection with the resting cysts of this form.

The interpretation that new structures are differentiated from or within the ribbon network is similar to Klein's (1929), but this investigator stated that the fine network ("engmaschiges Gitter") extends over the whole organism and later becomes wider in mesh. He did not observe the well-defined ribbon stage nor did he describe the gradual outgrowth of the looped fibrils from meridian to meridian. Furthermore, as will be shown later, there is no stage in this *Colpoda* during which the cytostome bears a fine network as described by Klein.

A different function or relationship than has heretofore been described for the intermeridional lines is suggested in some preparations. Instead of looped fibrils there appear to be "tongues" of an outflowing (?) substance made noticeable by their yellow or brownish color (Pl. 3 Fig 17). The periphery of this "tongue" appears as a fine thread or line and probably is idential with the looped fibrils already described. Nearly all the pro-intermeridians present this appearance in some preparations, and more often when creosote has been used as the clearing reagent. The final stages, on the other hand, show the intermeridians as lines or fibrils, with the inter-substance clear and inconspicuous as in the other preparations described and illustrated. It is thought that this substance being formed might be the new pellicle which only at first absorbs the silver nitrate. Whether or not the pro-intermeridians are underneath this substance or have any part in its formation are questions which cannot be answered at the present time. In position and general appearance the pro-intermeridians in the condition just described resemble the "infraciliature" of Chatton and Lwoff (1936).

It has been observed that in the region immediately next to the cleavage plane, no intermeridional fibrils are formed (Pl. 3 Fig. 16) until fission is completed. This fact suggests that the pellicle also might not be formed there until at this time.

Cytostome.

The old cytostome is entirely resorbed at an early stage of encystment. In relation to the reorganization of the meridians, this resorption usually is completed before the looped fibrils appear. The resorption is gradually progressive and many intermediate stages have been noted. The fibrils which line the mouth cavity (Pl. 2 Figs. 1 and 7) and the preoral ring are always the last to be resorbed, but within a short time the cytostomal region is no longer

recognizable because it has been entirely replaced by longitudinal meridians continuous with those situated immediately behind the former cytostome. The intermediate stages indicate that this condition may be brought about by a shifting of materials over this region from the region immediately posterior to it. At least it is true that the comparatively wide ribbons of the meridians intercepted by the old peristome are thinner the closer they approach the anterior end, and the old intermeridians appear to be likewise carried forward (Pl. 4 Fig. 24).

The stage has now been reached in which all meridians are continuous from pole to pole, so that this reorganizing ciliate visibly begins its redifferentiation as a radially symmetric cell (Pl. 4 Figs. 24 and 25). The anterior pole terminates in a tiny ring (or granule) about which is formed a polygon through the formation of new intermeridians. It can be clearly seen now that a few meridians do not extend directly from the polar ring but extend from the polygon (Pl. 4 Fig. 26) comparable with the relations that obtain at the posterior pole in this stage of division as well as in the differentiated organism (Pl. 2 Fig 4). The old contractile vacuole pore is still present in the posterior polygon at this stage (Pl. 4 Fig. 29), not being replaced with a smaller one until during the interval of the first fission.

During this preliminary stage of radial symmetry when the meridians extend from pole to pole, the first cleavage furrow makes its appearance. Structurally, in silver nitrate preparations, this is first recognizable when the meridians in the equatorial region become S-shaped. The pro-intermeridians have already appeared; the extent of their outgrowth varies between the various lots of cysts but is fairly constant in the same culture and when the individuals are nearly the same size. The first fission is completed before the second begins, a point which can be readily determined in permanent preparations because they are then highly transparent. The relative time of the completion of the first cleavage is also known from an observation made on living cysts from which the membrane had been removed. The halves were seen to separate completely and to lie at some distance from each other. (It is possible that a fine connection sometimes lingers, as known for ciliates which divide in the free-swimming stage.) Promptly after completion of the first fission a new furrow, also transverse to the longitudinal meridians, appeared in both halves, an event which occurred with clock-like precision.

The primordia of the new cytostome become visible during the first fission. A thickening appears on one meridian near the anterior pole of each cell being formed. This is meridian no. 1 (the stomatogenous meridian), and the thickening is the primordium of the ciliary field (Pl. 4 Fig. 26). Later thickenings appear, in progressive order, on the meridians to the left as well. These are the beginnings of the membranelle series and latticed fibrils of the cytostomal cavity. About this time also the second cleavage begins, and similar developing parts in the two posterior daughters can be seen along the deepening furrow. This suggests that these structures for the posterior daughters must have been forming earlier, although they are not meantime definitely recognizable. At first all the thickenings extend over a considerable length of the meridians (Pl. 4 Figs. 27, 28 and 30) but as differentiation continues they become shortened considerably. Parallel rows of granules (basal granules?) appear very early; before this time a distinct pattern seems to be lacking in the network.

The differentiation of the ciliary field precedes slightly that of the membranelles. The arrangement of granules in rows is perpendicular to the longitudinal meridians, and from eleven to eighteen rows have been counted in different individuals. Difficulty in determining the detailed later development of the field is experienced because a concavity soon appears therein, previous to formation of the cytostomal cavity. Pro-intermeridians being formed by meridian no. 1 along the outer (right) border of the ciliary field, result in about nine to eleven intermeridians. This large number in the young organism is of interest because the free-swimming forms commonly have only four.

Typically four meridians are involved in the formation of the membranelles. Of the many organisms examined, only one has been seen in which three meridians were concerned, but twenty in which five meridians formed the membranelles. Four of the latter organisms had thirteen instead of twelve meridians. The development of the membranelles into a structural unit proceeds in a definite series of steps (Pl. 4). Each of the participating meridians becomes distinctly curved to the right so that the anterior ends of the thickenings are now directed toward meridian no. 1. Each of the two meridians to the left, and possibly the third also, develops a transverse constriction which divides each primordium into unequal parts (Pl. 4 Figs. 28 and 30). The smaller, anterior portions become transformed into the network of the cytostomal cavity. The

arrangement of granules (basal granules?) in the membranelle primordia (the posterior part of the thickening) are in three longitudinal rows when they are first clearly visible and are larger and therefore more conspicuous than those found in the ciliary field. Each set of three rows of granules retain, for a considerable time, a connection with the meridian from which it was formed. Next the units of three rows of granules become closely approximated to each other (Pl. 4 Fig. 32), and the intermeridians which were forming in the intermeridional spaces again disappear (cf. Figs. 30 and 31, Pl. 4). At the same time the whole moves into a position opposite the ciliary field.

The ciliary field and membranelle series at this stage appear very much alike in general shape, size, and position (Pl. 4 Fig. 32) and do not in the least suggest the difference in their origin; for, as just described, the ciliary field is derived from one meridian, whereas the membranelles are derived from three, four, or five, but commonly, four meridians. The rows of granules of the membranelles are always more distinct, however.

The final developmental stage of the oral region is the completion of the cytostomal cavity, a procedure which also involves the formation of the preoral keel and ring. Although both ciliary field and membranelle series are carried inward by the invagination, their outer margins remain at the periphery. Now the membranelles rotate in a posterior direction so that in their definitive position they lie almost at right angles to the ciliary field. The extreme right end of this series lies deeper in the body and eventually lies under the posterior end of the ciliary field.

During the change in position of the membranelles the anterior pole becomes displaced in a latero-posterior direction along with the formation of the keel and the cytostomal cavity. The pole then comes to lie within the modified oral region (apical ring and mouth). The meridians (2—9) now extend directly from the apical ring, which is usually bisected with a connecting fibril (Pl. 4 Fig. 34). Meridians no. 1 and no. 2 impinge upon the mouth proper, while no. 11 becomes indirectly connected with meridian no. 10 (Pl. 2 Fig. 1) and thus to the preoral ring. It is difficult to trace the precise origin of the preoral ring because there are slight variations in the pattern around the anterior pole in these stages in different individuals. From a study of a considerable number of organisms and stages, the series shown in Plate 2—3 was selected as being typical.

This series of stages in the development of the cytostome has been found to take place also within the resting cyst, during the excystment period.

Contractile vacuole and cytoproct.

The old contractile vacuole pore is replaced with a smaller one some time during the interval of the first fission. At about this time also, a new contractile vacuole begins to function in the anterior daughter, as observed in the living organism. If another fission takes place, then the question arises: Is a new and still smaller contractile vacuole pore formed in each posterior daughter when new vacuoles arise in the anterior daughters? Structural evidence on this point in silverline preparations is difficult to obtain because of the small size of the new pores and the fact that they have not been observed in one organism at successive stages. The size of the pore and the size of the organism seem to be correlated, however.

The opening of the cytoproct apparently is formed anew at each functioning, for no permanent structures, such as the contractile vacuole pore ring, has been found. The locus of its formation nevertheless seems to be fairly well defined (Taylor and Furgason, 1938).

Location of silverlines.

It was considered important to determine, if possible, the exact position of the silverlines in the cortex. Free-swimming organisms (and cysts) crushed under the coverslip give ample evidence that the lines are very close to the periphery but this is also true of the ectoplasmic layer, which is comparatively thin in this ciliate.

If the lines represent grooves in the pellicle, one wonders why the China blue relief method does not reveal the characteristic pattern. Numerous China blue preparations were made but not a single specimen suggested it, altough the longitutinal body grooves were present in individuals retaining the normal body shape. When this relief method is used upon *Paramecium* the well-known surface pattern is beautifully delineated in the majority of specimens. On the other hand, in *Euplotes patella*, with its rigid pellicle, this same method does not show the polygonal silverline pattern of the ventral and dorsal surfaces.

Cross-sections of silver nitrate preparations, using the paraffin method, were made of free-swimming and encysted *Colpoda*

The sections, five microns in thickness, were mounted on slides with and without HAUPT'S fixative. The zone of silverlines is clearly visible in most of these sections. In free-swimming forms they appear to lie beneath a clear, translucent, thin pellicle. Whole mounts of free-swimming forms also show a clear layer outside the silverlines. Individuals are often oriented so that the intermeridional fibrils can be seen perpendicular to the slide surface, and in such favorable locations, a pellicle can be distinguished outside the fibril.

The evidence presented seems to indicate that the silverlines are beneath a pellicular layer, but because of the small dimensions involved, it has been considered desirable to continue this part of the study by employing other methods as well, and report the results later.

Discussion.

A detailed acount of the reorganization of the silverline system in *Colpoda duodenaria* during its fissions in quadrigenic cysts has been presented in foregoing paragraphs. It was noted that the observed structural changes include essentially (1) the complete resorption and redifferentiation of the silverline complex and (2) a striking reversion from the asymmetry of a fully differentiated cell to the more primitive radial symmetry at the initial stages of its redifferentiation.

Previous to any visible constriction in the plane of first fission, the meridional fibrils have disappeared with the formation of meridional ribbons, the mouth and mouth parts have vanished, a new anterior pole has appeared diametrically opposite the posterior pole and all the meridional ribbons now extend completely from pole to pole thus revealing a radially symmetric cell. Looped threads have also made their appearance along the right side of each ribbon and are proceeding across to the adjacent meridian gradually to replace the old intermeridional fibrils. Simultaneously the primordia of the new mouth parts, — the ciliary field and membranelles, become evident as widened anterior portions of the first and the twelfth, eleventh, tenth and ninth meridional ribbons respectively.

This resorption and redifferentiation of the old and new silverline systems mark the visible events of reorganization during the first fission of quadrigenic cysts of *Colpoda duodenaria*. The second fission, however, seems to transpire without a resorption of the structures redifferentiated during the preceding fission. One might conclude, therefore, that, perhaps due to the immediacy of second

fission, no reorganization then occurs. An alternative possibility, however, should not be overlooked. For example, it will be recalled that the looped threads which eventually form the new intermeridians appear to arise continuously throughout the periods of fission. This would indicate that redifferentiation, at least, is for these fibrils without interruption and so may suggest a continuous reorganization of protoplasmic constituents out of which new structures come to be formed. Thus, invisible constituents may undergo resorption and redifferentiation within the visibly differentiating membranelles which, therefore, persist troughout the period of second fission. An analogy to this is evident in the reorganization of the macronucleus of various ciliates in the migration of so-called reorganization bands during fission, yet leaving this organelle as such intact.

The remarkable reversion to radial symmetry in *C. duodenaria* at the beginning of redifferentiation, or what may properly be regarded as ontogenesis (Taylor, 1935) in the fission cycle, recalls the hypothesis advanced by Bütschli (1887, p. 1353) for the phylogeny of the ciliates in general. According to this hypothesis, in the primative ciliate the ciliary meridians converge in the anterior and posterior poles of the original polar axis, thus defining a radial symmetry. But since the circular mouth of this primitive ciliate (cf. *Holophrya*) precisely coincides with the anterior pole, the mouth interrupts all the ciliary meridians which thereby impinge symmetrically upon it. Now in spite of the many and varied evolutionary modifications responsible for the extraordinary diversity in the Ciliata of today, this primary relationship of mouth and ciliary meridians remains essentially unimpaired. In this connection, the most important of these modifications is the posterior elongation of the mouth on one side, — designated then the ventral side. Apparently correlated with the spiral course of the ciliary meridians, which is a general characteristic, this elongating mouth tends to spiral with a resulting asymmetry. Moreover, the elongating mouth tends in later phylogeny to foreshorten toward its posterior limits and, conspicuous for the holotrichs, this foreshortening leaves in its wake an apical ring. On this mouth complex, which includes the apical ring, all ciliary meridians now impinge so that the original relations of mouth and meridians remain unchanged.

However valid one may regard this interesting hypothesis for ciliate phylogeny, the ontogenetic redifferentiation of the mouth and silverline system in *Colpoda duodenaria* follows in general this sequence of events as detailed in foregoing paragraphs. An original

radial symmetry with diametric poles connected by all the meridional ribbons, a latero-posterior development and asymmetric invagination of the mouth and redifferentiating mouth parts, with the ciliary meridians finally impinging upon the apical ring and cytostome, — all this conforms in outline to the phylogenetic history proposed in BÜTSCHLI's hypothesis. At any rate, it would seem that a counterpart of the phenomena in *Colpoda's* reorganization way be found in other protistan forms.

Conclusions.

1. Reproductive cysts of *Colpoda duodenaria*, freshly prepared according to the silver nitrate methods of v. GELEI, provided the basis for this study of reorganization.

2. A preliminary survey of variation in the structure of free-swimming organisms, as revealed by these methods, showed that the structural changes leading to fission are confined to the encysted organism.

3. A detailed account is given of the reorganization of the intermeridians, whose primordia appear along the right side of each reorganizing longitudinal meridian. The pro-intermeridians, by a characteristic type of growth, proceed to cross the intermeridional space to the right and the old longitudinal meridians disappear before tho advance of the new.

4. Before the first fission furrow appears, the redifferentiating meridians initially extend completely from pole to pole. Since the poles are now diametrically opposite, the reorganizing cell thus exhibits radial symmetry.

5. The new ciliary field is the fist of the cytostomal primordia to appear, and is developed as a thickening in meridian 1 near the anterior pole.

6. The primordia of the new membranelles and lattice network of the cytostomal cavity are combined and appear as thickenings in the three, four, or five (commonly four) meridians to the left of meridian 1.

7. A transverse constriction separates the primordia of the membranelles from those which form the lattice network, the latter being nearer the anterior pole.

8. The membranelles become a structural unit secondarily by an elimination of the intermeridional spaces between them, with resorption of the intermeridians formed in these spaces.

9. The process of invagination by which the cytostomal cavity is formed is accompanied by the formation of the preoral ring in place of the anterior polygon, and appearance of the preoral keel.

10. The location of the silverlines in the cortex is considered and evidence from cross-sections of silver nitrate preparations is presented.

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Explanation of plates.

Plates 2—4.

All drawings were made with the aid of camera lucida, × 2400.

Plate 2.

Fig. 1. Left anterolateral view of free-swimming organism. Fixative: mercuric chloride-formalin-osmic acid. Ultra violet light reduction.

Figs. 2 and 6. Portion of silverline pattern of free-swimming organisms showing atypical intermeridians (probably pro-intermeridians). Fig. 2. Mid-region, left side; Fig. 6, right side. Mercuric chloride-formalin. Sunlight.

Fig. 3. Precystic organism, left posterolateral view. Mercuric chloride-formalin. Sunlight.

Fig. 4. Posterior end of precystic organism showing contractile vacuole pore within polygon, and origin of longitudinal meridians. Mercuric chloride-formalin. Sunlight.

Fig. 5. Early encysted stage before beginning of reorganization. Anterior view. Ciliary rings obscured by deeply stained border. Mercuric chloride-formalin. Sunlight.

Fig. 7. Cytostomal region in early stage of encystment. Ciliary field and membranelles separated (compare with Fig. 1). The ring at end of lattice network of flattened mouth cavity is opening of cytopharynx. Mercuric chloride-formalin-osmic acid. Ultra violet light.

Figs. 8—23. Reorganization of longitudinal meridians. The drawings were made from midregion of body or, after fission, along either side of fission furrow.

Fig. 8. Section of two longitudinal meridians, with interconnectives, of precystic organism. Fixative: mercuric chloride-formalin. Ultra violet light reduction.

Fig. 9. Earliest stage in formation of ringlets in region of old ciliary rings.

Fig. 10. "Cluster“ stage of meridians showing typical location of old ciliary rings. The left side of the meridian (right in drawing) is deepest part of body groove.

Fig. 11. Transition stage from "cluster“ to "ribbon“ stage.

Fig. 12. Same as above but note the chains of tiny rectangles with granules, interrupted by the old ciliary rings (side view) and old cilia. Compare with Fig. 11, in which similar structures are suggested.

Fig. 13. Completed ribbon. Network comparatively wide in midregion of meridians no. 1 and no. 12.

Plate 3.

Fig. 14. Earliest appearance of looped threads (pro-intermeridians). Section taken from small cyst (probably digenic).

Fig. 15. During first fission. Patches of loops with decrease in width of ribbon network.

Fig. 16. During first fission. Note absence of pro-intermeridians along ribbons in fission furrow.

Fig. 17. The pro-intermeridians appear as "tongues“ of an outflowing (?) substance. Preparation cleared in creosote.

Fig. 18. Note the location of smallest loops in relation to older ones. During first fission.

Fig. 19. Network of pro-intermeridians as found near end of first fission.

Fig. 20. Pro-intermeridional network in early period of second fission.

Fig. 21. Y-fibrils with long stems and fully differentiated intermeridians. Near end of second fission.

Fig. 22. Later stage of Y-fibrils.

Fig. 23. Meridians of fully differentiated daughter (1 of 4) ready for excystment. Compare with Fig. 8.

Plate 4.

Figs. 24—25. Stages in development of cytostome. Fixative: mercuric chloride-osmic acid-formalin. Ultra violet light reduction.

Fig. 24. Anterior pole. Early stage before first fission.

Fig. 25. Slightly later stage than shown in Fig. 24. Note origin of longitudinal meridians at polar ring.

Fig. 26. Anterior pole with polygon. Meridian no. 1 with primordium of ciliary field. First fission in progress.

Fig. 27. Anterior pole with developing ciliary field, and compined primordia of membranelles and lattice of cytostomal cavity to left of meridian no. 1. During second fission.

Fig. 28. Anterior end of one of four daughters. Early stage of membranelle rows and lattice network of cytostomal cavity.

Fig. 29. Posterior pole of stage shown in Fig. 26. Old contractile vacuole pore present; polygon reorganizing.

Fig. 30. A slightly later stage than shown in Fig. 28. Note unequal lengths of membranelle rows.

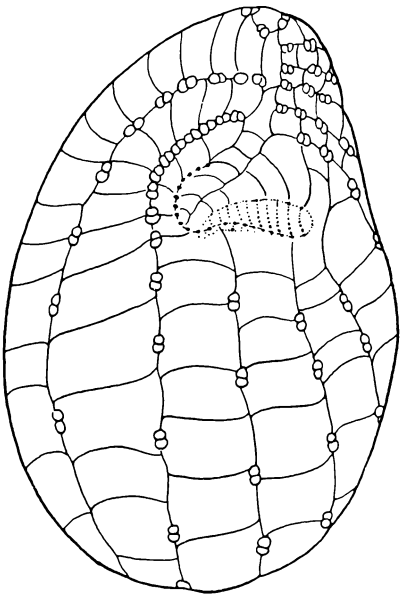
Fig. 31. Membranelle rows (in sets of 3 rows of granules with connecting silverlines) becoming approximated. Cytostomal cavity beginning to form.

Fig. 32. Membranelles have become a unit (12 rows of membranelles); cilary field lies opposite.

Fig. 33. Invagination of ciliary field and membranelles almost completed. Note preoral ring.

Fig. 34. Right end of membranelle series lies under posterior end of ciliary field. Pre-oral keel forming.

Fig. 35. Preoral ring of older, differentiated organism. Region flattened by pressure of coverslip; non permanent preparation in water. Compare with Fig. 1 Plate 2.



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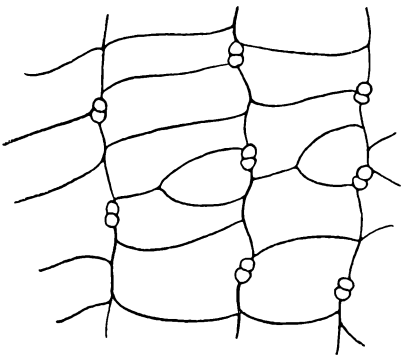

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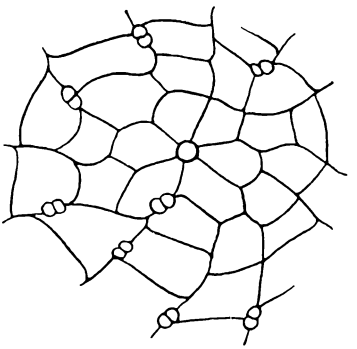

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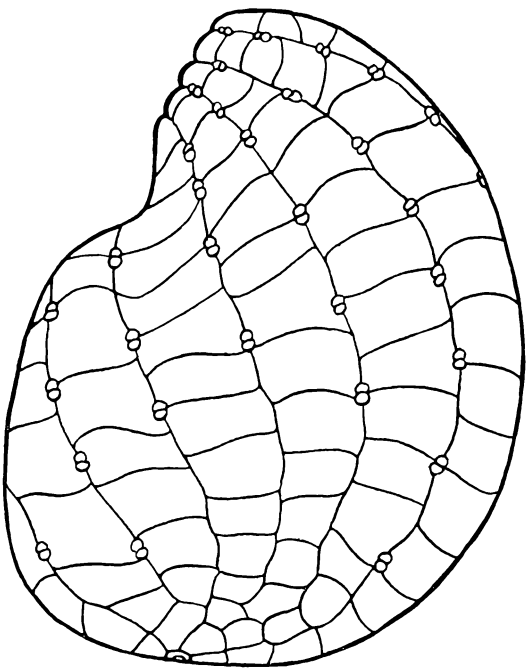

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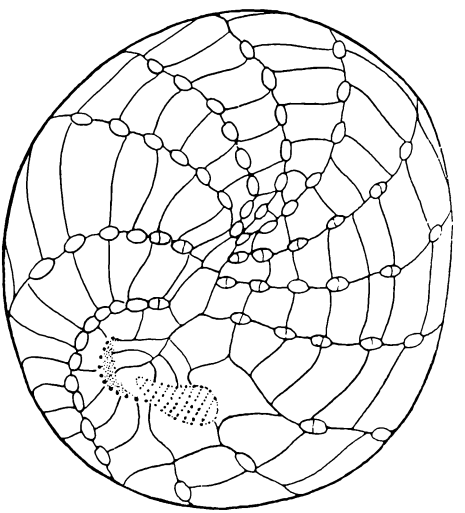

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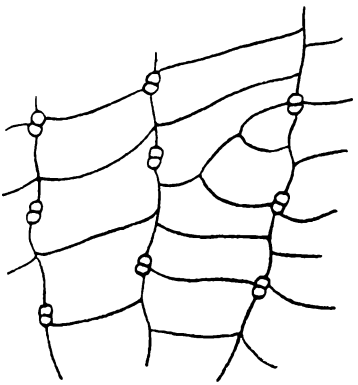

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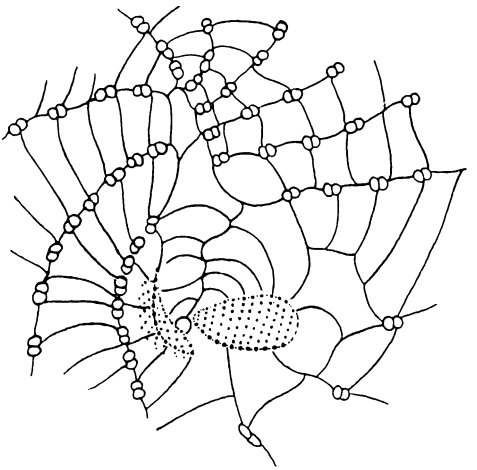

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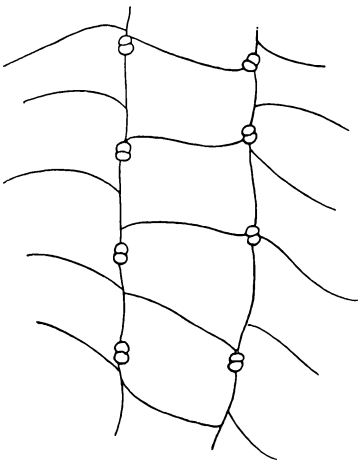

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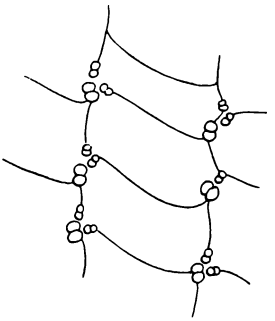

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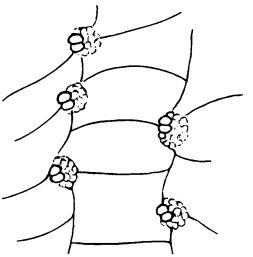

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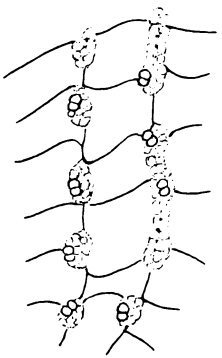

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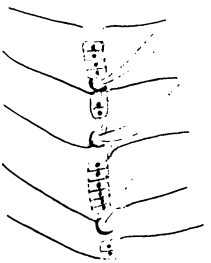

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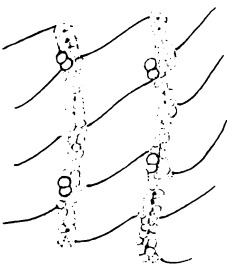

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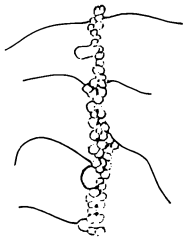

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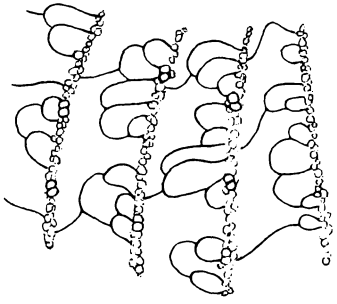

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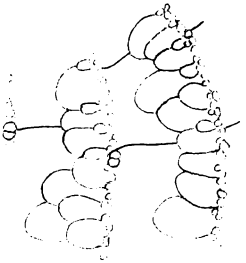

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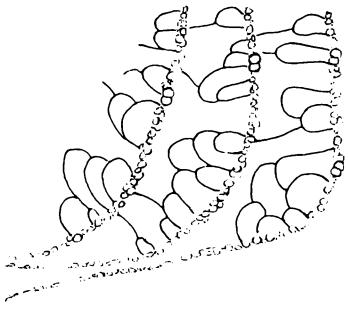

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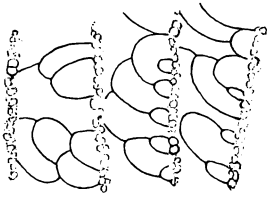

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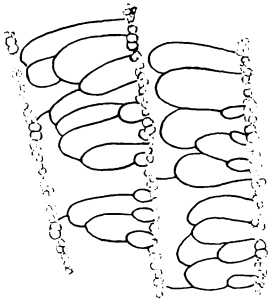

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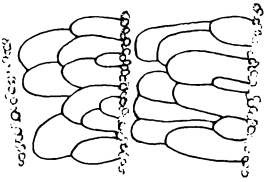

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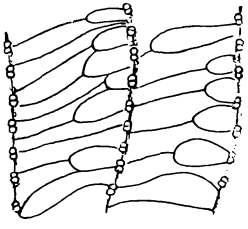

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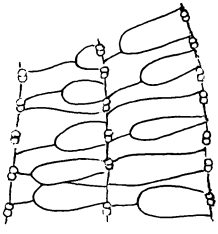

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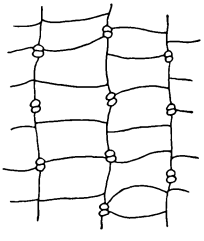

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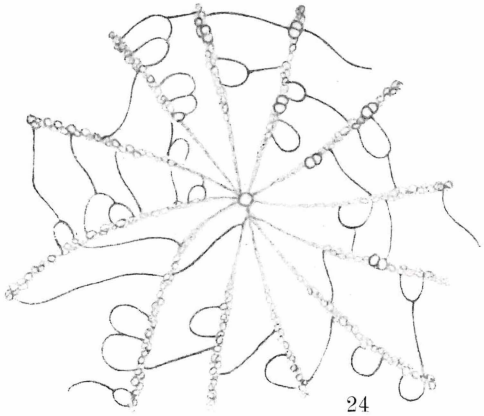

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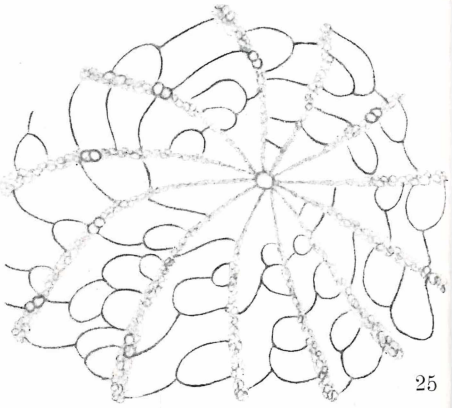

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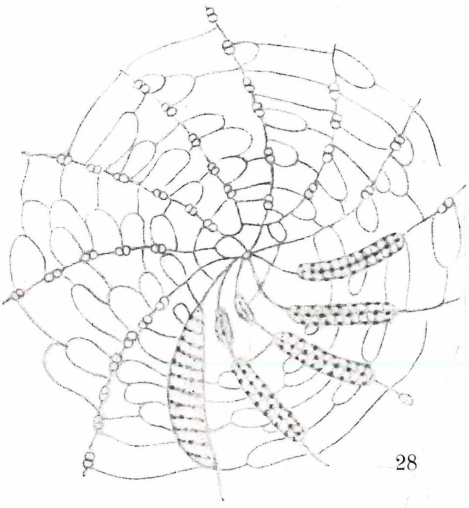

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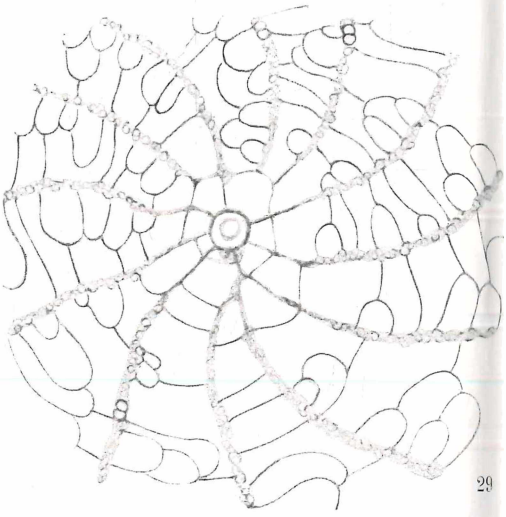

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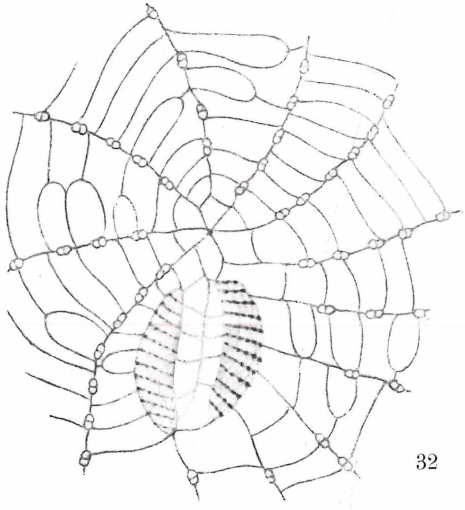

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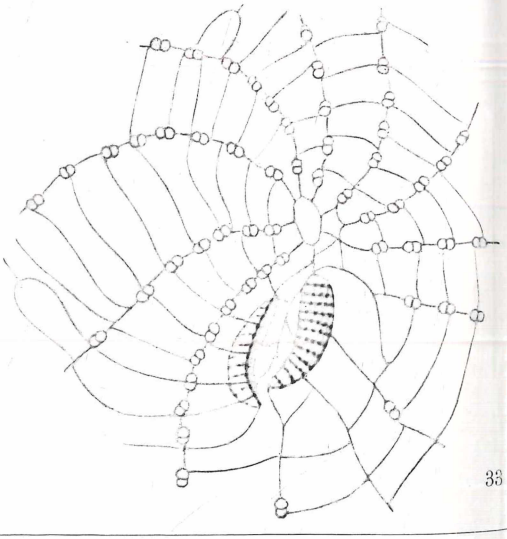

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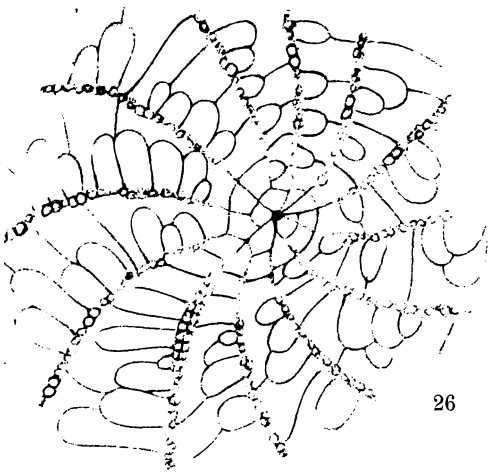

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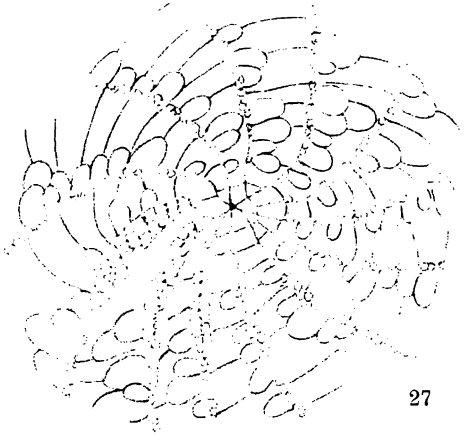

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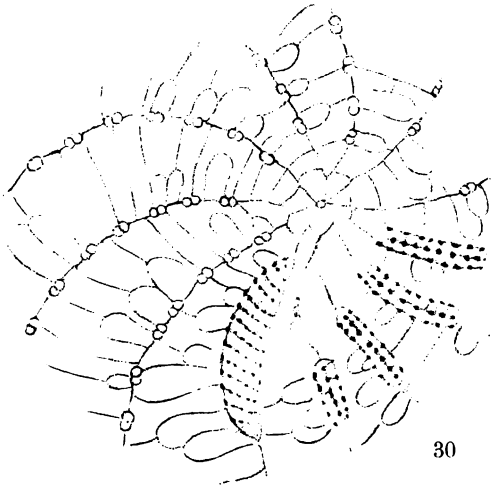

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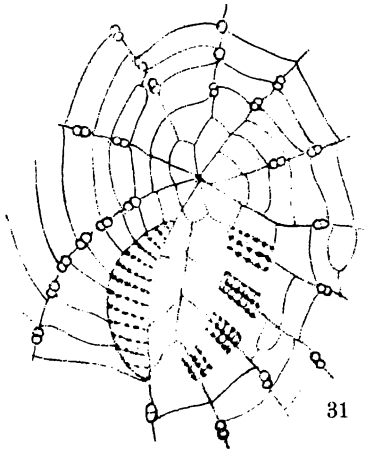

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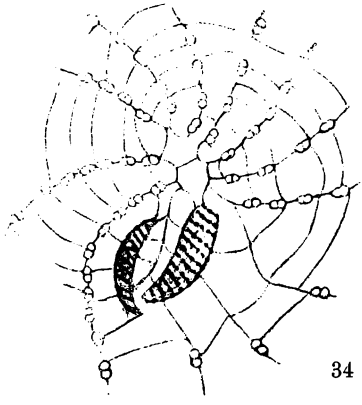

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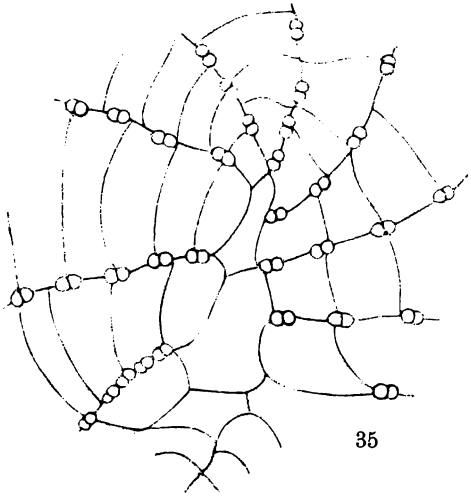

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