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Dedifferentiation and Redifferentiation in *Ichthyophthirius.*

I. Neuromotor System.

By

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(With 26 figures in the text.)

Introduction.

The portion of the fibrillar system attached to the motor organelles of the mature ciliate is bound together to form a single unit. SHARP (1914), the first to demonstrate the linkage of these fibrils and the ciliary structures in a single system, gave to this complex the name „neuromotor system".

The resorption and reorganization of this complex fibrillar apparatus of ciliates is a striking and clear cut example of cyclic changes in protoplasm. TAYLOR (1928) in studying the regeneration of cirri in *Uronychia* found that the whole differentiated ciliary apparatus is resorbed and reorganization is accomplished as a result of sub-visible protoplasmic reorganization. KLEIN (1926—1933) finds that the new portions of the fibrillar complex or silverline system are derived from pre-existing fibrils. The new basal granules and cilia are formed de novo on these fibrils. CHATTON and his collaborators (1921—1931) describe certain argentophilic granules, the infraciliature, which reproduce by fission and are continuous from one generation to the next, while the fibrils and basal granules are formed de novo under the influence of these granules of the infraciliature. Thus KLEIN and CHATTON describe a genetic continuity of one type or another in the neuromotor system of ciliates, while

TAYLOR considers that re-organization is complete during division or as a result of merotomy.

Ichthyophthirius was selected as an especially favorable form in which to study de-differentiation and re-differentiation of the neuromotor system. Division, de-differentiation, and re-differentiation are fully separated by the nature of the life cycle and hundreds of ciliates in the same stage of development may be taken from the same cyst. Large numbers of ciliates in any desired stage of the life cycle are thus available.

This investigation was designed to give information on the following points:

1. The structure of the neuromotor system in all stages of the life cycle.

2. Modifications of the activity of the cilia correlated with changes in the neuromotor system.

3. The relationship between the neuromotor system of the parent and daughter ciliates.

Materials and Methods.

Ichthyophthirius multifiliis FOUQUET, a holotrich infusorian parasitic in the skin of fresh-water fish was collected from fish of the Palouse River near Pullman, Washington, and maintained on fish kept in balanced aquaria. Heavily infested fish were placed in small glass dishes and the mature parasites collected as they left the host. These mature ciliates encysted and went through the cycle normally and the young ciliospores were used to infect clean fish. In this way the infection can be maintained indefinitely in the laboratory and artificial epidemics produced whenever desired. The speckled dace (*Apocope oscula carringtoni*) was found to live best under experimental conditions and support the heaviest infection of ciliates.

The ciliates were studied alive in hanging drops or in relatively large containers to test their motile ability. Their undisturbed activities are easily studied in the tail of young fish.

The best fixatives are SCHAUDINN'S fluid and ZENKER'S fluid. HEIDENHAIN'S iron haematoxylin and DELAFIELD'S haematoxylin are the most successful stains for fibrils. For silver-impregnation, the original drying method of KLEIN (1926) is best for the ciliospores, while the wet method described by LUND (1932) is the most satisfactory for the adults. The silver methods are somewhat more con-

venient and give more spectacular results, but the standard haematoxylin methods yield many facts not shown by the newer silver methods as pointed out by LUND (1932).

All drawings were made with a Leitz binocular microscope with apochromat objectives, and camera lucida.

Acknowledgments.

The author gratefully acknowledges a grant from the ELIZABETH THOMPSON Science Fund which materially aided this project. The fish were identified through the kindness of Professor LEONARD P. SCHULTZ, of the Department of Fisheries, University of Washington, Seattle.

Life Cycle.

The life cycle of *Ichthyophthirius* is in two distinct parts, the parasitic feeding stage during which growth takes place and the encysted astomatous stage during which division takes place resulting in the formation of many small ciliospores, the infective stage. Dedifferentiation occurs immediately after encystment and before division. The mouth is resorbed within an hour after the commencement of the secretion of the cyst. The macronucleus loses its elongate sausageshape and rounds up. The first division starts shortly after this and is usually finished before the cyst wall is completed. In the next three or four divisions cleavage is synchronous in all the daughters, regularly giving rise to 16 or 32 celled cysts. After this each daughter divides more and more independently until 200—1000 ciliospores are produced. The plane of cytoplasmic cleavage is perpendicular to the ciliary rows and therefore transverse with respect to the original longitudinal axis. The body cilia are continuously active during this process and are not sloughed as stated by ZACHARIAS (1892). The old ciliary rows are lengthened and new ones intercalated in response to the greatly increased surface area, but no re-differentiation is started until after the divisions are completed.

Normal division without encystment and division on the host is frequently described although repeatedly denied by many other authors. STILES (1898) observed division without encystment and considered it to be an alternative method of reproduction. However, by the use of the isolation method the cycle was traced with individuals of known history and it was found that those ciliates which divide without encystment never go beyond three or four divisions

before death. STILES (1898), NERESHEIMER (1908) and others point to the presence of two or more parasites within the same blister on a host as proof of division on the host. This does not follow, since the parasites migrate over relatively large distances. NERESHEIMER (1908, text. fig. D) shows a parasite in which the macronucleus is bilobed and interprets it as division. This figure labeled „Reproduktion der verlorenen originalzeichnung nach dem Gedächtnis" is the only positive evidence of division on the host. HAAS (1932), using some of Professor NERESHEIMER's original preparations, found all cytological evidence against the possibility of division on the host and his findings were corroborated in the present investigation.

Re-organization of the young ciliates starts after division and while they are yet within the cyst. Nuclear re-organization then takes place (HAAS 1933). At the same time the new mouth begins to form in the middle of the ventral side. If the ciliates are freed by tearing the cyst at this time, they assume a spherical shape (Fig. 13). However, within the cyst, re-organization continues and when the ciliospores rupture the cyst wall they emerge as the typical banana-shaped organism with the mouth rudiment in the concave surface. During their free-swimming life, which may continue for as long as 72—96 hours, ciliary rows are added around the mouth and the whole oral area sinks in. The ciliospore does not differentiate beyond this point until it is able to penetrate the skin of a fish. Soon after this occurs, the mouth becomes functional, completes its differentiation along with the rest of the body and the ciliate assumes its adult form.

Differential growth.

The growth of the different parts of the ciliate is markedly unequal producing a gradual shift in the position of the mouth. The earliest mouth rudiment is located in the middle of the ventral side (Fig. 16). The elongation which is characteristic of the infective ciliospore is most pronounced in the region posterior to the mouth so that only one-third to one-quarter of the ciliospore is now anterior to the mouth (Fig. 18). This differentiation between the rates of growth of the part anterior to the mouth and the part posterior to the mouth continues through the whole growth period so that in the adult ciliate (Fig. 1) less than one-twelfth of the total length is anterior to the mouth. This extreme shift has given rise to persistent statements that the mouth is terminal although ZACHARIAS (1892) and many later authors have shown that this is a mistaken interpretation.

Origin of fibrils and basal bodies.

The problem of the differentiation of the complex neuromotor system of the adult is in its simplest form the problem of the differentiation of new fibrils, basal granules, and cilia. New ciliary rows are being formed throughout the whole life cycle but are seen most abundantly and most clearly in the ciliates artificially freed from the cyst.

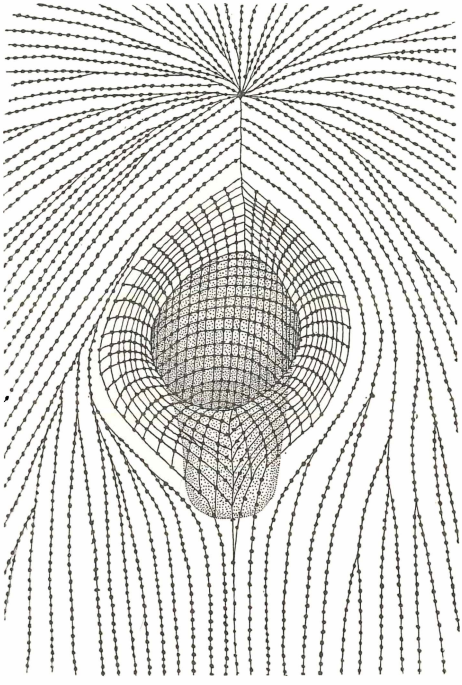


Fig. 1. Oral region and anterior field region of adult ciliate. Wet "silver-line" method. × 1500

The first sign of growth in the neuromotor system, exclusive of the oral region, is the appearance of small lateral projections from the longitudinal ciliary fibrils midway between the basal granules (Fig. 2). A very delicate fibril next appears connecting the tips of each of the projections. The lateral projections gradually disappear and the new ciliary fibril (Fig. 3) is freed except at the ends. Basal granules of new cilia arise after the fibrils are formed (Fig. 4). Small granulations are first noticed and these then grow to their adult size. In rapidly differentiating forms, such as those just excysting, every ciliary row is forming a new one, and each new row extends nearly the full length of the body. In older ciliospores (24—72 hrs.) fewer and shorter rows are being formed and in many cases these are merely short loops extending around one or two basal granules.

The oral fibrils are much more highly developed than the fibrils of the body and show in addition to the above method of multiplication a method by which new fibrils bud out from an established fibril but are attached to it only at one end (Fig. 12). The proximal end of each fibril being the oldest section, the largest basal granules

are formed there. From this region the granules grade down in size until at the distal end the fibril is bare. All stages of differentiation of basal granules are thus visible on the same fibril.

Both types of multiplication described above are essentially the same. New fibrils bud out from established fibrils, and basal granules are specializations of these fibrils and are not formed from the division of old basal granules. The new ciliary elements of the neuromotor system arise from pre-existing ciliary elements.

CHATTON and his collaborators (1921—1931) have described a dual structure in the neuromotor system-black argentophilic granules which show a continuity throughout the life cyle. These are the

Fig. 2.

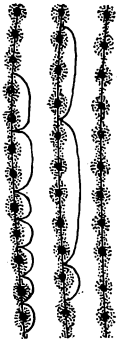


Fig. 3.

Fig. 4.

Figs. 2—4. Formation of new longitudinal ciliary lines in young ciliospores. Dry "silver-line" method. Fig. 2. From an encysted form showing earliest stages. Fig. 3. From a ciliospore 24 hours after excystment. Fig. 4. Origin of new basal granules.

granules of the infraciliature. From these granules are formed secondary granules and fibrils which they identify with the basal granules and silver-line system of KLEIN (1926, 1932). KLEIN (1932), however, finds that this network is permanent and the basal granules (often compound) are formed de novo from this network.

In *Ichthyophthirius* it is clear that it is from fibrils already in existence that the new fibrils form and as in KLEIN'S description the basal granules form on them. The basal granules are simple in all cases, neither multiple as described in many cases by KLEIN, nor dual as described by CHATTON. New portions of the neuromotor system, both fibrils and basal granules, are formed as growths from the old fibrils alone.

Arrangement of fibrils of the neuromotor system.

The short cilia, or somatic cilia, which cover the body are located on longitudinal rows which are linked together anteriorly and to some extent posteriorly by small centers, the anterior field (Fig. 1) and posterior field. These two centers are connected by a fibril, the suture fibril, which marks the ventral side. The suture fibril is interrupted and divided into anterior and posterior segments by the fibrils of the oral region. Accessory suture fibrils, which are ciliated, are arranged concentrically on both sides of the oral region and terminate anteriorly and posteriorly in the suture fibrils.

The lip of the oral opening is bounded by the outer peristomial fibrils which are linked to the suture fibrils. The circular fibrils which line the walls of the oral cavity (Fig. 1) are called the inner peristomial fibrils. Radial fibrils intersecting the two sets of peristomial fibrils at right angles form an additional co-ordinating set of fibrils.

Behavior of fibrils during the division cycle.

Throughout encystment the ventral suture fibril is retained even though the rest of the oral fibrils are completely resorbed (Fig. 5). The anterior portion of the fibril shows no ciliary bodies although from it many ciliary rows arise at an angle. These rows extend around both sides of a small lenticular space in the pellicle which marks the former position of the resorbed mouth and fuse posteriorly with the posterior suture fibril. The posterior suture fibril usually shows ciliary granules.

The first external evidence of division is the appearance of a shallow furrow at the equator (Fig. 13). It is always perpendicular to the ciliary lines. The portions of the longitudinal fibrils lying in the furrow become indistinct and light-brown in color after impregnation rather than the normal jet-black. They become very broad and pale and then fade out completely. The free ends of the fibrils are brought into contact as the furrow deepens and they fuse along the suture lines thus re-establishing a morphological and a physiological linkage. This linkage, however, is not complete at this time for fibrils which end in the anterior and posterior fields do not establish a linkage during the division stages, but only after the whole division cycle is finished.

Each division thus divides each fibril of the ventral suture region as well as each of the main somatic fibrils. The original

orientation is preserved even though the mouth parts and the anterior and posterior fields are de-differentiated and resorbed. The orientation marked by the ventral suture region is thus perpetuated throughout the division cycle and serves as the basis for re-organization of the young ciliospore.

The linkage of the fibrils in the anterior and posterior fields is completed soon after the division cycle is ended. The anterior field in particular shows a complex structural differentiation. A large, solid hyaline knob devoid of cilia is seen in living infective ciliospores (Fig. 17) and in the adult parasites. A portion of this knob is clearly seen in haematoxylin or silver preparations (Figs. 1, 16, 18) as a small dark body in which the somatic and suture fibrils unite. HAAS (1933) interprets this body as a sort of perforatorium. A comparison of figures 17 and 18 will show that this basophilic body or anterior field, is only a small part of the whole perforatorium apparatus, the hyaline cap being invisible after fixation and staining. The hyaline cap performs the function of a wedge in the penetration of the host's tissue, the fibrillar center, or anterior field beneath it being a co-ordinating center. This function is clearly seen in the behavior of ciliates freed by tearing the cyst wall. Ciliates of all sizes down to 60—80 μ are slow and clumsy in their movements and free themselves from their neighbors very slowly, even though no division strands are left, rolling this way and that in their efforts toward locomotion. This size range includes ciliates in all stages up to the last division and all lack the anterior and posterior field systems. Young ciliates 40 μ or less in diameter include only those which have completed the last division. Although these still hold their awkward spherical shape, they boil out when the cyst wall is torn, progressing rapidly in straight paths which are changed only when solid obstructions are met. They also show the slow-revolving movement around the main longitudinal axis which is characteristic of the differentiated ciliospore. The ciliates thus show a co-ordinated activity of the somatic cilia only when the longitudinal ciliary fibrils are linked by the anterior and posterior fields.

Origin of the oral fibrils.

The oral fibrils begin to differentiate almost immediately after the final division has taken place. A small fibril, the oesophageal loop, forms as a posterior extension of the anterior suture fibril

(Fig. 6, 16). Simultaneously new fibrils grow out from the suture fibril within the lenticular area bordered by the accessory suture fibrils (Fig. 6). These are linked posteriorly in a small vacuolated

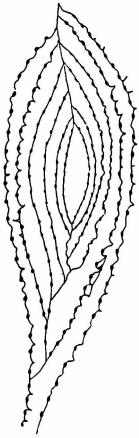


Fig. 5.

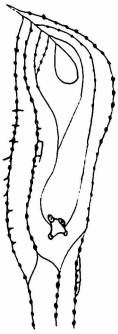
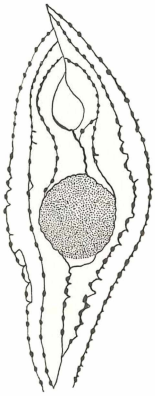


Fig. 6.



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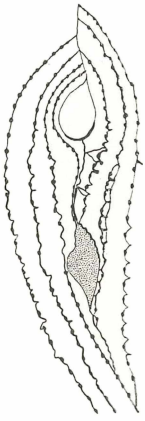


Fig. 8.

Fig. 9.

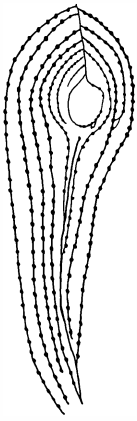


Fig. 10.

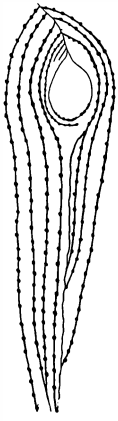


Fig. 11.

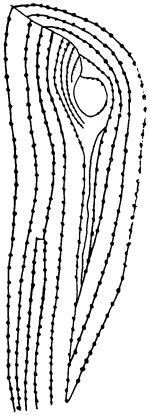


Fig. 12.

Figs. 5—12. Re-differentiation of the oral region. Dry "silver-line" method. Fig. 5. Suture system between divisions. Cyst in 16—32 cell stage. × 620. Fig. 6. First appearance of the oesophageal loop and origin of posterior scar. From a torn cyst. × 1500. Fig. 7. Posterior scar fully developed. Peristomial fibrils beginning to form. From torn cyst. × 1500. Fig. 8. Reduction of posterior scar. Just after excystment. × 1500. Fig. 9. Posterior scar completely resorbed. Just after excystment. × 1500. Figs. 10—12. Active formation of outer peristomial fibrils. 24 hours after excystment. × 1500.

area which is strongly argentophilic. These vacuoles fuse and form a large area which appears brown after impregnation (Fig. 7) and is bounded by fibrils linked anteriorly and posteriorly to the suture system. A heavy non-ciliated fibril (Fig. 7), the temporary central fibril, forms a direct connection between this posterior scar and the oesophageal loop. A new outer peristomial fibril then forms on both sides of the anterior suture fibril near the loop and extends posteriorly to connect with the fibril around the posterior scar. The scar is then resorbed, first forming an irregular, elongate area (Fig. 8) and then disappears completely (Fig. 9). No suggestion of the function of this posterior scar has been discovered. The outer peristomial fibrils detach themselves from the scar as it is resorbed and connect directly with the posterior suture. An additional outer peristomial fibril forms on the right side just within the one formed previously and grows posteriad to connect with the apex of the scar area (Fig. 8). In this manner, one or two additional fibrils are added on each side and in one of these the old temporary central fibril is incorporated (Fig. 9) by the time the posterior scar is completely resorbed.

Excystment most commonly occurs at this stage of differentiation, but may occur at any period in the differentiation of the mouth. Excystment is apparently due to a mechanical break caused by the pressure of the ciliospores inside and this would vary both with the strength of the cyst and with the force exerted by the ciliospores.

That part of the pre-oral suture lying between the last row of body cilia and the anterior portion of the oesophageal loop becomes a differentiation center for the outer peristomial fibrils. Each fibril as it grows out follows the curve of the right side of the oesophageal loop (Figs. 10—12). Posteriorly it meets and fuses with a new central fibril forming at the same time between the two middle rows of body cilia. Then successively between this outer peristomial fibril and the oesophageal loop new fibrils grow out (Fig. 12). Fibrils also grow out on the left side during the latter part of this process.

The center of differentiation then shifts to the oesophageal loop. Basal granules form in it while the outer fibrils are growing. Additional fibrils are budded off by the oesophageal loop and become the inner peristomial fibrils (Fig. 18). A heavy enlargement is then formed near the anterior end of the oral opening effectively

linking together the inner peristomial fibrils. This thickening is the earliest sign of the neuromotorium.

The radial fibrils begin to form soon after two or three of the inner peristomial fibrils have been formed. This new set of fibrils grows out from the oesophageal loop (Fig. 18) and expands radially to intersect each of the oral strands at the places where their basal granules are located. These radial fibrils form first in the middle of the right side of the mouth and then new fibrils are added anteriorly and posteriorly. Each fibril terminates in the outermost peristomial fibril. The outermost peristomial fibril and the adjacent body fibril on each side at this time become markedly heavier than the other fibrils and are thus easily recognized.

Ciliary rootlets begin to grow inward from the basal granules as the radial fibrils are formed. They are relatively short at this time and form only a thin fibrillar layer in the ectoplasm around the oral structures.

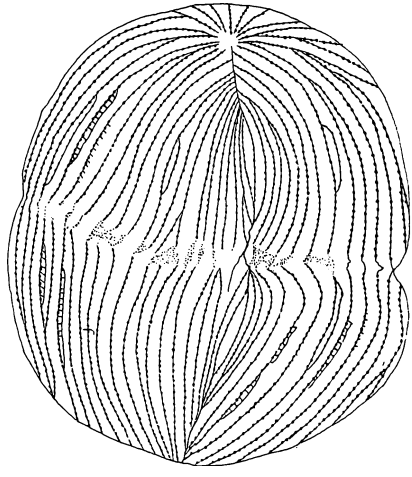


Fig. 13. Division in 32—64 cell stage. Suture system divided in two. Absence of fibrillar connections in the anterior field well marked. × 620.

Changes in the form of the oral region accompany the differentiation of the fibrils. The region of the oesophageal loop sinks in to form a shallow pit (Fig. 14) and this invagination is accelerated as the oral cilia are formed and commence their strong beat. At the same time the wall of the mouth near the motorium begins to show a thickening. This is the primordium of the oesophageal plug, which is described later in connection with the adult mouth.

The gullet breaks through only after the oral fibrils are well differentiated (Fig. 19), usually 48—72 hours after excystment. The cilia grow more numerous, longer, and more active and beat actively into the gradually deepening oral pit. It seems likely that the pressure from the ciliary beat is a mechanical factor responsible in part at least for the formation of the oral invagination. The breaking through of the gullet is merely a continuation of this process.

Oral region of the adult.

The mouth parts rapidly strengthen and enlarge with the start of active feeding. Many additional rows of circular and radial fibrils are formed and the motorium becomes a bilobed structure (Fig. 19). The basal granules of the oral cilia sink further beneath the pellicle. The thick homogeneous layer of ectoplasm in which the basal granules lie forms a rigid supporting matrix for the mouth. The ciliary rootlets continue to grow and their free ends tend to collect in bundles which form the beginnings of the heavy oesophageal strands.

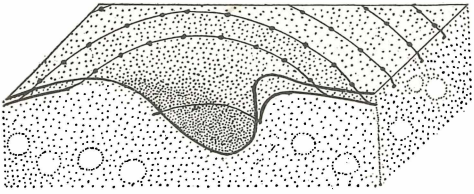


Fig. 14.

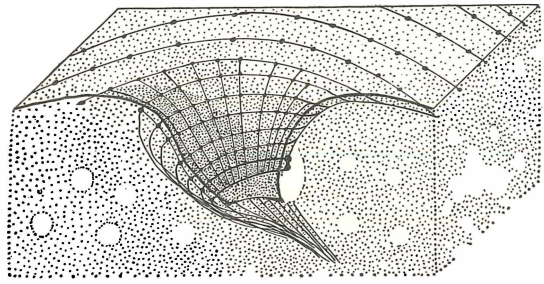


Fig. 15.

Figs. 14 and 15. Reconstruction of oral region of young ciliates. Cilia omitted. Fig. 14. Oral pit just forming in ciliospore from a torn cyst. (Com pare with Fig. 16.) Fig. 15. Mouth parts of young ciliate just after penetrating the skin of the host. (Compare with Figs. 18 and 19.)

The oral region reaches a stage of mature differentiation (Figs. 1, 20—26) 48—72 hours after the parasite penetrates the fish. The only change occurring after this time is the growth of the mouth accompanied by an increase in the number of cilia and ciliary rows.

The mature mouth is a steep-sided, nearly cylindrical pit with a rounded or conical bottom (Fig. 24). The gullet is blocked in non-feeding specimens by an expansion of one wall which forms a valve-like plug (Fig. 20).

The inner peristomial fibrils have increased greatly in number and now form a closely-set series of loops around the oral pit. The outer peristomial fibrils which are linked to the anterior and posterior suture fibrils (Fig. 1) form the lip of the oral opening. The radial fibrils cross the inner peristomial fibrils and terminate in the outermost peristomial fibril. Two heavy basophilic rods,

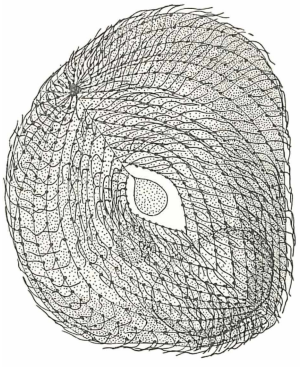
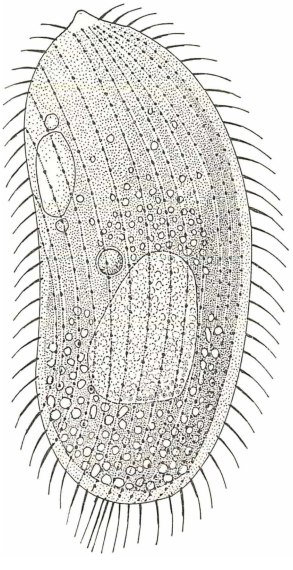


Fig. 16.



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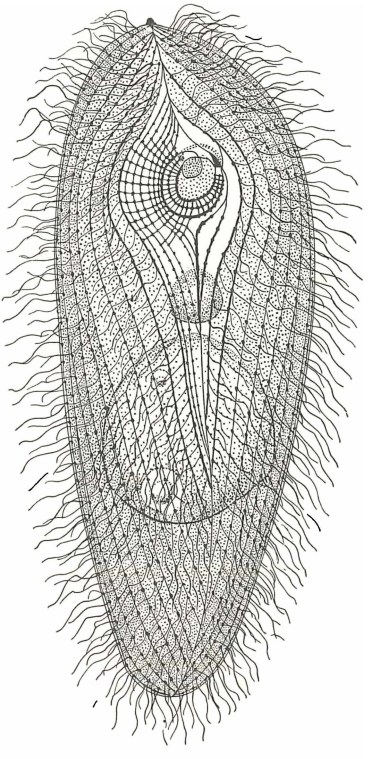

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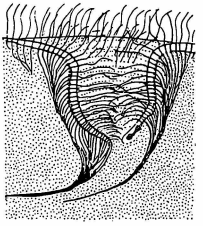

Fig. 19.

Figs. 16—19. Re-differentiation of the oral region. Fig. 16. First appearance of the oesophageal loop. Anterior field system completely differentiated. (Compare with Fig. 6.) × 1500. Fig. 17. Lateral view of live ciliospore just after excystment. Perforatorium a large, hyaline knob on anterior end. × 1500. Fig. 18. Formation of radial fibrils in oral region. From ciliospore 72 hours after excystment. SCHAUDINN's fluid, DELAFIELD's haematoxylin. × 1500. Fig. 19. Oral region of young ciliate 24—48 hours after penetration of the skin of the host. The split of the motorium to form a double body well shown. Ciliary rootlets beginning to combine to form large oesophageal fibrils. Section, 5 μ, × 1500. ZENKER's fluid, HEIDENHAIN's haematoxylin.

each attached to a heavy oesophageal fibril, are located near the oesophageal plug. These are the neuromotoria and are derived from the single body of the 72-hour ciliospore. The beginning of the split of the single neuromotorium is shown in Fig. 19.

The cilia of the oral region become considerably longer than those of the body, and at the same time the basal granules also become larger and sink further beneath the surface (Figs. 19, 24). Since they are set very close together this growth results in an elongate shape. The radial and peristomial fibrils are carried to the outer ends of the basal granules during this growth. The

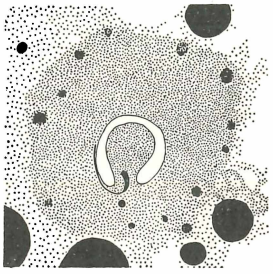

Fig. 20.

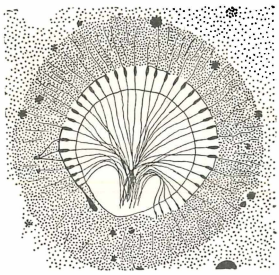

Fig. 21.

Figs. 20—21. Cross sections through the gullet region of a mature parasite. 5 μ. ZENKER's fluid, HEIDENHAIN's haematoxylin. $\times$ 3000. Fig. 20. Region of the eosophageal plug. Inner lobe of the motorium and inner oesophageal strand leading from it. Fig. 21. Region of outer motorium. An inner peristomial fibril runs from the neuromotorium to the basal granules. Ciliary rootlets extend to the oesophageal strands.

region between the fibrils and the pellicle is filled with a dense homogenous ectoplasm which aids in giving considerable rigidity to the whole oral region. The cilia are simple structures and are not grouped in membranelles as suggested by TEN KATE (1926).

The ciliary rootlets grow enormously during the maturation of the ciliate and finally extend across the ciliate (Fig. 26). Fifty to onehundred individual ciliary rootlets combine to form each large oesophageal strand (Fig. 22) which turns sharply and continues into the endoplasm parallel to the main axis of the oral pit. The ciliary rootlets are best developed in the region of the inner peristomial fibrils, less well developed in the region of the outer peristomial fibrils, and are not found at all in the region of the ordinary body cilia.

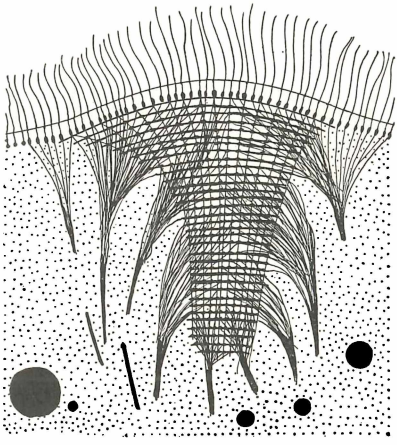

Fig. 22.

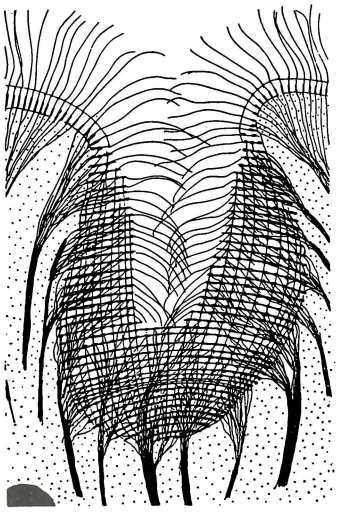

Fig. 23.

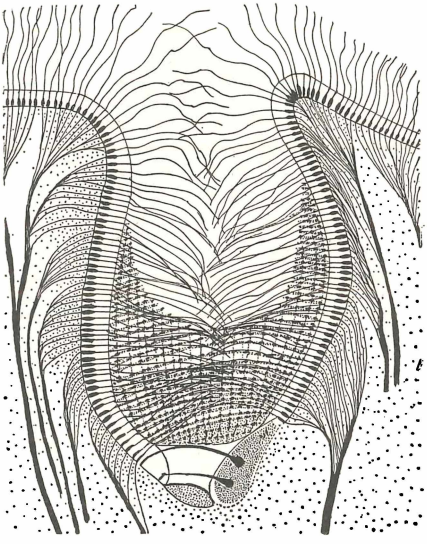

Fig. 24.

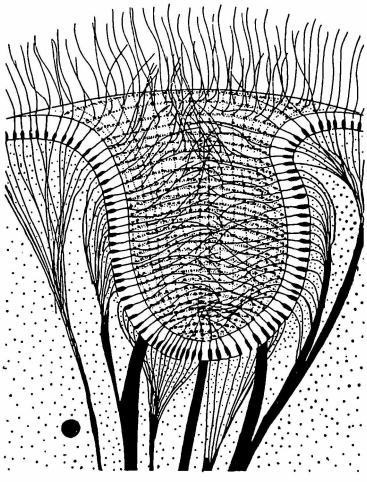

Fig. 25.

Figs. 22—25. Serial longitudinal sections through the oral region, showing the ciliary rootlets, oesophageal fibrils, and neuromotoria. The large black bodies in the endoplasm are protein granules. ZENKER's fluid, HEIDENHAIN's haematoxylin. 5 μ. $\times$ 3000.

Transverse connections between the ciliary fibrils are present only in the oral region (Figs. 1, 15, 18). They are never present in any stage of the life cycle between the fibrils of the ordinary somatic cilia. This is similar to the conditions found by LUND (1933) in *Paramecium*.

Feeding activities.

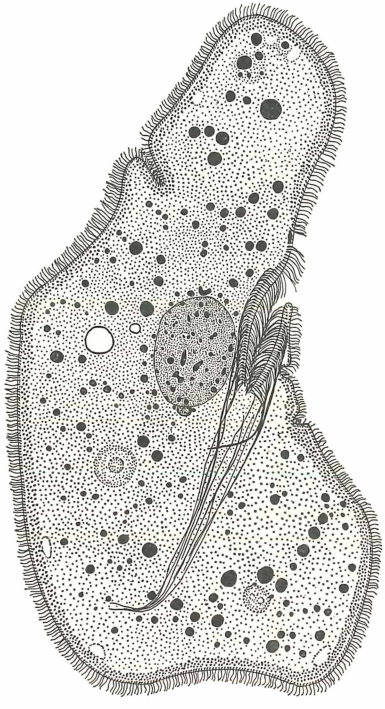

Fig. 26. Longitudinal section of a mature ciliate within the epithelium of a fish, showing the fully developed oesophageal fibrils. ZENKER's fluid, HEIDENHAIN's haematoxylin. 5 μ. $\times$ 320.

The normal undisturbed feeding activities of *Ichthyophthirius* may be easily observed in the fins and tail of parasitized fish.

The young parasites migrate actively through the skin with a definite boring motion. The ciliate revolves in a clockwise direction (in anterior view) with the non-ciliated tip of the anterior field used as a wedge to force the tissue apart. The powerful beat of the cilia of the body force the ciliate forward. The ciliary beat is very powerful and often forces wrinkles and other distortions in the soft body. The oral region on the other hand is rigid and as the ciliate forces itself against the epithelium of the host the beat of the powerful oral cilia continually scrape off cells and forces them into the mouth of the ciliate. The ciliate almost literally eats its way through the epithelium. One young ciliate, 50 μ long, covered a distance of 150 μ in less than 10 minutes.

The larger ciliates migrate less actively when undisturbed. They keep rotating on any axis within the blister formed by their activities and the cells scraped off by their cilia are eventually taken up by the mouth.

There was absolutely no evidence that the ciliate injured the host by other than mechanical means. The mitochondria, GOLGI apparatus, nucleus, membranes etc. of the skin cells are at all times perfectly normal until they lie within the digestive vacuoles of the ciliate.

Discussion.

The fibrils connecting the basal granules of the cilia in the Infusoria have been known for many years. ENGELMANN (1880) was probably the first to suggest a possible neuroid function. SHARP (1914) was the first to demonstrate that the fibrils connecting the cilia are bound together in a single, unified system which he called the "neuromotor system". KLEIN (1926) demonstrated fibrils connecting ciliary granules which he believed to be hitherto undiscovered and named this the "silver-line system". CHATTON and his collaborators (1921—1931) using a similar method, describe certain bodies, the "infraciliature" from which they claim the silver line system is formed. All these concepts, varying in details, assign a neuroid function to the network of fibrils connecting the motor organelles. Since SHARP was the first to advance this concept, his terminology has been followed in this paper.

The neuroid interpretation of the neuromotor system rested first on purely morphological grounds. The first experimental evidence was that of TAYLOR (1920) who showed that when the main fibrils of *Euplotes* are cut the co-ordinated action of the neuromotor system is destroyed. MAC DOUGALL (1928) excised the neuromotorium of *Chlamydodon* and confirmed TAYLOR's results. Additional evidence of a new sort is furnished by this study of the re-organization of the fibrillar system in *Ichthyophthirius*. Normal swimming movements are not found until the morphological linkage of neuromotor fibrils is accomplished. The interruption of the fibrillar system of ciliates, whether caused by natural or experimental factors, produces a characteristic disorganization in ciliary activity. The neuromotor fibrils are the actual conductile elements of the ciliates and are coordinated in the oral region by the neuromotorium and in the general body ciliation by the anterior field.

The resorption of all of the external motor organelles during division or encystment has been known in Hypotrich ciliates since the observations of STEIN (1854, 1859). CALKINS (1911), DEMBOWSKA (1925), TAYLOR (1929) and others have shown that this resorption

also occurs after a part of the motor system has been injured by cutting. These highly specialised ciliates are unable to modify their motor organelles to fit in with the changed conditions produced by division, encystment, or artificial deformity. In the less specialised Holotrich ciliate *Ichthyophthirius*, encystment and division result in resorption of only the most highly specialised portions of the neuromotor system — the oral region and the anterior and posterior fields. The less specialised parts — the body system and the suture system are then rearranged without resorption into a form appropriate for the young ciliospore. Differentiation is thus reversible in the less specialised parts which are re-organized without complete disintegration. On the other hand, a high degree of specialisation carries with it a loss in plasticity, differentiation becomes irreversible, and resorption becomes necessary. The oral parts of *Ichthyophthirius*, and the whole group of cirri and membranelles in the Hypotrichs (TAYLOR, 1929) are discarded when a need for re-organization becomes apparent. De-differentiation involves resorption only of those parts in which differentiation has been carried on to such a point that differentiation is irreversible.

Re-differentiation involves both a re-arrangement of old parts and the formation of new parts which will meet the needs of the young ciliates. TAYLOR (1929) suggests that the formation of new parts is a condensation process dependent on the general protoplasmic organization. However, the history of the fibrils, which TAYLOR did not trace, shows that a part of the neuromotor system of the re-organised ciliate consists of portions of the old neuromotor system merely re-molded to fit the young form, along with parts which arise by growth and differentiation from the old fibrils. The de-differentiation and re-differentiation of the neuromotor system is centered in the neuromotor system itself.

The different parts of the neuromotor system show different powers of organization. The fibrils of the ordinary somatic rows form only additional somatic rows — a problem of simple reproduction. The suture fibril alone exhibits the capacity of forming fibrils which are differentiated into new and complex structures — the oral region, anterior field system, and posterior field. The center of differentiation within this fibril is not fixed but shifts during development. The anterior tip is active first in the development of the anterior field system, then the differentiation center shifts to the posterior end of the fibril and the oesophageal loop and then

the outer peristomial fibrils are formed. The oesophageal loop is the next center and the inner peristomial fibrils and the radial fibrils are formed. In all cases the basal granules and the cilia are differentiation products of the fibrils themselves.

The visible processes of re-differentiation are accomplished by the activity of definite portions of the permanent neuromotor system. This localization of activity is strikingly similar to the "organizer" of the amphibian gastrula. The whole protozoan cell enters into the process of re-organization, but specific parts of this re-organization are accomplished by the direct activity of small active portions of the cell. De-differentiation involves the destruction of many highly specialised structures, but the fundamental specialisation of each part, visible even with relatively crude morphological methods, is retained and forms the basis of new structures. The gross rhytmic cycle of de-differentiation and re-differentiation which has such spectacular manifestations in the ciliates is the product of the smaller cycles of each system of organelles. They in turn are the product of many cycles involving portions of each system.

Conclusions.

1. De-differentiation involves resorption of organelles only when these are too specialised to be utilised in the formation of the young ciliate. Less specialised portions are retained and form the basis of the neuromotor system of the young ciliates.

2. Re-differentiation is accomplished by the activity of the neuromotor fibrils. All new fibrils are growths from old ones. Basal granules and cilia are differentiated on the new fibrils.

3. The suture system serves as an organizing center in the re-differentiation of the oral system and the anterior and posterior fields.

4. Co-ordinated action of the cilia is not re-established until the neuromotor fibrils are connected by the anterior field system.

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