Fission in *Isotricha prostoma.*

By

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(With plates 11 u. 12.)

In a previous study the writer has intimately discussed certain features of the interkinetic phase of the structure of the holotrichous ciliate *Isotricha prostoma*, symbiotic in cattle. In the material used in that investigation an abundance of animals in various stages of division was noted. This paper undertakes to describe binary fission on the basis of this material. The technique for microscopical examination was the same as that described previously (1929). In some cases thin sections of five microns were cut.

So far as the writer is aware no study of the division process has been given in the Isotrichidae previously, save that by Dogiel and Fedorowa (1927) in *Dasytricha ruminantium.*

The earliest stages in the fission of *Isotricha* were seen in relatively few individuals in certain collections. Most of the later phases were obtained in greater numbers in other collections. In these, upward of 60 % of the *Isotricha* present were in some stage of division. Since fission stages are rare in the majority of collections from cattle and their appearance is signalized by a general "mitotic flare" in the greater number of ciliates of the rumen it is evident that the extraordinary outburst of reproductive activity is due to some undetermined stimulus, possibly of an external nature. Not only are the species of *Isotricha* present but also division figures in the other ciliates are abundant; collections which contain stages of *Isotricha* in division also show similar phases of the Ophryoscolecidae. In all these only the division of neuters was observed.

Externally, the beginnings of binary fission may be first determined by a change in form. The animal loses it usual olive-shape and tends to become irregularly ovoidal, as the process of plastotomy continues, and eventually reaches a figure 8. A constriction appears in the middle around the cytosome, dividing the animal by a shallow furrow into two more or less unequal parts. As this ditch deepens and constricts the cytoplasm, the animal becomes separated into halves. The anterior half becomes ellipsoidal in outline and is a slightly irregular globe compressed from above downward, while the posterior half is subconical, usually as a broad bluntly angled cone of approximately 60^0, rounding into the flattened posterior side of the upper half of the animal. The furrow, which by its progressive constriction from outside to the center strangulates the cytoplasm, continues to grow progressively smaller in diameter, causing, in later stages, the assumption of a figure 8 form by the dividing pair. This form is at first that of a broad eight and later that of a narrow eight. In this stage the two halves are connected by a narrow stalk-like bridge which forms the sole connection between them. Later stages are found when the bridge is lost and the two animals are connected only by the undivided macronucleus. The writer has seen somewhat similar stages in the division of *Paramaecium caudatum* so that this is apparently a general feature of the process among the *Holotricha*.

An important and interesting observation lies in the distinction between the anterior and posterior daughters. This distinction may persist for some length of time even after separation. The anterior daughters are more globular in form than the more elongated posterior daughters. They do not differentiate an oral apparatus quite so soon as the posterior daughters. A posterior invagination in relation to the macronucleus is also retained for some time, lending to a ready identification. The posterior daughters, on the other hand, are usually longer than wide, but not always, and the naked, spirally twisted end of the macronucleus projects outside through a sort of "birth pore". A mouth is regenerated sooner than in the anterior daughter and there is, of course, no posterior invagination. It is noteworthy that the anterior daughter usually carries with it the remnant of the old, rejected oral apparatus which becomes ghost-like as it fades away, while a new one is redifferentiated. The process as described above is outlined in Pl. 11 Figs. 1—14.

Internal changes of course take place in the cytoplasm during the process outlined in the previous description. The furrow appears early on the surface across the middle of the animal as a lightly staining area composed of large alveoli derived from the cortical cytoplasm. This division band is probably due to the excessive solation of the alveolar protoplasm of this region of the body. Following this local change of the cytoplasm there is an extension of fluidity. The evidence for this resides in the easy assumption of various and often even bizarre forms by the animal at this or related phases (Pl. 12 Fig. 5), and by the increase in size of the peripheral alveoli all over the body. The cytoplasm always retains its dense, heavily-staining appearance during the process. Similarly the boundary membrane retains its identity during the division period. The endoplasm, however, appears to undergo considerable modification. The cortical and medullary portions lose their distinctness entirely and take on a very coarse, but morphologically uniform alveolar structure. The endoplasmic alveoli become irregularly globular and thin-walled. As the process continues and the endoplasm becomes increasingly uniform there is some evidence of protoplasmic translocation demonstrated by coagulated lines of streaming indicative of regular movements of material from the centre toward each end of the dividing individual. As fission proceeds the loosely organized peripheral endoplasmic alveoli tend to assume their interkinetic appearance and a cortex is redifferentiated from the dense central medulla; this portion of the cytoplasmic process begins in the late figure 8 stage and draws to an end some time before the two daughters separate. This dedifferentiation and redifferentiation of the endoplasma is suggestively indicative of the profound lability of protoplasmic reorganization.

Probably the most spectacular of the internal changes accompanying binary fission in *Isotricha* are those of the macronucleus. The first change seen is a noticeable elongation and straightening up into a rod-like form. The elongation is accompanied by a consequent decrease in diameter. Rarely individuals show a marked condensation and thickening up of the macronucleus. The succeeding developments are the formation of a transverse constriction across the middle of the macronucleus, and as the two daughter animals separate, the pulling out of this very narrow rod-like central area into a spring-like spiral. This spiral is often quite long and forms for some time the only connection between the two daughter animals. After the separation of the two daughters this narrow, spring-like rod is retained by the posterior daughter, from which it projects anteriorly through an aperture in the cuticle to the exterior (Pl. 12 Fig. 3). Gradually, it loses its coiled character, becomes rod-like and eventually is pulled back into the endoplasm, leaving the "birth pore" to heal.

The finer structure of the macronucleus undergoes certain changes in the course of development outlined in the preceeding paragraph. For some time during the first half of the process it retains its granular structure. Later, but before the two halves begin to constrict, the chromatin substance becomes organized into a definite number of rods which run lengthwise in the macronuclear membrane. These are undoubted so-called somatic chromosomes. In one very favorable longitudinal section obtained (Pl. 12 Fig. 7) there were clearly twelve of these rods, the cut ends of which are shown in the accompanying camera lucida drawing. A less favorable and obviously earlier stage is drawn in Pl. 12 Fig. 4. In this instance eleven of the rods are clearly shown and possibly the twelfth was sectioned away and lost. For this individual these chromosomes appear as bands. Their rod-like structure is probably a product of a later condensation. When the two halves of the macronucleus have begun to separate these chromosomes are lost and the substance of the nucleus becomes densely granular, a condition retained during the larger part of the life of the individual. A reconstruction zone is not found, and manner of the division of the somatic chromosomes is yet wholly unknown.

The interpretation of the function of the somatic chromosomes, which have been reported from several, unrelated ciliates — in none so clearly as in *Isotricha prostoma* — is naturally difficult. Nothing like them is suggested in *Dasytricha ruminatium* by DOGIEL and FEDOROWA (1927). It is quite possible that they are related in some way to agametic inheritance and afford a chromosome basis for somatic variations without the complicated process of conjugation or endomixis neither of which phenomena has yet been described in *Isotricha prostoma.*

The micronucleus of *Isotricha prostoma* is roughly oblately sphaeroidal during interkinesis. It begins to show the first indications of division rather late, not much earlier than after the macronucleus is strung out between the two halves of the animal in the middle of the narrow figure 8 stage. It escapes earlier from the macronuclear pocket which encapsulates it and lies freely in the cytoplasm, not however, moving very far away from the side of the macronucleus.

The earliest two daughter micronuclei seen in my material were still connected by a thread of nuclear wall material; one daughter was in each of the two daughter animals. The micronuclei in this stage are very narrowly pear-shaped. The micronuclei soon disconnect, at first retaining the pear-shaped and later rounding up in a globular form. The sphaeroidal micronuclei now move freely in the cytoplasm, eventually reaching a position at each end of the macronucleus, a location retained until the two daughter animals separate. Only at a later stage does the micronucleus come to its oblate sphaeroid form characteristic of the interkinetic phase, and reside in a pocket.

In the material studied there was little opportunity to observe the changes within the micronucleus during division. In the majority of instances the chromatin material was dense and compact, staining a homogeneous black with iron-haematoxylin. In early phases, during the elongation of the macronucleus, just after the micronucleus has been freed from its pocket at the side of the macronucleus two pear-shaped bodies or chromosomes are found within the nuclear membrane (Pl. 12 Fig. 2). Neither their earlier or their later history has been satisfactorily traced. A diffusely staining mass is found during the figure 8 stage of the animal. If there two bodies are really chromosomes and it is not improbable that they are, there is a very interesting possibility that we are confronted with a condition suggesting haploidy. However, only an accurate study of conjugation will serve to settle this very intriguing question and *Isotricha prostoma* is apparently a satisfactory form for the purpose of such study.

The complex nucleo-suspensory apparatus, recently redescribed by the writer (Campbell, 1929), behaves during fission as a metaplastid. Only in a few instances has it been possible, however, to trace its behavior. It collects, as a loose bunch of strings, around the rejected oral apparatus, and of course, loses all its connections with the boundary membrane. This rejected system becomes less and less chromophile, fading out ghost-like, as the division process continues. The apparatus, together with the oral structures, become functionally inactive and are lost to the animal very early, at least previous to the assumption of the figure 8 stage. A new nucleo-suspensory apparatus is redifferentiated only after the two daughter animals have been separated for some time. The same is evidently true of the oral apparatus and the oral fibers, but the writer has not been able to make out fully their source or progress of development.

Bibliography.

CAMPBELL, A. S. (1929): The structure of *Isotricha prostoma.* Arch. f. Protistenk. Vol. 66 p. 331—339 Pl. 10—12.

DOGIEL, V. and FEDOROWA, T. (1927): A note on the reproduction of *Isotricha* (*Dasytricha*) *ruminatium.* Arch. russe de Prot. Vol. 4, 1—2 p. 75—82 figs. 1—11 (in Russian with English summary).

Explanation of Plates.

Plates 11—12.

Plate 11.

A series of whole mounts of *Isotricha prostama* showing successive steps in fission. 196 : 1.

Plate 12.

All 470 : 1.

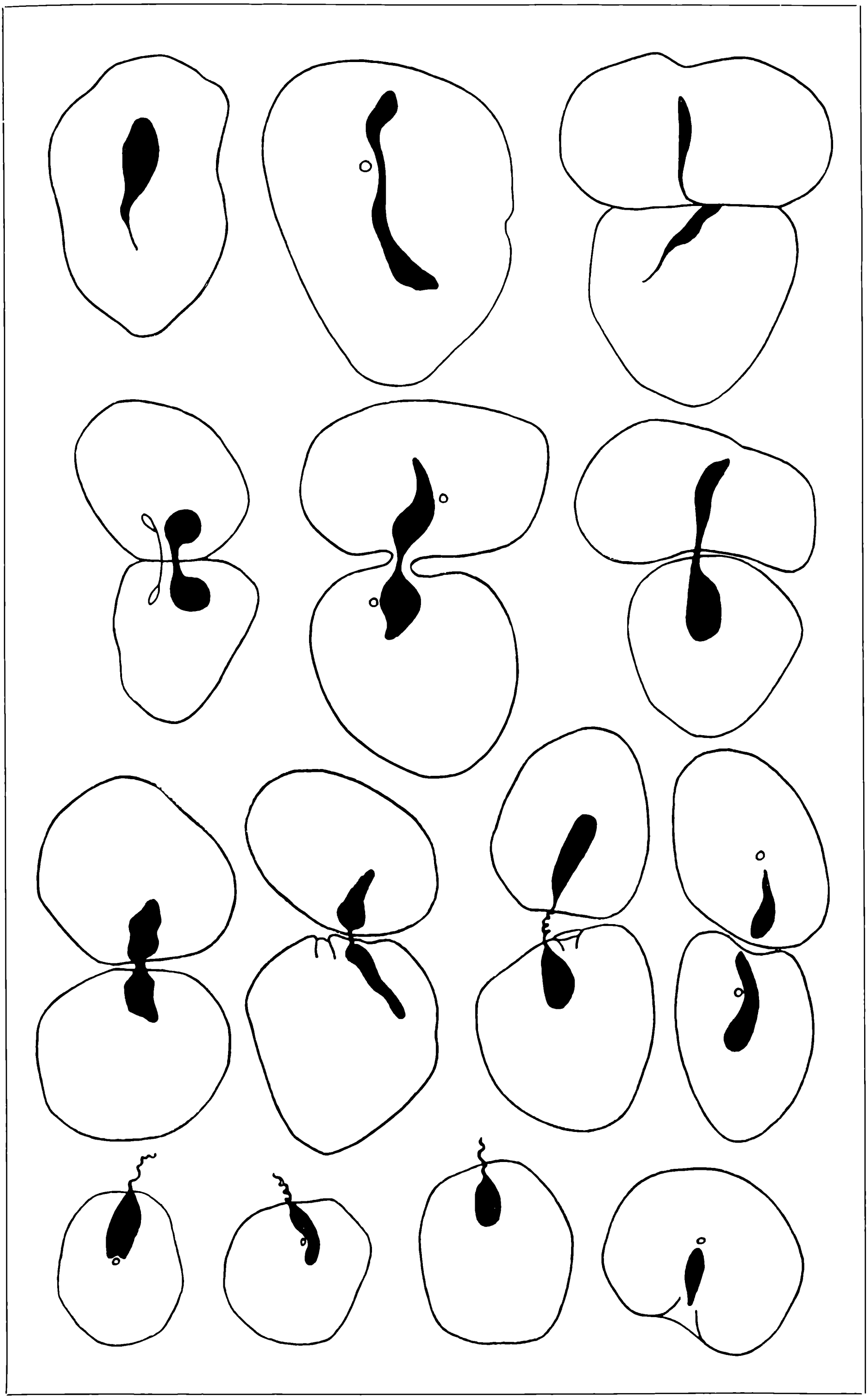
Fig. 1. A sagittal section showing the 12 somatic chromosomes.

Fig. 2. A sagittal section showing the macronucleus spiralling between the two daughters.

Fig. 3. Detail of micronucleus with two chromosomes.

Fig. 4. An earlier stage than fig. 1. Sagittal section.

Fig. 5. Bizarre form, whole mount.



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