Über das Vorkommen von Ergotamin in spanischem Mutterkorn

Das Ergotamin wird nach STOLL[1] zweckmässigerweise aus einem hiezu geeigneten Mutterkorn zentraleuropäischer Provenienz gewonnen. In spanischem Mutterkorn, das vorwiegend Ergotoxin-Alkaloide enthält, wurde jedoch das Ergotamin bisher nicht aufgefunden. Ein analytisches Trennungs- und Nachweisverfahren für einige Mutterkornalkaloide, unter anderem für Ergotamin, mittels Verteilungschromatographie wurde von CARLESS[2] angegeben, der in den von ihm untersuchten drei Proben von spanischem Mutterkorn kein Ergotamin nachweisen konnte.

Wir haben das von uns zur papierchromatographischen Trennung und quantitativen Bestimmung der einzelnen Mutterkornalkaloide ausgearbeitete Verfahren[3] auch auf die Analyse von über hundert Proben von spanischem Mutterkorn angewendet und konnten das Ergotamin in sämtlichen Proben auffinden, und zwar in einer Menge von 3 bis 8% der gesamten Alkaloide. Der durchschnittliche Gehalt an Ergotamin von mehreren Tonnen spanischen Mutterkorns wurde mit etwa 5% der gesamten Alkaloide ermittelt.

Nach Abtrennung von den übrigen Alkaloiden durch Gegenstromverteilung nach HELLBERG[4] konnten wir rund 70% der analytisch gefundenen Ergotaminmenge als Phthalat[5] isolieren. Aus 2 kg spanischer Mutterkornware wurden so 120 mg Ergotaminphthalat erhalten.

Die Angabe von CARLESS[2], dass in dem spanischen Mutterkorn etwa 20% der Alkaloide als Ergosin vorliegen, in anderen europäischen Mutterkornvorkommen dagegen weniger, steht in guter Übereinstimmung mit unseren Ergebnissen.

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Summary

Ergotamine, which usually is obtained from Central European ergot of rye, has been found for the first time to occur in Spanish ergots in an amount of 3–8% of the total alcaloids.

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Electron Microscope Study of Intact Tentacles and Disc in *Tokophrya infusionum*

Tokophrya infusionum is a small (17–50 μ in diameter) fresh-water protozoan belonging to the class (or order[1]) Suctoria. The adult form of the organism is sessile, being supported on a slender stalk which in turn is fastened to the substrate by an attaching disc. During this stage of its life, it feeds on living ciliates, such as *Tetrahymena pyriformis*[2], which come into contact with the adhesive ends of one or more of its long tentacles. After the prey is secure and paralyzed[1] the tentacles function as small tubes by means of which the contents of the prey are drawn into the body of Tokophrya.

The adult reproduces by endogenous budding, forming a succession of ciliated embryos which, after leaving the brood pouch within the parent body, metamorphose to the mature, sessile form. The parent organism retains its identity and continues to reproduce for a period of several days to several weeks before it dies. This latter feature makes Tokophrya a useful organism for studies of structural and physiological changes associated with aging[2].

Considerable interest surrounds the morphology and function of the feeding apparatus or tentacles of these protozoa as well as that of the device by which the organism adheres to a substrate and remains stationary during feeding. In some Suctoria[3] the tentacles are of two types: (a) the prehensile, which serves to capture the prey, and (b) the feeding type, through which the cytoplasm of the prey is drained into the predator. In Tokophrya, however, as in most Suctoria, both functions are performed by a single structure. This has the form of a very slender protrusion from the body of the organism, about 15–50 μ in length and $\frac{1}{2}$ μ in diameter, with a rounded knob at its end. The stalk[4] with its attaching disc is likewise a very slender extension (about 1 μ thick) from the body but nonetheless is a tough one capable of holding the organism against the tugs and pulls of many newly captured ciliates. The functioning of neither of these organelles is well understood, partly because important structural details are too small for light microscope resolution. For this reason it seems of interest to describe a few observations made on their fine structure by electron microscopy. These are preliminary to a more extensive study by these methods, of changes associated with aging.

For the purpose of these investigations Tokophrya was cultured in microdrops on either film-coated (formvar) cover glasses or electron microscope grids. Where cover glasses were used the organisms were transferred by way of the thin film to the grid by procedures outlined earlier for cultured tissue cells[5]. Where the culturing was done directly on coated grids this was unnecessary. For this latter procedure stainless steel grids were coated with formvar film under asceptic conditions and as many as 20 grids were picked up on a single cover glass. A small drop of yeast medium[6] containing Tokophrya and Tetrahymena was placed on each of the grids and the whole preparation mounted over a large well-slide. Since enough food was supplied in each drop, the Tokophrya reproduced very quickly and the young organisms settled down on the coated grid and adhered to the formvar by their attaching discs. When after 24 to 48 h sufficient organisms of the appropriate stage had accumulated in the microcultures they were fixed in vapors of 2% OsO_4 and then washed and dried, by the same procedure as that used for cultured tissue cells[5]. The dry preparations were shadowed with gold, or gold manganin, and examined.

Tentacles. In the light microscope under oil immersion the tentacle of *Tokophrya infusionum* appears as a

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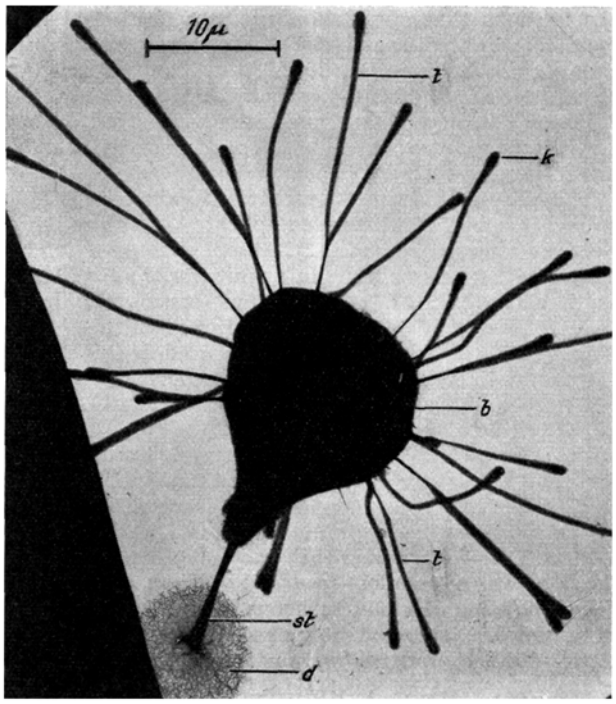

Figure 1.–Low power electron micrograph of a whole, intact *Tokophrya infusionum*. The body (*b*) is too thick for electron penetration and therefore no internal structure can be seen. *t* = tentacle; *k* = knob; *st* = stalk; *d* = disc. Magnification ×1720.

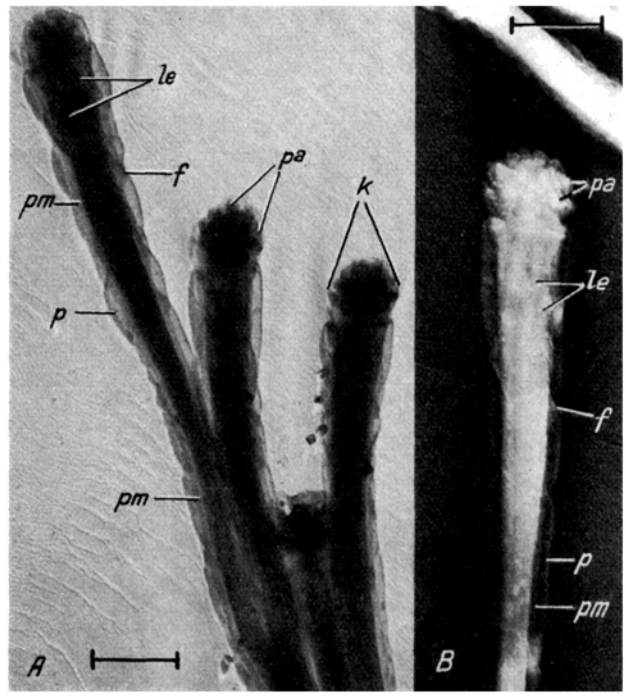

Figure 2*A*, *B*.–Higher power of the distal part of the intact tentacles, gold shadowed. *pa* = papillae; *le* = longitudinal elements; *f* = fold; *p* = pellicle; *pm* = plasma membrane; *k* = knob. Magnifications ×10,700 (*A*) and ×11,500 (*B*).

slender, straight, homogeneous projection with a rounded knob at its distal end. Except for the knob, which is a little broader, the tentacle seems to be uniform in diameter (about 0·5 μ) throughout its whole length. However, the electron microscope study of whole unsectioned tentacles disclosed that its very distal end, close to the knob, differs appreciably in diameter from the rest of the tentacle (Fig. 1 *t*, Fig. 2). Thus while the major part of the tentacle measures about 0·5 μ the distal end is about 1 μ. The internal structure of the tentacle is not too well defined in these whole-mount preparations, but is shown to better advantage than in the electron microscope study performed on tentacles of *Discophrya piriformis*[1]. It exceeds also the remarkably detailed description achieved by means of light microscopy by COLLIN[2]. He was able to distinguish three layers in a tentacle: (1) a pellicular sheath covering the whole tentacle, (2) a fluid cortical plasma, and (3) a wall surrounding the canal of the tentacle. The electron microscope study[3] of the unsectioned, whole tentacle shows that it is indeed covered by a pellicular sheath (Fig. 2*p*) which appears to be folded (Fig. 2*f*). Folds have been observed previously by light microscopy in feeding Suctoria[4] and were ascribed by some investigators to the cortical plasma and not to the pellicle[5]. Below the pellicle a plasma membrane can be detected which probably corresponds to COLLIN's cortical plasma (Fig. 2*pm*). The central component of the tentacle, described by Collin as the wall surrounding the canal of the tentacle, appears to consist of a number of longitudinal elements bundled together (Fig. 2*le*). The diameter of each is about 120 mμ. The number of these in a tentacle varies throughout its length. At the base there seems to be only one such element while at its distal end, just behind the knob, there may be as many as four or more. The width of the tentacle increases with the number of longitudinal elements. During feeding the tentacle shortens and broadens, and it seems probable that this is due to the contraction of these longitudinal elements. At the same time the pellicle, which has fixed dimensions, is obliged to develop folds (Fig. 2*f*).

The end or knob (Fig. 1*k*, Fig. 2*k*) of the tentacle is about 1 μ long and 1 μ wide and appears to have a complex structure. It obviously consists of a tuft of papillae, averaging nine in number (Fig. 2*pa*), each about 130 mμ in diameter. It is probable that the papillae are related to the longitudinal elements as terminations of them. The pellicular sheath ends at the base of the knob and thus leaves the papillae free. Since the swimming prey becomes attached only to the knob of the tentacle it is assumed that the knob is covered with a sticky substance; and there is in fact a suggestion of this in the nature of a homogeneous material surrounding the dried papillae.

Disc. The body of the adult Tokophrya is supported by a stalk (Fig. 1*st*, Fig. 3*st*) which terminates in a disc (Fig. 1*d*, Fig. 3). The stalk is about 8–20 μ long and only about 1 μ thick but is too dense for electron penetration and therefore no internal structure can be seen. The disc by which Tokophrya is held on the substrate is about 8–12 μ in diameter and is very thin and flat. The firmness of its attachment is attested by the

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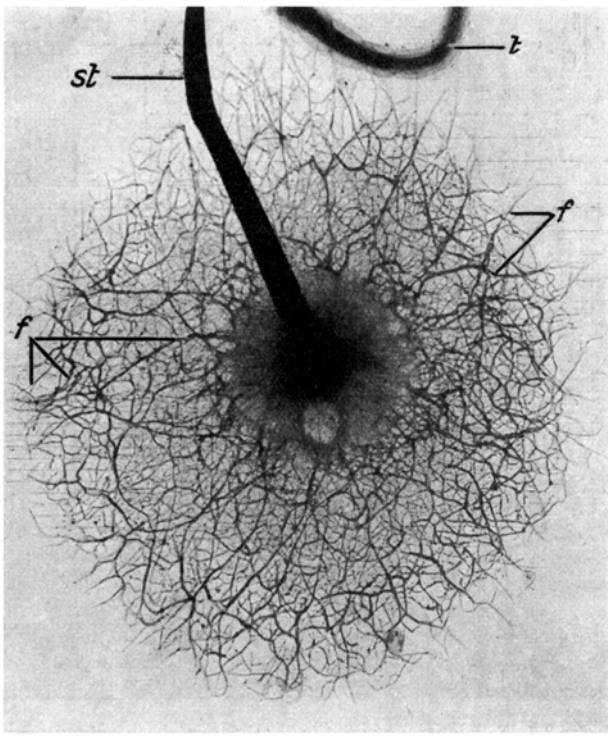

Fig. 3.–Higher power of intact attaching disc and stalk. *f* = fibrils; *st* = stalk; *t* = part of tentacle. Magnification × 6750.

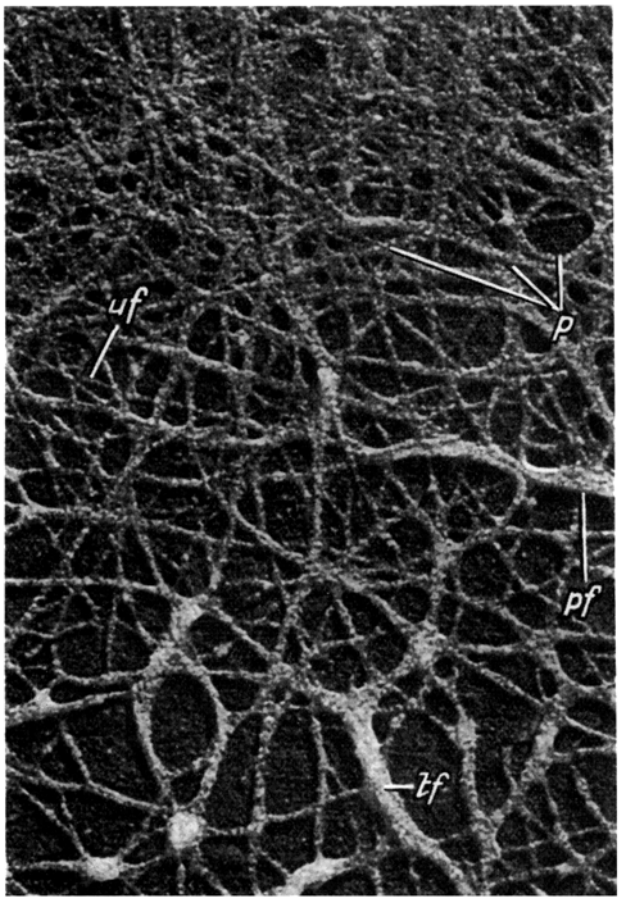

Fig. 4.–High magnification of part of disc, gold manganin-shadowed. *uf* = unit fibril; *tf* = twisted fibrils; *pf* = parallel-running fibrils; *p* = suggestion of periodicity. Magnification × 42,100.

observations that one Tokophrya may feed on ten or more ciliates at the same time, all struggling to escape, and yet not be wrenched loose from its fixed support. Even after the organism dies and disintegrates, the disc with the stalk remains in place, and in an old culture one can see a dense accumulation of discs on the walls of the culture vessel.

This very thin organelle, structureless under the light microscope, presents a very complicated structure in the electron microscope (Fig. 3). It consists of a felt or meshwork of innumerable, fine, unit fibrils about 150 Å in diameter (Fig. 4 *uf*). There is no limiting membrane around the disc and the fibrils end free at the periphery. Single fibrils are best seen and measured in this region. In micrographs taken at higher magnifications there is scattered evidence of periodicity in the structure of the unit fibrils. The spacing is about 120 Å (Fig. 4 *p*). It is difficult, to measure the length of the unit fibrils because they are frequently intertwined and tangled. Some are twisted to form ropes (Fig. 4 *tf*); others run parallel in bands (Fig. 4 *pf*), resembling the cellulose fibers of plants[1]. It is conceivable that they are formed in much the same way as cellulose in *Acetobacterium xylinum*[2] by polymerization of a homogeneous substance, the future stalk and disc, secreted from the embryo at the time it settles down to undergo metamorphosis.

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Zusammenfassung

Elektronenmikroskopische Untersuchungen am sessilen Süsswasser-Protisten *Tokophrya infusionum* zeigen besonders komplizierte Strukturen der «Tentakel» und der «Haftscheibe». Die Tentakel sind von zwei Membranen umgeben und umschliessen eine Anzahl von Längselementen. Ihre Spitzen sind aus einer grösseren Zahl von «Papillen» zusammengesetzt. Die Haftscheibe besteht aus einem reichen Fibrillennetz.

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[3] Supported by a grant from the National Heart Institute, U.S. Public Health Service.

The Visualization of the Granulated Mitochondrial Inner Body through Treatment of Isolated Mitochondria with Xylene[1]

In a previous communication it was shown[2] that isolated rat liver mitochondria suspended in distilled water and dried down on Formvar films *in vacuo* at 0°C are an excellent object for electronmicroscopical studies of mitochondrial structure. In such preparations the mitochondria revealed themselves as composed of three main constituents: (1) a granulated inner body, (2) a very finely granulated matrix material and (3) a membrane surrounding the mitochondrion.

[1] This work is a part of an investigation supported by a grant from The Swedish Cancer Society.

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