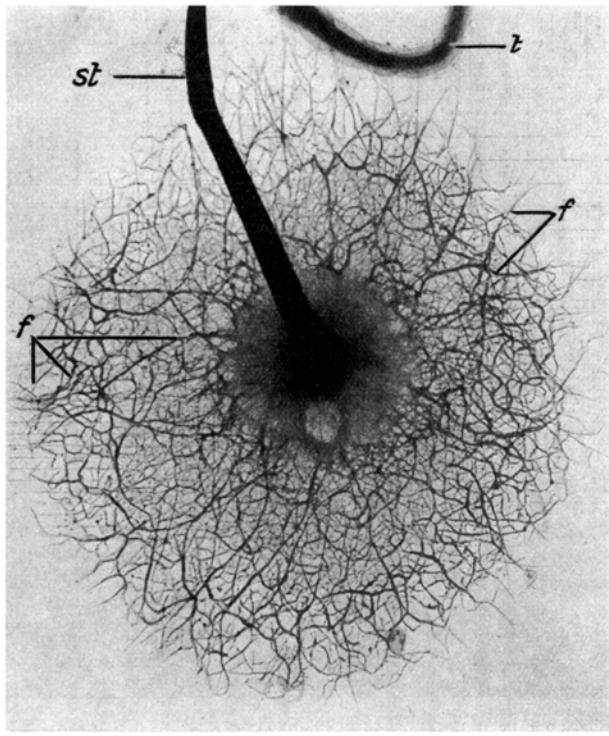

Fig. 3.–Higher power of intact attaching disc and stalk. *f* = fibrils; *st* = stalk; *t* = part of tentacle. Magnification × 6750.

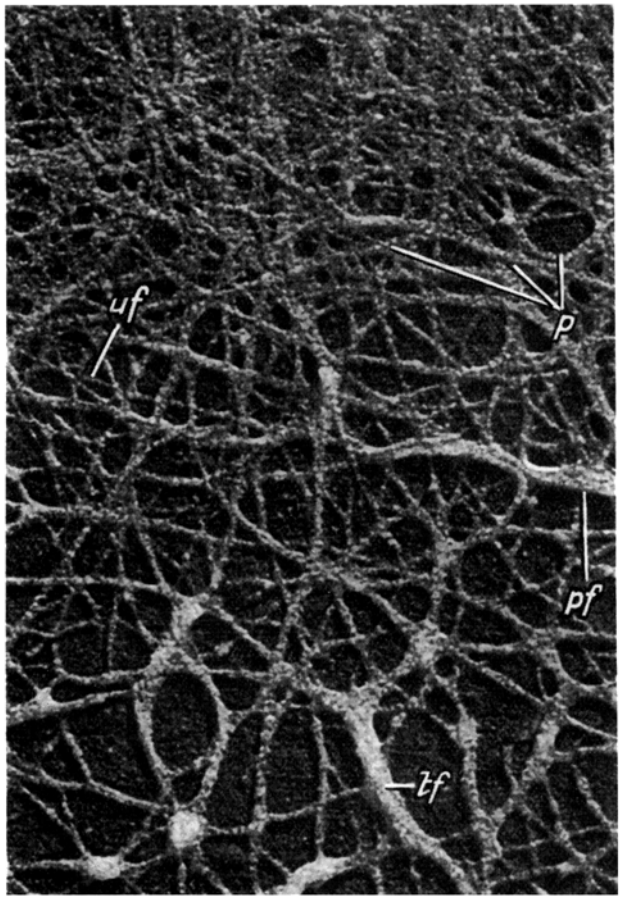

Fig. 4.–High magnification of part of disc, gold manganin-shadowed. *uf* = unit fibril; *tf* = twisted fibrils; *pf* = parallel-running fibrils; *p* = suggestion of periodicity. Magnification × 42,100.

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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Laboratories of the Rockefeller Institute for Medical Research, New York 21, N.Y., June 28, 1954.

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.

[1] K. MÜHLETHALER, Biochim. Biophys. Acta *3*, 527 (1949); Z. Zellforsch. *38*, 299 (1953).

[2] K. MÜHLETHALER, Biochim. Biophys. Acta *3*, 527 (1949).

[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.

[2] G. GLIMSTEDT, S. LAGERSTEDT, and K. S. LUDWIG, Exp. Cell Res. *7*, 1954 (in press).

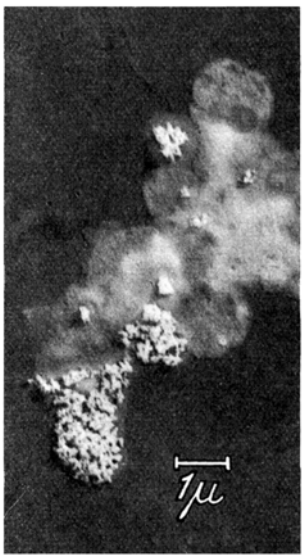

Fig. 1.

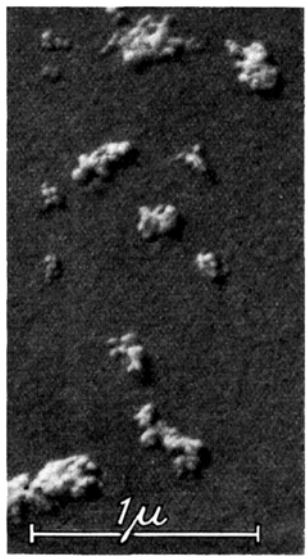

Fig. 2.

Fig. 1.–Mitochondrial preparation dried at 0°C, treated with xylene for 20 min. The clearly shown granular inner body is restricted to the outlines of the mitochondria.

Fig. 2.–Smear of inner body granules, prepared as described in text. The individual granules have a diameter of approx. 300 Å.

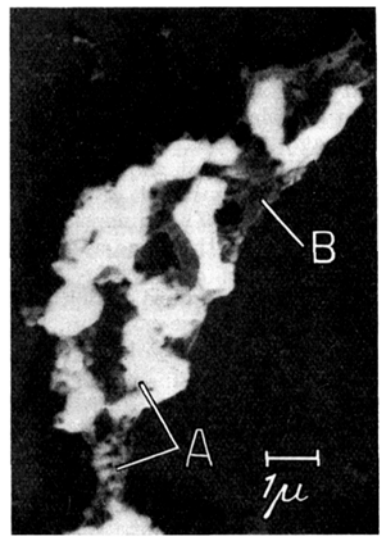

Fig. 3.

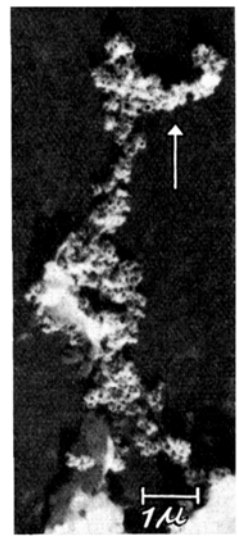

Fig. 4.

Fig. 3.–Frozen-dried mitochondrial suspension from 0·88 *M* sucrose solution. *A* = Inner bodies. *B* = membranes.

Fig. 4.–Same preparation as in Figure 3 but after 20 min treatment with xylene. The inner body now shows the granular structure with the granules forming a cross-striation at several places (f.i. as indicated by the arrow).

In earlier investigations[1] of isolated mitochondria after OsO_4 fixation the inner body was found to be composed of equal-sized granules, each approx. 0·1 μ in diameter. However, some doubt was felt about the correctness of the dimensions given, as granules could occasionally be observed showing a disc-like appearance, possibly based on smaller components within the single granule[1]. The discovery of the matrix material[2] gave an explanation of the difficulties previously met with, as matrix substance, when covering the inner body, obviously impaired the optimal conditions for detailed observation. In a series of investigations testing the effect of different fixatives and solutes on the directly dried mitochondria[3], it was found that treatment with xylene revealed the ultrastructure of the inner bodies in a rather interesting way, which will be reported here.

Rat-liver mitochondria, isolated from 0·88 *M* sucrose solution by differential centrifugation, and then suspended in distilled water, were dried on Formvar films on the screens as previously described[2]. Drops of xylene covering the preparations were left there in a closed PETRI dish for 5, 10, 20, 40, and 80 min. After withdrawal of the xylene, the preparations were dried *in vacuo*. The observations were made after shadow-casting at 30° angle with palladium in a SIEGBAHN-SCHÖNANDER electron microscope at 50 kV.

After 5 min xylene treatment, the granules earlier[1] observed with a diameter of 0·1 μ were clearly seen. After 20 min, however, the individual granule was resolved into smaller units (Fig. 1). Further increase of the time of treatment did not change the picture with reference to the inner bodies. If the xylene was carefully withdrawn, the position of the granules closely followed the outlines of the mitochondria. After washing of the preparations with xylene in a definite direction, it was found that the granules had been removed from their original localization and now formed smears of granular material from the mitochondria and in the direction of the stream of xylene. In such preparations it was easy to measure the sizes of the individual granules, which was found to be of the magnitude of 300 Å (Fig. 2).

To be sure that the material here described as inner bodies really belonged to the inner parts of the mitochondria, and also to get better preservation of the intact mitochondria but without preliminary fixation, trials were made to investigate frozen-dried mitochondria before and after xylene treatment. For this purpose drops of mitochondrial suspensions in 0·88 *M* sucrose solution as well as in distilled water, were placed on screens, covered with Formvar films. The screens were immersed in propane, previously cooled by liquid nitrogen to about – 190°C, and, after the freezing, dried in an apparatus according to GLICK-MALMSTRÖM[1] at – 80°C. To remove the heaps of sucrose covering the mitochondria from 0·88 *M* sucrose solutions, the mitochondria were fixed in vapour from 70% alcohol[2] during 5 min and then washed with 3–4 drops of distilled water. After drying *in vacuo* some of these preparations, together with a number of those directly frozen-dried from distilled water, were treated with xylene as described above. The others were directly shadowcast and used as controls.

From the point of view of preserving the morphological relations between the components of the intact mitochondrion, the attempts to freeze-dry the distilled water suspensions were not too successful. It was quite obvious that the mitochondrial spheres, in which form

[1] G. GLIMSTEDT and S. LAGERSTEDT, Kungl. Fysiograf. Sällsk. Handl. N. F. *64*, 3 (1953); Kungl. Fysiograf. Sällsk. Förhandl. *23*, 1 (1953); Anat. Anz. *100*, Erg. H. 97 (1954).

[2] G. GLIMSTEDT, S. LAGERSTEDT, and K. S. LUDWIG, Exp. Cell Res. *7*, 1954 (in press).

[3] G. GLIMSTEDT, S. LAGERSTEDT, and K. S. LUDWIG (to be published).

[1] D. GLICK and B. G. MALMSTRÖM, Exp. Cell Res. *3*, 125 (1952).

[2] G. GLIMSTEDT, S. LAGERSTEDT, and K. S. LUDWIG (to be published).

the mitochondria appear in distilled water preparations[1], burst, probably as a result of the freezing and drying. On the pictures the three main components i.e. membranes, inner bodies and matrix substance, are easily recognized, but their mutual relationships are hard to ascertain, as they form fragments scattered over the fields of view. After xylene treatment, however, the inner bodies were found to be composed of smaller granules, just as after drying at 0°C. The dimensions of these granules were also found to be the same as in the directly dried ones, i.e. 300 Å.

On the contrary, in those mitochondria which had been frozen-dried from 0·88 *M* sucrose solution, the mitochondrial membranes were obviously broken up, but with localization of the inner parts of the mitochondria largely retained (Fig. 3). There were no signs of swelling of the membranes, as their remnants fit quite well the dimensions of the mitochondria present. The inner structure is revealed partly as a homogeneous mass, sometimes showing a cross-striation, and partly as composed of granules approx. 0·1 μ in diameter. After treatment with xylene, the granules were visualized as composed of smaller granules of approx. 300 Å diameter (Fig. 4). In places where the original morphology of the inner parts of the mitochondrion probably was retained, these granules were seen to give rise to a cross-striation of the mitochondria. The preparation method here employed is too crude as yet to allow a detailed study of the mutual relations of the granules.

It is surprising that the mitochondrial membranes, which in this type of preparation are seemingly not distended, and where the parts shown to the observer seem to be rather intact, do not show any lamellar structures protruding into the mitochondria. This is not in agreement with the observations[2] in thin sections of embedded material, where a cross-striation of the inner parts of the mitochondrial body is attributed to intramitochondrial membranes, entirely[3] or partly[4] going across the mitochondria. Any membranes in this sense were not found in sectioning isolated mitochondria[5]. On the other hand, a cross-striation of the mitochondrial body has repeatedly been observed in isolated material, as well as after fixation of mitochondria with OsO_4 in 0·88 *M* sucrose solutions[6]. The absence of intra-mitochondrial membranous structures in the present material could not be attributed to any damage due to the washings with water after fixation in alcoholic vapour, as shown in experiments performed in this laboratory[7]. The possibility remains that the cross-striation observed in sectioned material may not correspond to membranes within the mitochondria, but to the periodic arrangement of material with different electron-scattering power perpendicular to the long axis of the mitochondria. This hypothesis would find support in the observations on the arrangement of the granules forming the inner body in the present investigation, especially as the dimensions here found are in good agreement with those reported for the distances between the intramitochondrial cross-striations. The question is receiving further attention in this laboratory.

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Department of Histology, University of Lund, Sweden, July 16, 1954.

Zusammenfassung

Die Autoren stellen mit einer speziellen Technik Präparate von aus Rattenleber isolierten Mitochondrien her, die mit Xylol nachbehandelt werden. Dadurch wird der Innenkörper der Mitochondrien einer eingehenden elektronenmikroskopischen Untersuchung zugänglich. Der Innenkörper besteht aus einzelnen Granula, die einen Durchmesser von 300 Å haben. Die Anordnung der Granula ergibt eine charakteristische Querstreifung des Innenkörpers.

[1] K. W. CLELAND, Nature *170*, 497 (1952). – J. L. FARRANT, R. N. ROBERTSON, and M. J. WILKINS, Nature *171*, 401 (1953). – J. W. HARMAN, Exp. Cell. Res. *1*, 394 (1950). – H. U. ZOLLINGER, Amer. J. Path. *24*, 569 (1948); Schweiz. Z. Path. Bakt. *11*, 617 (1948).

[2] F. S. SJÖSTRAND and J. RHODIN, Exp. Cell Res. *4*, 426 (1953). – G. E. PALADE, Anat. Rec. *114*, 427 (1952).

[3] F. S. SJÖSTRAND and J. RHODIN, Exp. Cell Res. *4*, 426 (1953).

[4] G. E. PALADE, Anat. Rec. *114*, 427 (1952).

[5] R. WEBER, Z. Zellforsch. *39*, 630 (1954).

[6] G. GLIMSTEDT and S. LAGERSTEDT, Kungl. Fysiograf. Sällsk. Handl. N. F. *64*, 3 (1953); Kungl. Fysiograf. Sällsk. Förhandl. *23*, 1 (1953); Anat. Anz. *100*, Erg. H. 97 (1954).

[7] G. GLIMSTEDT, S. LAGERSTEDT, and K. S. LUDWIG (to be published).

[1] Research Fellow aided by the "Stiftung für biologisch-medizinische Stipendien", Basle (Switzerland). Present address: Institute of Anatomy, University of Basle.

Un cas nouveau de chromosomes sexuels multiples dans le genre *Gerbillus (Rodentia – Muridae – Gerbillinae)*

J'ai fait connaître[1] les conditions chromosomiques chez trois espèces de *Gerbillus: G. campestris* et *G. garamantis* ont respectivement 56 et 54 chromosomes, les mâles étant dotés d'un couple *X–Y* du type habituel pour la sous-famille; l'*X* est métacentrique, l'*Y* submétacentrique, les deux hétérochromosomes étant de grande taille. Chez *G. pyramidum*, le nombre diploïde est de 40. A la méiose, il y a formation facultative d'un quadrivalent sexuel, le couple *X–Y* pouvant s'associer à un bivalent autosomique. L'interprétation du cas est facile et se fonde sur l'hypothèse d'une petite translocation entre l'un des bras court d'un autosome et le chromosome *X*.

Ainsi, au total, quatre cas de chromosomes sexuels multiples ont été décrits chez des Mammifères. Voici le cinquième.

Gerbillus gerbillus OLIVIER (les sujets étudiés proviennent du sud de l'Algérie et m'ont été donnés par le Dr F. PETTER du Muséum de Paris) est doté de 43 chromosomes, probablement tous métacentriques, ce nombre ayant été établi par l'analyse des cinèses spermatogoniales du mâle (Fig. 1). L'un des éléments est immédiatement reconnaissable à sa taille atteignant 10 μ et à sa forme asymétrique, le centromère séparant deux bras dont l'un est six à sept fois plus long que l'autre. Cet *X* diffère beaucoup de celui de tous les autres *Gerbillinae* étudiés dont l'*X* est un métacentrique.

Le nombre impair 43 laisse supposer que la femelle a 44 chromosomes, ce qui signifierait une digamétie mâle de type *X–O*. Il n'en est rien: des «squashes» d'ovaire permettent d'obtenir des figures correctes de mitoses dans les cellules folliculaires: ces divisions (Fig. 2) montrent 2 *X* et, au total, 42 chromosomes. Ceci implique l'existence de chromosomes sexuels multiples et le schéma: $\delta : X - Y_1Y_2 \cdot \varphi : X - X$.

[1] R. MATTHEY, Arch. J.-Klaus-Stift. Vererbungsforsch. *27* (1952); Rev. suisse Zool. *60* (1953); Caryologia *6*, 1954).