J. ULTRASTRUCTURE RESEARCH **29**, 343–349 (1969)

The Spermatozoon of Arthropoda

VI. Ephemeroptera[1]

B. BACCETTI, R. DALLAI, AND F. GIUSTI

Istitute of Zoology, University of Siena, Siena, Italy
Received April 21, 1969

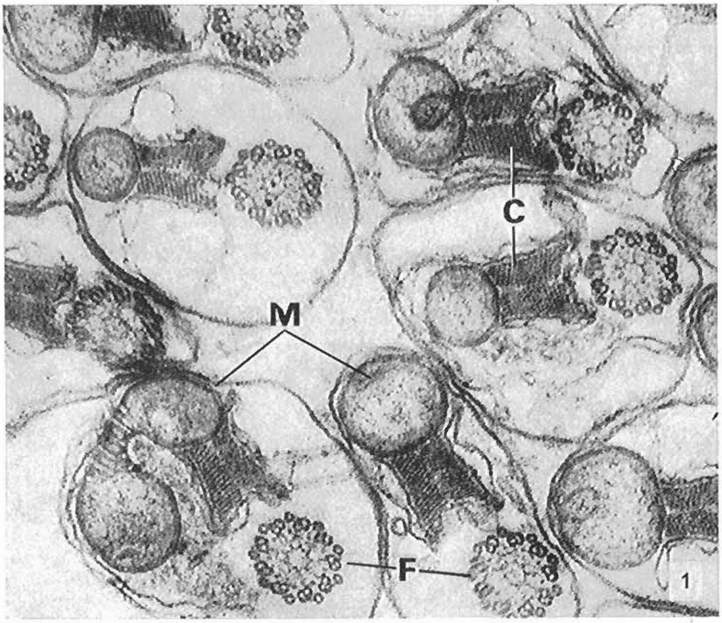
In this work the spermatozoon of *Chloëon dipteron* (Ephemeroptera) is examined. The three most important features are that (a) the acrosome is very brief; (b) the mitochondrial derivative is represented by a long mitochondrion with transverse cristae and by a crystalline mass enveloped in a separated membrane; (c) the axial filament of the tail lacks the two principal central units and the central sheath, and is therefore, of the 9+9+0 pattern. This type of flagellum has not previously been described.

Among Arthropoda, the insects have achieved the highest degree of adaptation to terrestrial life. In this regard they have developed highly flexible devices of internal fertilization, the basic structure of the spermatozoon being modified from 9+2 (as in Collembola) to 9+9+2 (*2*), an aquisition which is independently acquired by other phyla with internal fertilization. From this pattern separate branches evolve toward the biflagellarity achieved in rhyncotoids (*4*) to total loss of the flagellum in scale insects (*14*), giant flagella in *Sciara* (*13*), and flagella 9+9+1 in *Culiseta* (*9*). Formula 9+9+0 has been hitherto unrecorded. It is interesting to discover its presence now in an order, the Ephemeroptera, which is highly aberrant in other respects.

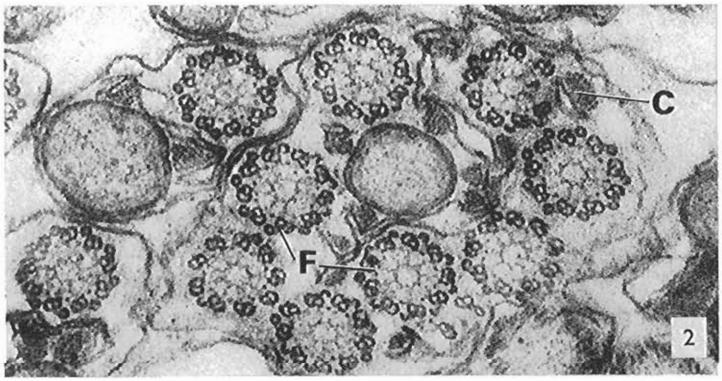
MATERIALS AND METHODS

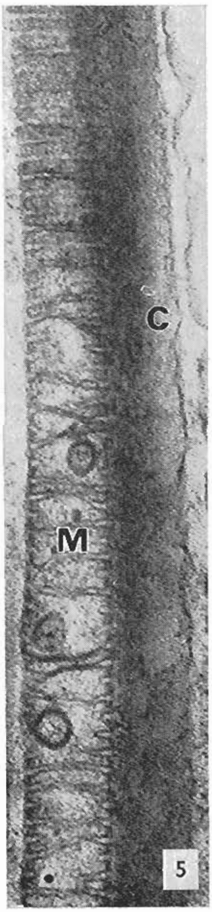
The present research was carried out on *Chloëon dipteron* L. The testes were fixed *in vivo* in 2.5% Hoyle-buffered, pH 7.2, glutaraldehyde for 20–40 min, postfixed for 5–10 min in 1% osmium tetroxide in Hoyle buffer, pH 7.2, dehydrated, and embedded in Epon. Sections cut on an LKB II and Reichert ultramicrotomes were collected on grids and usually stained with 1.5% uranyl acetate and lead citrate. Carbon-coated sections were examined in a Siemens Elmiskop IA 125 kW of the Institute of Zoology of the University of Siena.

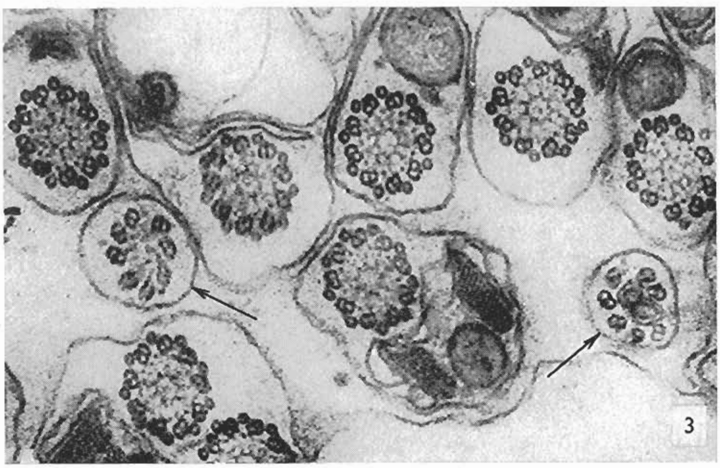
[1] Research performed under C.N.R. contract.


C
M
F
1


N
F
4


C
F
2


C
M
5


3

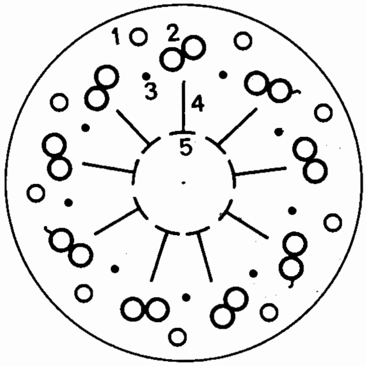


FIG. 6. Schematic drawing of the axial filament complex of the spermatozoon of *Chloëon dipteron*. *1*, accessory fibers; *2*, double fibers; *3*, thin filaments; *4*, Afzelius rays; *5*, secondary fibrils

OBSERVATIONS

In the *Chloëon dipteron* spermatozoon there is a brief, apically rounded, bilaminar acrosome, less than 0.3 μ long, 0.16 μ thick, surrounded by a membrane of ~80 Å (Fig. 7). The nucleus, which is cylindrical, is surrounded by a thin membrane (about 80 Å) which is separated from the chromatin material by a light interspace 200–300 Å wide. Chromatin material (Fig. 4) appears rather electron opaque and tightly packed. Many clear vesicles are noted, approximately 300 Å in diameter, exhibiting an opaque central body about 150 Å across. Immediately behind the nucleus is the centriole, but the adjunct centriole is missing. The tail is composed of the flagellar axial filament and mitochondrial complex.

The flagellar axial filament (Figs. 1, 8) is about 0.2 μ in diameter and in transverse section seems to be composed of 9 apparently hollow outer accessory fibers 160 Å in diameter, 9 doublets either bearing incomplete arms or, in most cases, lacking them, 9 well evident Afzelius rays, and 9 secondary fibrils. Both the main central fibers and central sheath are missing. The large secondary fibrils and the rays form a star-

---

FIG. 1. Cross sections of the middle piece in several spermatozoa of *Chloëon dipteron*. The axial filament complex (*F*) is of the 9+9+0 pattern; the mitochondrial derivative consists of a normal crested mitochondrion (*M*) and two paired crystalline bodies (*C*). ×60,000.
FIG. 2. Cross sections of a more caudal portion of the sperms. The crystalline bodies (*C*) of the mitochondrial derivative are smaller and separated each other. In some axial filament complex (*F*), some of the outer fibers are lost. ×60,000.
FIG. 3. Terminal cross sections of sperm tails. In some cases (arrows) the axial filament complex consists only of the 9 double principal fibers. ×60,000.
FIG. 4. Longitudinal section of the centriole region. *N*, nucleus; *F*, axial filament complex. ×60,000.
FIG. 5. Longitudinal section of a more caudal region. Note the crested region of the mitochondrial derivative (*M*) and the crystalline body (*C*). ×60,000.

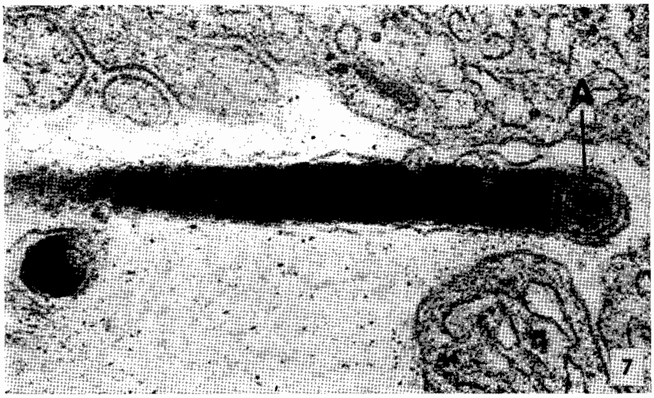


Fig. 7. Cross section and oblique sections of the acrosome (A) region in *Chloëon* spermatozoon. ×60,000.

shaped structure occupying the central core of the axial filament and surrounded by 9 thin filaments about 50 Å in diameter, aligned with the 9 accessory fibers and immediately below the doublets. Proceeding toward the tail end (Fig. 2), the 9 accessory fibers, Afzelius rays, secondary fibers (Fig. 3), and finally the doublets, are seen to disappear. The doublets seem to disappear one by one; in fact, images of 9, 8, 7, and only 3 doublets are observable.

In the mitochondrial complex are found a single mitochondrion and two crystalline structures lying external to it. The whole complex is wrapped in a membrane formed by two electron dense layers, approximately 80 Å in thickness, separated by a light space of varying dimensions (100–150 Å), which sometimes seems strikingly dilated, above in all relation to the sides of the paracrystalline bodies. The mitochondrion (Figs. 1–3) situated in front of the flagellum originates immediately below the nucleus, where it appears to measure about 0.2 μ. It increases in size as it extends toward the central part of the tail, reaching a maximum diameter of 0.28 μ and then tapering again to about 0.12 μ. In longitudinal section (Fig. 5) it is slightly spindle-shaped and shows numerous inner cristae parallel to one another, about 150 Å apart; these are interrupted along the central axis, thus forming a ring. However, the crystalline axis present in many insects is absent.

Between the cristae spheroid vesicles are visible (Fig. 5); these are approximately 600 Å in diameter, surrounded by a membrane 80 Å thick, and transparent to the electron beam. The center of each vesicle contains a thick electron dense material, and membrane fragments are occasionally present. These vesicles are likely to originate from invaginations of the mitochondrial membrane, to which they often adhere.

The two crystalline structures, like the mitochondrion, are seen to arise immediately

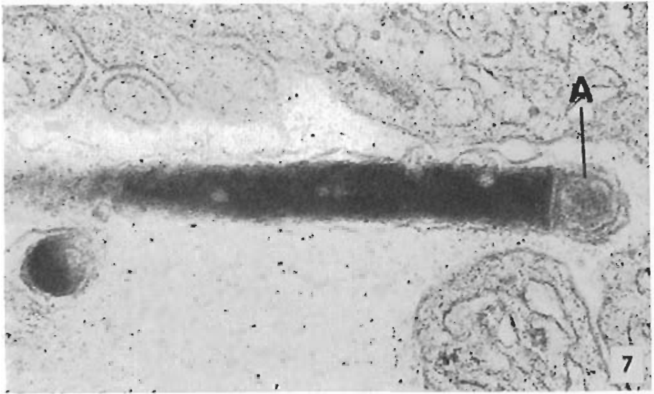


Fig. 7. Cross section and oblique sections of the acrosome (A) region in *Chloëon* spermatozoon. × 60,000.

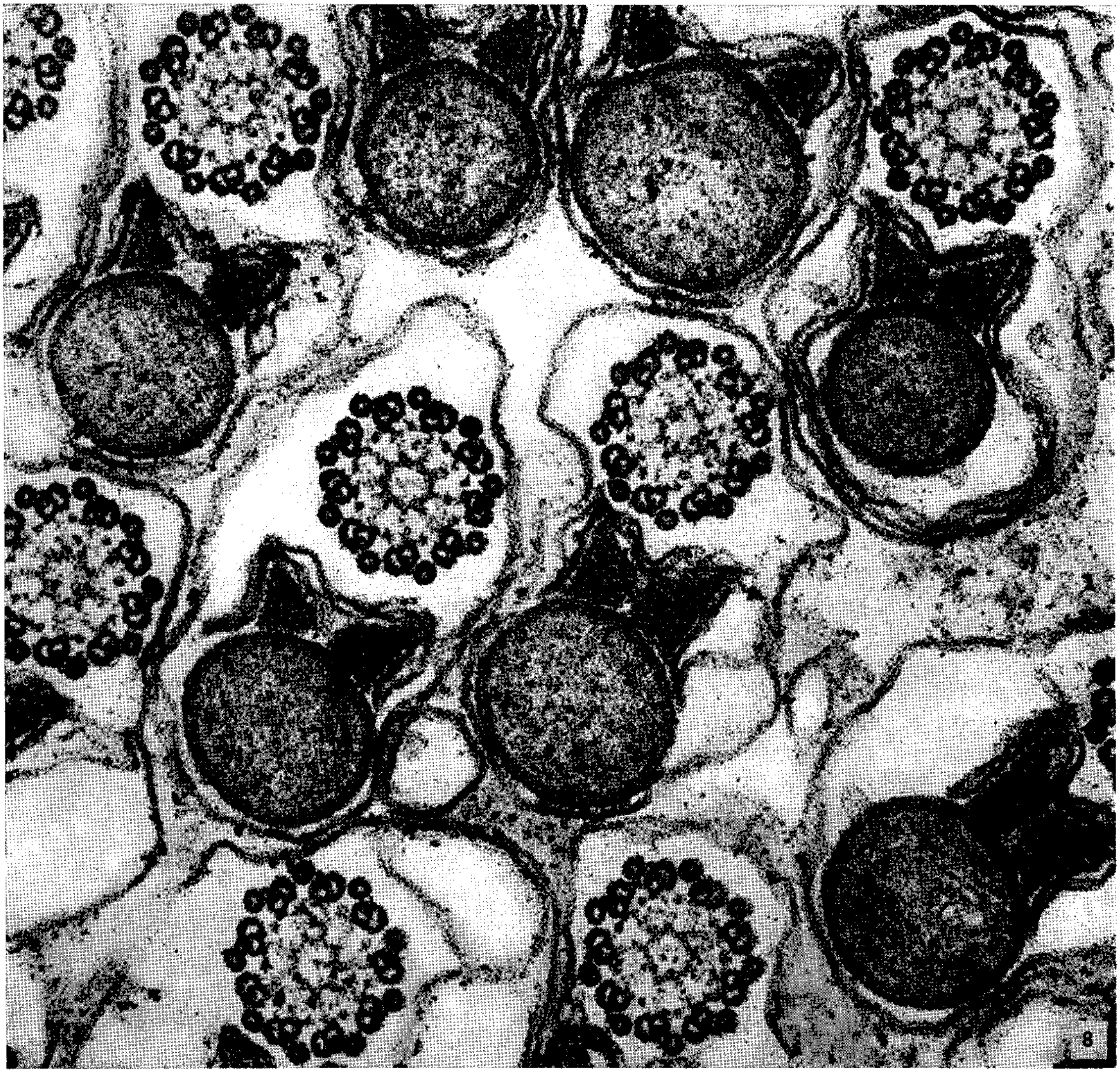


Fig. 8. High magnification of the axial filament complex. x 120,000

beneath the nucleus. In transverse section their overall pattern is comparable to an arrow tail and seems to vary in size. Where it begins, the entire crystalline complex is about 0.12 μ in height and the same in width, while in the direction of the median zone of the tail, it has a height and width of 0.18 μ. Subsequently, these two structures separate and the distance between them increases until by the end of the tail they

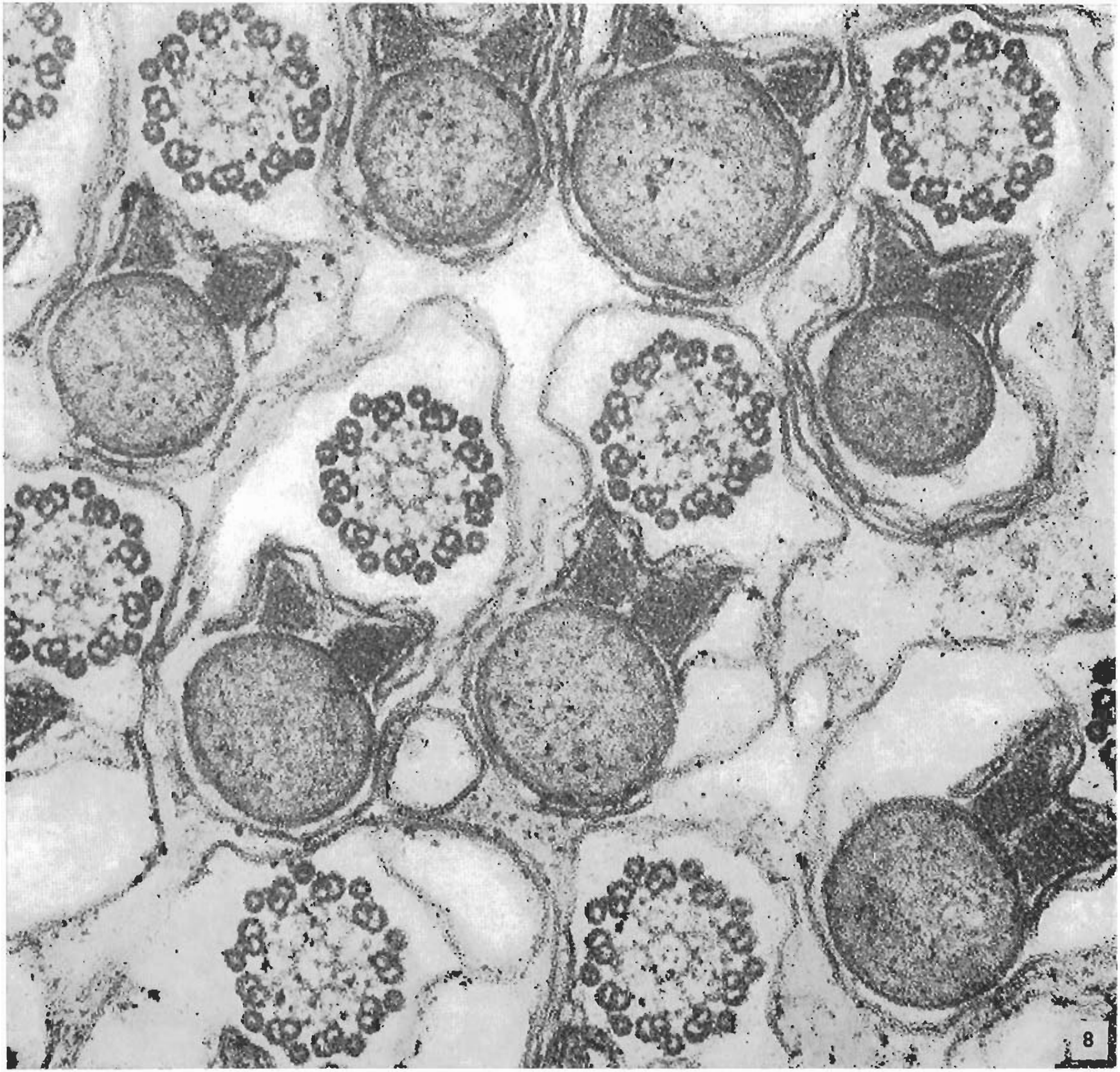


Fig. 8. High magnification of the axial filament complex. x 120,000

become very slender and disappear. They are made up of a material organized in crystalline form, arranged according to a period of approximately 160 Å, exactly like the proteinaceous material which in insects permeates either fully or in part the mitochondrial derivatives.

DISCUSSION

The spermatozoon of the ephemerid under investigation is noteworthy owing to a number of characteristics that single it out from the types thus far known. It has a very brief acrosome, a 9+9+0 flagellum, retains an isolated normal mitochondrion and a crystalline proteinaceous complex within a single membrane. Judging from some characters it is rather primitive, while other features make it appear aberrant.

The littleness of the acrosome is a relatively widespread phenomenon in certain insect orders. It has been observed, for example, in *Drosophila* (BAIRATI and PEROTTI, in press). The acrosome is totally lacking in a coleopteran (*11*), in Neuroptera (*3*), and in Trichoptera (*5*). The flagellar axial filament of the 9+9+0 type is a curiosity worth noting. Cases of 9+1 have so far been reported in many platyhelminths (*8*, *15–19*) and a 9+0 pattern in a diatom sperm (*12*), a eugregarine gamete (*10*), an annelid (*1*), and a fish (*7*). Among 9+9+2 sperms, only the dipteran *Culiseta* shows a 9+9+1 array (*9*). The present study is the first to show a tail with the formula 9+9+0. The Ephemeroptera are the most primitive in the pterygote insect order, and also strongly aberrant. Since Thysanura are already endowed with a stable 9+9+2 pattern (*6*, *20*), one could suppose that in the Ephemeroptera the flagellum has secondarily lost its doublet of central fibers. Within the mitochondrial complex, the separation of the crystalline mass from the long mitochondrion which preserves all its cristae intact right into maturity is also certainly peculiar. The most evolved insect sperms show all of the mitochondrial mass transformed into crystals; in other instances, crystal and cristae coexist (*2*). The situation we describe here is unprecedented. Maintenance of the normal structure in the single mitochondrion which runs throughout the entire length of the flagellum is perhaps a highly primitive character.

REFERENCES

1. AFZELIUS, B. A., *Proc. 5th Intern. Congr. Electron Microscopy, Philadelphia, 1962* M 1. Academic Press, New York, 1962.
2. BACCETTI, B., *Atti 7th Congr. Nazl. Ital. Entomol., Bologna*, 32 (1967).
3. BACCETTI, B., DALLAI, R. and ROSATI, F., *J. Microscopie* **8**, 233 (1969).
4. —— *ibid.* **8**, 269 (1969).
5. —— *J. Ultrastruct. Res.* (in press).
6. BAWA, S. R., *J. Cell Biol.* **23**, 431 (1964).
7. BOISSON, C., MATTEI, X. and MATTEI, C., *Compt. Rend. Acad. Sci.* **264**, 2909 (1967).
8. BONSDORFF, C. H. VON and TELKKA, A., *Z. Zellforsch. Mikroskop. Anat.* **66**, 643 (1965).

9. BRELAND, O. C., GASSNER, G., RIESS, R. W. and BIESELE, J. J., *Can. J. Genet. Cytol.* **8**, 759 (1966).
10. DESPORTES, J., *Compt. Rend. Acad. Sci.* **263**, 517 (1966).
11. FURIERI, P., *Redia* **48**, 179 (1963).
12. MANTON, G. and VON STOSCH, H. A., *J. Roy. Microscop. Soc.* **85**, 119 (1966).
13. PHILLIPS, D. M., *J. Cell Biol.* **30**, 499 (1966).
14. ROBISON, W. G., *J. Cell Biol.* **29**, 251 (1966).
15. ROSARIO, B., *J. Ultrastruct. Res.* **11**, 412 (1964).
16. SATO, M., OH, M. and SAKODA, K., *Z. Zellforsch. Mikroskop. Anat.* **77**, 232 (1967).
17. SHAPIRO, J. E., HERSHENOV, B. R. and TULLOCH, G. S., *J. Biophys. Biochem. Cytol.* **9**, 211 (1961).
18. SILVEIRA, M. and PORTER, K., *Protoplasma* **59**, 240 (1964).
19. TULLOCH, G. S. and HERSHENOV, B. R., *Nature* **213**, 299 (1967).
20. WERNER, G., *Z. Zellforsch. Mikroskop. Anat.* **63**, 880 (1964).