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REACTION OF SURFACE LAMELLA OF MOTH SPERMATOZOA TO VINBLASTINE

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SUMMARY

Previous ultrastructural studies appear to indicate that the lacinate appendages (highly elaborated laminar structures which cover the surface of moth spermatozoa) may be intracellular derivatives of transient microtubules found in the elongating spermatids of these insects. Additional support for this theory is supplied by the present study in which testes of the warehouse moth *Ephestia cautella* were treated *in vivo* with the antimitotic agent vinblastine sulphate. Solutions containing 10^{-5} M vinblastine caused the lacinate appendages to become poorly resolved, and at 10^{-3} M they disappeared. This concentration-dependent response of the appendages to vinblastine resembles that of tubulin-containing structures.

INTRODUCTION

Lacinate appendages (Fig. 1, p. 355) are highly elaborated structures covering the surface of the eupyrene (nucleated) spermatozoa of moths. They consist of radially arranged laminar structures, which appear in transverse sections as rays of alternating electron-opaque and electron-lucid areas projecting from the body of the cell (Phillips, 1971). In a previous paper, dealing with the differentiation of these appendages, we proposed that they may be derivatives of transient intracellular microtubules found in the elongating spermatids, although they appear in the cell surface and lack any tubular structure (Friedländer, 1976).

The characterization of the main microtubular component, the heterodimer protein subunit named tubulin, is based on its property of binding specifically to certain compounds called antimitotic agents. Among these are found the vinca alkaloids which are able to bind to tubulin, to depolymerize microtubules and to induce polymorphic forms of tubulin-like paracrystals, macrotubules, rings, etc. (Wilson & Bryan, 1974; Wilson, 1975; Hinkley, 1976).

To find out whether the laminar structures of the lacinate appendages respond to vinblastine treatments in the same way as tubulin-containing structures, we treated *in vivo* males of the warehouse moth *Ephestia cautella* with vinblastine sulphate.

MATERIALS AND METHODS

A culture of *Ephestia cautella*, reared in our laboratory, was used in all the experiments. The abdomen of pupating larvae was cut open, and the operated area was covered with insect saline (Peacock, 1966) containing either 10^{-5}, 10^{-4} or 10^{-3} M vinblastine sulphate (Sigma Chem. Co., St Louis, U.S.A.). Other pupating larvae, whose operated area was covered with

the same saline solution but without vinblastine sulphate, were used as controls. The dissected insects were placed in a humid box for maintaining constant concentration of vinblastine sulphate. After 14 h the testes were removed and fixed in 3 % glutaraldehyde in 0·2 M phosphate buffer, pH 7·2, for 3 h. Subsequently, they were postfixed in 1 % OsO_4 in the same buffer for 1 h, dehydrated and embedded in Epon. The ultrathin sections for electron-microscopy were stained with ethanolic uranyl acetate and lead citrate.

RESULTS

The lacinate appendages always coat the head of the mature spermatid (Fig. 1), but may be absent from the flagellum. Therefore, we mainly studied transverse sections through the heads of spermatids of 3 distinct stages of differentiation, namely early, middle and mature. The stages were determined according to the appearance of the chromatin and the extranuclear components of the spermatid head.

Differentiation of the eupyrene (nucleated) spermatids of the control testes follows the pattern reported earlier for the normal spermiogenesis of *Ephestia* (Friedländer, 1976). In the *early* elongating spermatids the nucleus contains wavy threads of chromatin, which run parallel to the long axis of the cell. Sleeves of parallel and regularly spaced microtubules encircle the nucleus. In the *middle* elongating spermatids the chromatin becomes increasingly condensed. A rod, oval in transverse section, appears close to the plasma membrane and parallel to the nucleus. The perinuclear microtubules increase in number and assemble together, forming rays which radiate from the nucleus to the plasma membrane (Fig. 3).

In the mature spermatids the nucleus is partially covered with a multi-membranous sleeve, which is open at the area facing the rod and projects to the sides like wings. The paracrystalline rod acquires the shape of a half oval or a comma, with the concave side close to the nucleus. The perinuclear microtubules are no longer seen, and the lacinate appendages appear at the sites where these microtubules were previously found, i.e. close to the external face of the multimembranous sleeve (Figs. 1, 6).

The flagellum of the middle spermatid contains an axoneme having the typical 9+2 pattern, 2 mitochondria derivatives, and non-axonemal microtubules running parallel to the axoneme. An extracellular electron-opaque rod, the reticular appendage, is attached to the flagellum. In the flagellum of the *mature* spermatid the non-axonemal microtubules are no longer seen, and the lacinate and reticular appendages cover its surface. Lacinate appendages, however, may sometimes be absent from the flagellum.

Testes treated with solutions containing 10^{-5} or 10^{-4} M vinblastine sulphate display typical crystals and macrotubules in the cytoplasm of somatic cells, spermatocytes and early spermatids (Fig. 2). In the early and middle spermatids most of the perinuclear and the non-axonemal microtubules withstand the treatment, but they appear together with macrotubules. The macrotubules appear either as a layer or as aggregates. The lacinate appendages of the mature spermatid are still observed, but their ultrastructure appears poorly resolved (Figs. 6, 7). In the flagella the lacinate appendages may be either separated from the cell (Fig. 10), or may be replaced by numerous filaments filling the space between the flagella (Fig. 9). The reticular appendages, however, appear unaltered (Fig. 9). No changes were observed in the microtubules of the axonemes at any stage of development. Some testes did not react as described above, as their

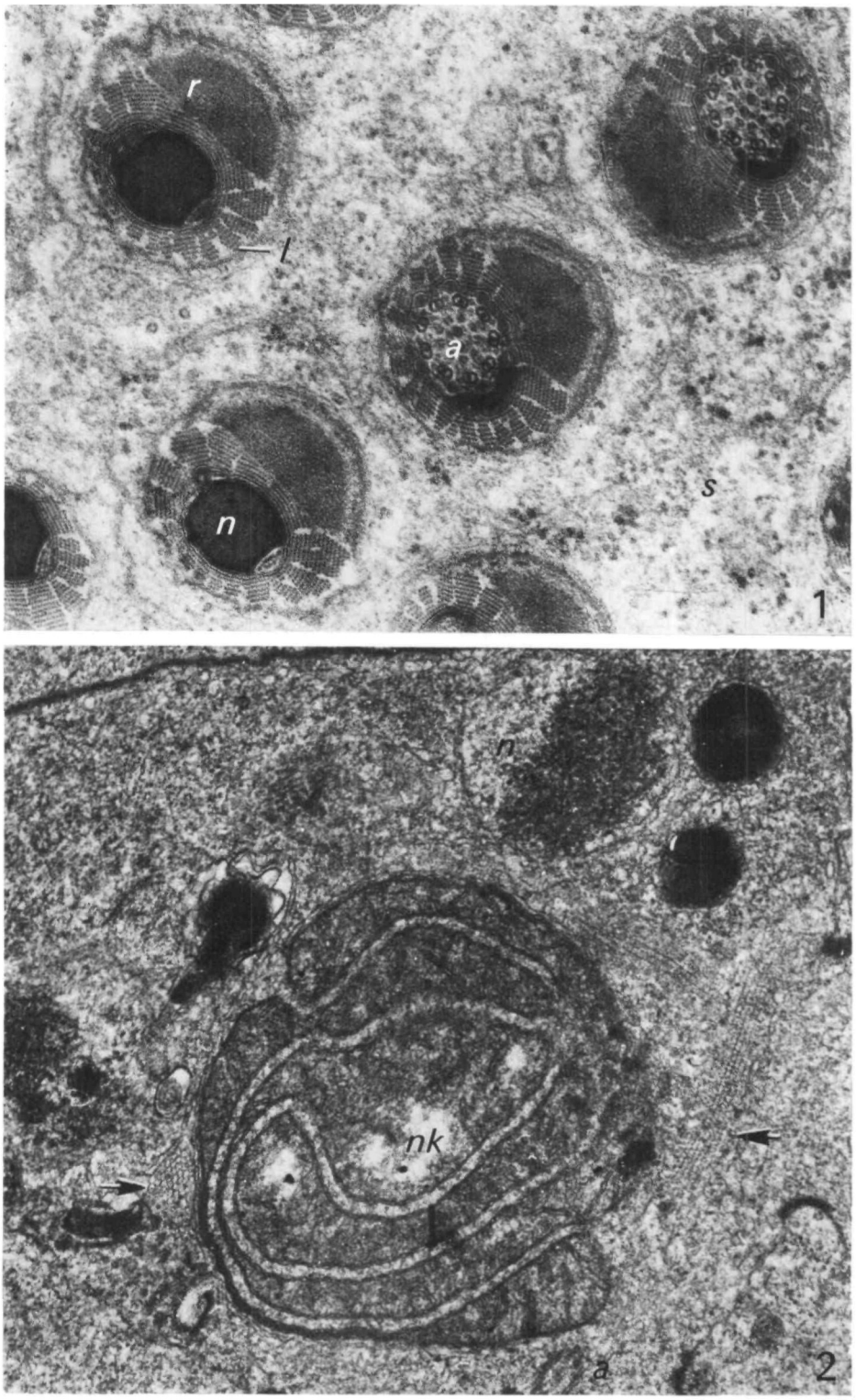


Fig. 1. Eupyrene mature spermatids of untreated testes. Transverse sections through the nucleus (*n*) and axoneme (*a*). Note the half-oval shape of the rod (*r*). *l*, lacinate appendages; *s*, supporting cell. × 55000.

Fig. 2. Early spermatid treated with 10^{-5} M vinblastine, showing paracrystals (arrows). *a*, axoneme; *n*, nucleus, *nk*, Nebenkern. × 30000.

microtubules resemble those of the control testes, lacking any crystal or macrotubule. Other testes show unaltered spermatid microtubules, but contain somatic cells and spermatocytes with paracrystals and macrotubules. Still other testes show altered and unaltered cells together in the same cyst (Fig. 10).

Testes treated with 10^{-3} M vinblastine sulphate contain early and middle spermatids having only a few of the innermost perinuclear microtubules (Figs. 4, 5). Both the head and the flagellum contain numerous macrotubules. Late spermatids, having a comma-shaped rod in transverse sections, lack the lacinate appendages found in the comparable control spermatids. The nucleus, rod and the multimembranous sleeve adjacent to the nucleus appear within an empty space delimited by the plasma membrane of the supporting cells. In addition to these structures, the empty space may contain ribbons of an electron-opaque material which, like the lacinate appendages, are close to the external face of the multimembranous sleeve which covers the nucleus (Fig. 8). In the flagellum we found the axoneme, the mitochondria derivatives, and the reticular appendage. Although we searched carefully, no lacinate appendages could be found either in the head or in the flagellum of mature spermatids.

DISCUSSION

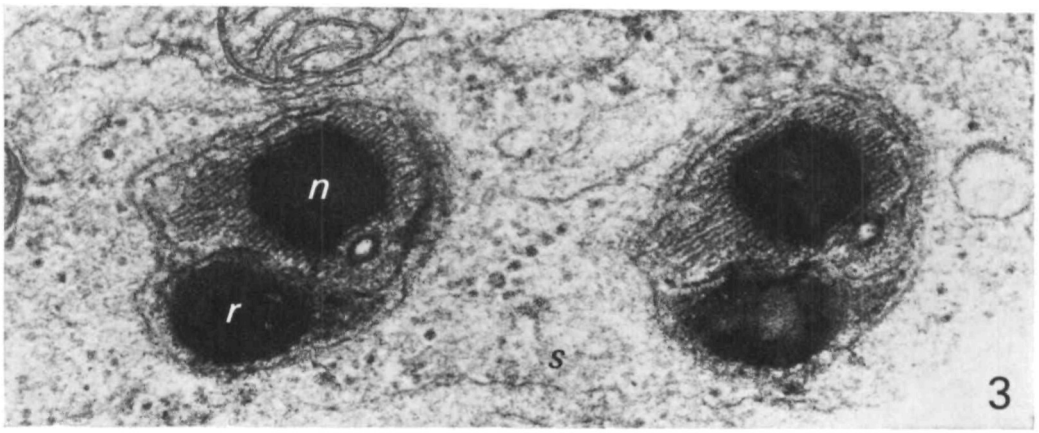
The lacinate appendages, which coat the surface of the mature eupyrene spermatids, appear to be microtubular derivatives, although they lack the structure of any of the known polymorphic forms of tubulin. Two types of data support this conclusion: (1) ultrastructural analysis of the morphogenesis of the lacinate appendages, and (2) the effects of the alkaloid vinblastine on the microtubules and lacinate appendages *in vivo*.

In the mature spermatids the lacinate appendages replace transient perinuclear microtubules of the early and middle elongating spermatids. They are structures which appear at the surface of the mature spermatid only after the original plasma membrane has been cast off (Friedländer, 1976), being therefore different from the extracellular structures which appear outside the original plasma membrane of spermatozoa of certain species (Baccetti, 1972) and other animal cells as well (Luft, 1976). Lacinate appendages differ profoundly from the cellular membranes and, in particular, they lack the typical trilamellar conformation of membranes (e.g. Crane & Hall, 1972).

Fig. 3. Transverse sections of control middle spermatids. The perinuclear microtubules form rays radiating from the nucleus (*n*). The rod (*r*) is oval. *s*, supporting cell. × 55000.

Fig. 4. Transverse sections of middle spermatids, comparable to those of Fig. 3, treated with 10^{-3} M vinblastine. Only some of the innermost microtubules withstood the treatment (arrows). Numerous macrotubules, or paracrystals, replace the microtubules. × 55000.

Fig. 5. Middle spermatids, comparable to those of Figs. 3 and 4, treated with 10^{-3} M vinblastine. Longitudinal sections through the rod (*r*) and paracrystals or macrotubules (left) and through the rod (*r*), nucleus (*n*) and macrotubules (right). *s*, supporting cell. × 55000.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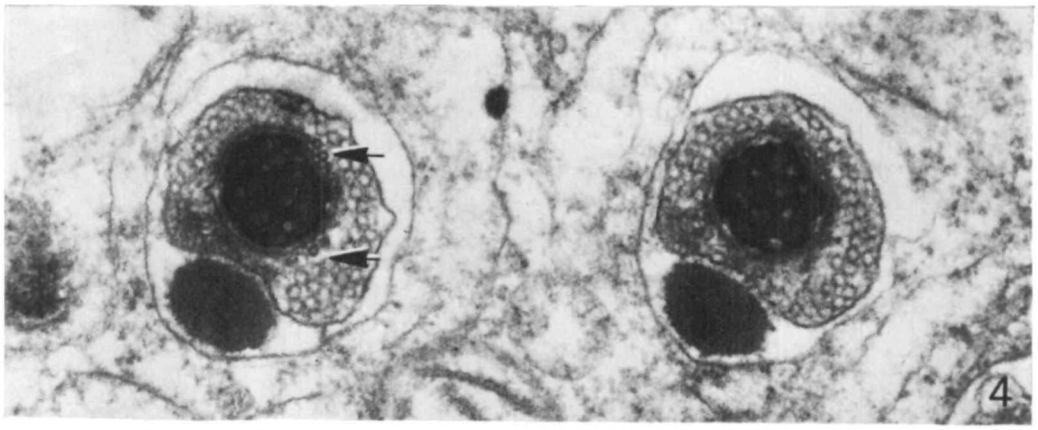
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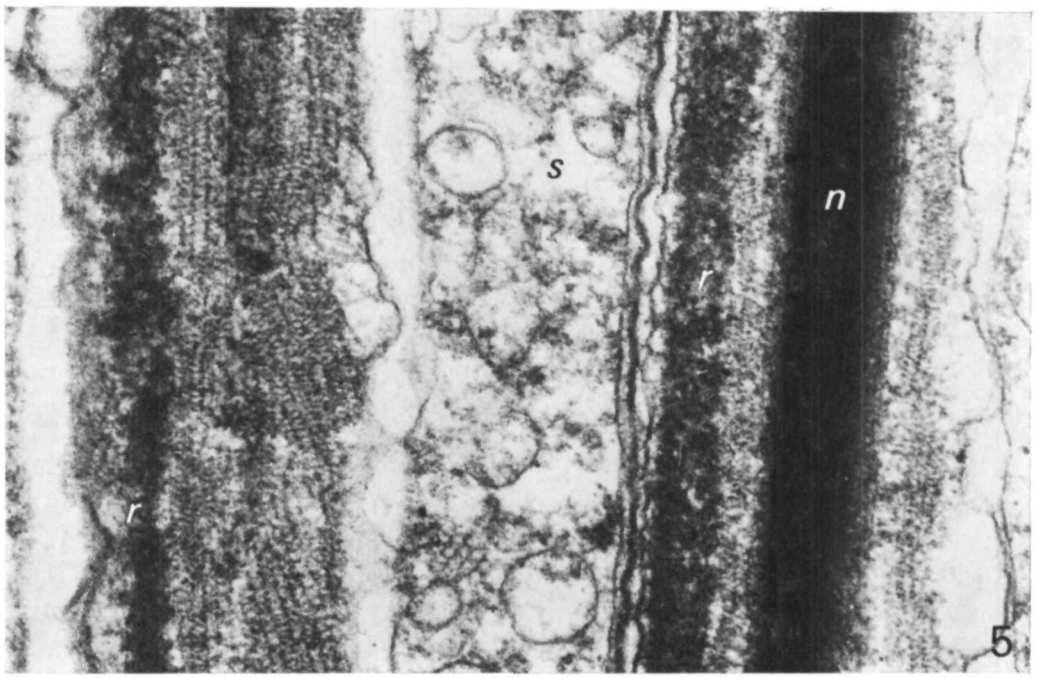
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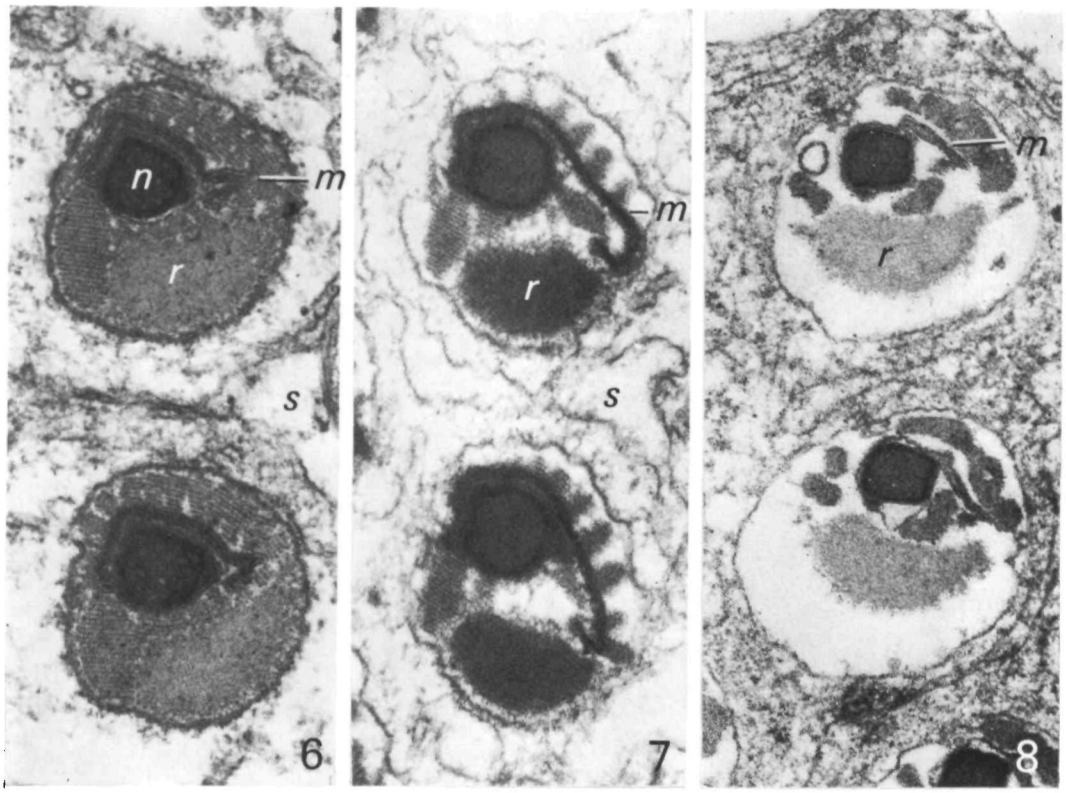
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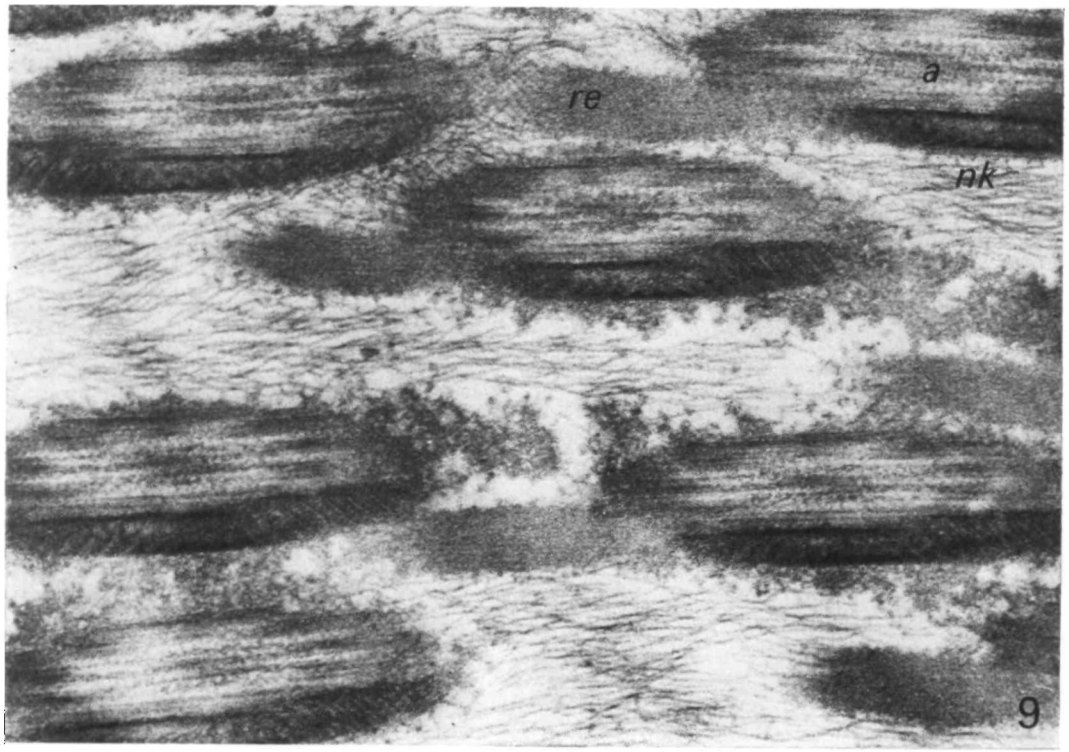
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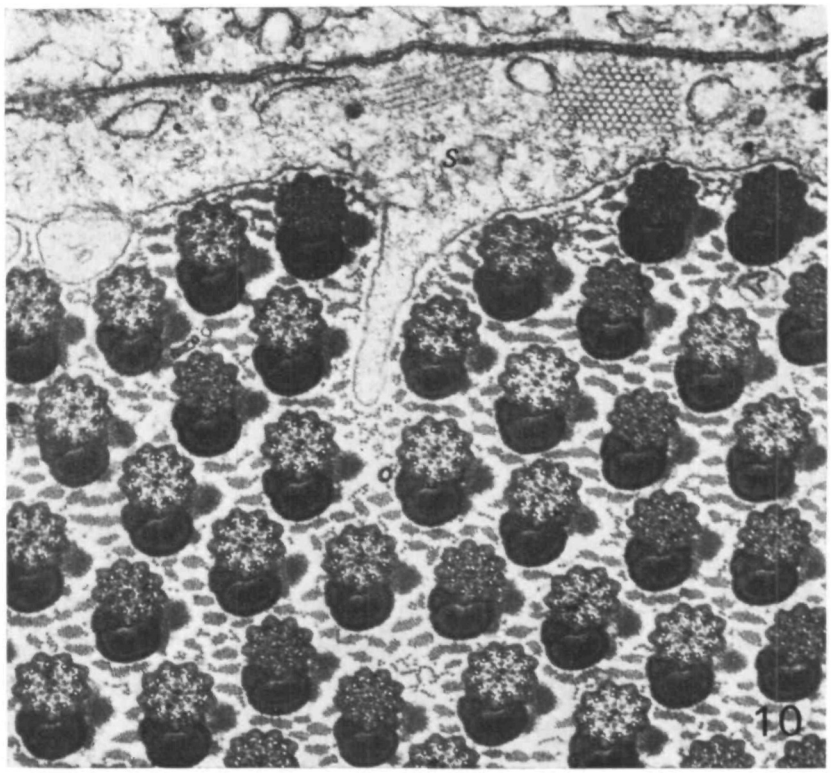

Fig. 10. Transverse sections through flagella of mature spermatids treated with 10^{-4} M vinblastine. The lacinate appendages are separated from the body of the cells. Paracrystals are present in the supporting cells (*s*). Note the electron-dense material which appears in the axoneme of numerous cells, but is absent from the axoneme of other cells of the same cyst. × 35000.

The lacinate appendages disappear after the *in vivo* treatment with vinblastine. The control solution, lacking vinblastine, induces no changes in the appendages. Moreover, the effect of vinblastine is concentration-dependent, since low concentrations cause the appendages to appear poorly resolved, while high concentrations disrupt them. It appears, however, that the response of each testis, and even that of each cyst of the same testis, to the low concentrations of vinblastine depends also on factors other

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Figs. 6, 7. Transverse sections of mature spermatids treated with 10^{-5} M (Fig. 6) and with 10^{-4} M (Fig. 7) vinblastine. The lacinate appendages appear poorly resolved. *m*, multimembranous sleeve; *n*, nucleus; *r*, rod; *s*, supporting cell. × 55000.

Fig. 8. Transverse sections of mature spermatids treated with 10^{-3} M vinblastine. Note the lack of lacinate appendages, the ribbons of electron-opaque material close to the external face of the multimembranous sleeve (*m*) and the comma-shaped rod (*r*). × 55000.

Fig. 9. Oblique sections of flagella of mature spermatids treated with 10^{-4} M vinblastine. Note the lack of lacinate appendages and the numerous filaments filling the space between the flagella. *a*, axoneme; *nk*, Nebenkern derivatives; *re*, reticular appendage. × 55000.

than the concentration of the solution. Among these might be the physiological condition of the insect at the beginning of the experiment, the damage caused by surgery, damage caused by the vinblastine to non-testicular tissues, the actual concentration of vinblastine reaching the testis, etc. The variability of these parameters may explain the variability of the results obtained with the low concentrations of vinblastine. On the other hand, the fact that high concentrations of vinblastine produce the same results on the lacinate appendages in all the cysts of the treated animals, appears to indicate that at high concentrations the effect is direct and unrelated to factors other than vinblastine.

The effects of vinblastine on the different groups of microtubules are also concentration-dependent. For example, most of the perinuclear microtubules of the middle elongating spermatids withstood the treatment with 10^{-4} M vinblastine, but only a few of them withstood treatments with 10^{-3} M vinblastine. Both the direct action of vinblastine on microtubules and tubulin and the concentration dependence of the reaction of vinblastine with tubulin, are well documented (Wilson, 1975; Bhattacharyya & Wolff, 1976*a*). It seems, then, that lacinate appendages react to vinblastine similarly to tubulin-containing structures.

The existence of additional natural polymorphic forms of tubulin such as the lacinate appendages should not be surprising. Indeed, the tubulin pool of the cell is already known to be distributed among different configurations, namely (*a*) isolated microtubules, (*b*) structures made of microtubules like axonemes and mitotic spindles, (*c*) soluble tubulin (Sluder, 1976) and (*d*) membrane-bound tubulin (Bhattacharyya & Wolff, 1976*b*). There are also induced polymorphic forms of tubulin, like macrotubules, paracrystals, rings, etc. which have not been encountered in untreated cells. It might well be that additional forms of tubulin have not yet been recognized, since they lack clear morphological markers of the tubulin-containing structures, as in the case of the lacinate appendages.

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