THE EFFECTS OF VINBLASTINE SULFATE ON THE MICROTUBULAR ORGANIZATION OF THE OVARY OF RHODNIUS PROLIXUS

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INTRODUCTION

It has been well established that the trophocytes, or nurse cells of heteropterous ovaries, contribute a variety of cytoplasmic components to the developing oocytes (Bonhag, 1958). Hemipteran ovaries are of the telotrophic type in which the nurse cells are confined to the apical trophic-chamber of each ovariole and are connected to developing oocytes, during their early stages of differentiation, by means of long nutritive or trophic cords. The central region of the trophic chamber is syncytial and is referred to as the trophic core. The fibrillar nature and basophilic staining of the trophic cords have been well documented; however, only a few investigators have concerned themselves with an ultrastructural analysis of this important corridor of cellular transport (Hamon and Folliot, 1969). This study is concerned with (a) the morphology of the trophic cords of the ovaries of the insect Rhodnius prolirixus and (b) the effects of the alkaloid vinblastine sulfate—a drug that complexes microtubular protein—on the microtubular components of the cord.

MATERIALS AND METHODS

For general cytological studies ovaries were excised from CO2-anesthetized adult animals that had been...
previously maintained at 28°C in a humid incubator. Ovaries from fed and unfed animals were fixed for both light- and electron-microscopy. For light microscopy, ovaries were fixed in Bouin's solution, embedded in paraffin, and stained with Heidenhain's iron hematoxylin. For electron microscopy, ovaries were prefixed in the formaldehyde-glutaraldehyde mixture of Karnovsky (1965) in 0.1 M sodium cacodylate. After washing in 0.1 M sodium cacodylate buffer, the tissue was postfixed in a 1% solution of osmium tetroxide buffered with 0.1 M phosphate buffer at pH 7.4. Subsequently, the tissue was dehydrated in a graded series of ethanol, infiltrated, and embedded in Epon (Luft, 1961).

Anesthetized animals were injected abdominally with 0.05 ml of a 1 × 10^{-4} M solution of vinblastine sulfate dissolved in insect Ringer's, and the wound was sealed with dental wax to prevent leakage of body fluids or injected solution. Controls were injected, in a similar manner, with 0.05 ml of insect Ringer's without vinblastine sulfate. Ovaries were then excised 1, 3, 8, and 24 hr postinjection and prepared for electron microscopy as indicated above. Thin sections were cut on a Porter Blum MT-2 microtome and stained with uranyl acetate followed by the lead citrate stain of Venable and Coggeshall (1965) and observed in an RCA-EMU-3H or a Philips 200 electron microscope. Thick sections (1 μm) were stained with toluidine blue.

RESULTS AND DISCUSSION

The trophocytes in the emerged adult activate the previtellogenic phase of oocyte development (see Masner, 1968). Wigglesworth (1936, 1964) has demonstrated that this aspect of egg development is independent of hormonal control in *Rhodnius prolixus*. The trophic tissue sustains the process of oogenesis until the hormonal factors act upon the follicular tissue and initiate vitellogenesis (Wigglesworth, 1936).

The normal histology of the ovariole is illustrated in Fig. 1. The apical region consists of the trophic chamber (*T*) harboring trophocytes and shows a tangential cut of the central trophic core (*TCO*). Emanating from the trophic core (*TCO*) are trophic cords, one of which is seen at *TC*. These trophic cords pass through the prefollicular tissues (*PT*) to oocytes (*O*) with which they are in cytoplasmic continuity. Such cords remain associated with developing oocytes for some time, as is illustrated by the arrows in Fig. 4.

The trophic cords are bounded by a unit membrane which is confluent with the oolemma. The membrane of this rather long cytoplasmic bridge lacks the conspicuous dense region on its cytoplasmic side which has been seen girdling shorter nurse cell-oocyte bridges (Anderson and Huebner, 1968; Steinert and Urbani, 1969).

In both fed and unfed animals, the cytoplasm within the trophic core and cords is characterized by the presence of many microtubules (Fig. 5, *MT*), and copious quantities of ribosomes and mitochondria (Fig. 5, *M*). The microtubules are oriented longitudinally to the long axis of the cord, and mitochondria and ribosomes are interspersed between them (Fig. 5). Some of the fascicles of microtubules within the trophic core curve and deviate slightly from the long axis. The microtubules presumably constitute the physical basis for the fibrillar nature of the cords in *Rhodnius*. Perhaps this distinguishing characteristic of the trophic cords of *Rhodnius* is a general one for telotrophic ovaries, since Hamon and Folliot (1969) report similar findings in the Homopteran *Auchenorrhynchus* sp. Investigators working with insects possessing polytrophic ovaries have not reported microtubules within the bridge (Steinert and Urbani, 1969).

Hamon and Folliot (1969) suggest that microtubules in the telotrophic nutritive cords may be
specializations for cytoplasmic movement, transport of metabolites, or support. In *Rhodnius*, it appears that all three of the forementioned functions may be important. Due to the length of the bridges the cytoskeletal role of the microtubules presumably is accentuated, a function which in short cytoplasmic bridges may be performed by the dense regions. The utilization of microtubules both in the production of cell form (Overton, 1966) and maintenance of cell form (Tilney, 1968) is not unusual.

Tilney (1968) and others have implicated the role of microtubules in the transport of cytoplasmic constituents. An interesting similarity exists between the structure of the trophic cords of *Rhodnius* and that of axons, in that both extend over great distances, both exhibit flow of materials along their length, and both possess microtubular elements (see Weiss, 1969).

The nuclear activity of trophocytes as shown by Anderson and Beams (1956) and by the labeling experiments of Vanderberg (1963) suggests that the nurse cells produce RNA and proteins which are subsequently transferred via the cords to previtellogenic oocytes. The production and transport of components such as DNA, RNA, and protein from nurse cells to oocytes have also been reported in the literature for other insect ovaries (Bier, 1963; Platova, 1967). These studies, in conjunction with our findings, suggest that in the elongated trophic cords, microtubules are presumably important in cytoplasmic transport with the directional flow of mitochondria and ribosomes being similar to that indicated in the polychaete *Dioptera cuprea*, i.e., from nurse cell to oocyte (Anderson and Huebner, 1968).

In other systems possessing cytoplasmic flow where one finds an elongated structure supported in part by microtubules, it has been experimentally possible to demonstrate this flow by constriction (see Weiss, 1969). The dependence of this flow on microtubules has been shown by using agents which destroy microtubular structures, e.g., alkaloids (Tilney, 1968). One of the agents used to destroy microtubular morphology has been the alkaloid vinblastine sulfate (VLB). The alkaloids VLB and colchicine have been shown to break down normal microtubule structure, resulting in the formation of fibrillar aggregates or, in the case of VLB, crystals having fibrillar or tubular substructure. The effects of these chemicals on microtubules have been studied in starfish eggs (Malawista and Sato, 1969; Malawista et al., 1969), fibroblasts, and leucocytes (Bensch and Malawista, 1968, 1969), nerve tissue (Wisniewski et al., 1968), HeLa cells (Robbins and Gonzales, 1964), on microtubular proteins in vitro using rabbit brain homogenates (Bensch et al., 1968), and on HeLa cell and pig brain homogenates (Marantz et al., 1969).

Exposure of *Rhodnius* ovaries in vivo to VLB \((1 \times 10^{-4} \text{M})\) for 1, 3, 8, and 24 hr resulted in the appearance of filamentous aggregates in both the trophic core and cords (Figs. 2, 3, 6, 7). With progressively longer exposure times there is a decrease in the number of microtubules and an increase in the number of aggregates, suggesting that the aggregates arise from the breakdown products of the microtubules.
of microtubules. Fig. 9 demonstrates that microtubules which normally appear in the follicle cells (Fig. 8, MT) disappear upon treatment, indicating that the aggregates (Fig. 9, AG) may be microtubular protein. This result is in agreement with the observations of Malawista and Sato (1969), who found that crystal formation in the oocytes of the starfish *Pisaster ochraceous* is presumably a function of concentration and incubation time. This is to be expected, since VLB functions not only in the induction of the crystals but presumably becomes part of its structure also (Malawista et al., 1969).

The studies of Wisniewski et al. (1968) and Bensch and Malawista (1969) using colchicine and VLB suggest that during treatment there may be a dissociation of microtubules into protos fibrils which are subsequently reorganized into tubular elements. The aggregates formed in our experiment appear to be composed of a fine filamentous material (Fig. 3). Perhaps this reflects the initial organization into fibrillar complexes which may transform into crystals having a tubular substructure at a later time. This possibility is presently being tested. After 24 hr exposure some ovarioles of *Rhodnius* still possess a few microtubules. This could be due to biochemical differences in microtubules, resynthesis of microtubules, or simply may indicate that a higher concentration or longer exposure time is required to bind all the microtubular protein.

Upon treatment with VLB the trophic core becomes structurally disarranged and contains aggregates, microtubules, and many trophocyte nuclei, which were formerly restricted to the periphery of the syncytium. This is reminiscent of the chaotic array of neurotubules and filaments seen after 2 days' treatment in nerve (Wisniewski et al., 1968). Tortuous cell processes from apically and peripherally located membrane-bounded cells and prefollicular tissue now protrude into the trophic core. Numerous mitochondria congregate in the core area adjacent to the prefollicular tissue (Fig. 7, M) and in individual trophic cords (Fig. 2). The latter remind one of the ultrastructure of constricted axons (Weiss, 1969) in which Friede (see Weiss, 1969) noted the accumulation of respiratory enzymes proximal to the ligature. These were shown to correspond to mitochondrial accumulations (see Weiss, 1969). The inhibition of this axonal flow with colchicine treatment by Kreuzberg indicates the role of microtubules in normal axonal flow (cited by Weiss, 1969). In the trophic core and cords of ovarioles treated with VLB the mitochondria are no longer separated spatially by the microtubules; consequently they tend to accumulate between the aggregates, emphasizing the number present in the cord (Figs. 2 and 7). The presence of this large population of mitochondria within the cords indicates a possible role of the trophic tissue, i.e., it may supply mitochondria for the differentiating oocyte. The presence of filamentous aggregates in the trophic cords presumably impedes the flow of cytoplasm from the nurse cells and is reminiscent of the damming up of axoplasm of ligated sciatic nerves (Weiss and Hiscoe, 1948; Weiss, 1969). This, in addition to the movement of material to the center of the core, normally kept peripherally by the central tubule bundles, may result in a damming up of cytoplasmic constituents in the trophic chamber. Nevertheless, the presence of mitochondria and ribosomes in both the normal (Fig. 5) and treated tissue (Figs. 2, 7) suggests that these organelles normally pass along this corridor. The overall disorderly dispersion of cytoplasmic components, upon VLB treatment, indicates the importance of microtubules to the structural integrity of the ovariole. The nature of the aggregates formed is similar to that of the fibrillar aggregates seen by others (Wisniewski et al., 1968). Tubular substructure was not seen in the filamentous aggregates (see Fig. 3).

It is interesting to note that trophocyte cytoplasm also was found to harbor filamentous aggregates (Fig. 6, arrows). Whether this merely reflects the presence of microtubules or suggests that a portion of the metabolic machinery of the trophocyte is the production site of the microtubular proteins required for the core and cords cannot be determined from these data. If these cells are critical to pool synthesis or factors effecting monomer-polymer equilibrium, then further experimentation, as suggested by Tilney (1968), may help to determine the factors which influence the equilibrium and perhaps microtubule control mechanisms. It would be of interest to examine the effect of protein-synthesis inhibitors on the normal distribution and site of aggregate formation.

The normal cytology and experimental data of this report indicate the presence of numerous microtubules within the trophic tissue of the ovaries of *Rhodnius prolixus* and suggest their importance in the functional and structural integrity of this system. Vinblastine sulfate treatment results in the
Figure 8  A portion of the cytoplasm of a follicle cell illustrating the presence of microtubules (MT) and nucleus (N). × 34,000.

Figure 9  An electron micrograph of follicle cells treated with VLB for 3 hr. Note the absence of microtubules and the appearance of filamentous aggregates (AG) and nucleus (N). × 12,800.
formation of filamentous aggregates in the trophic tissue. If these crystals could provide a means of isolating microtubular protein as suggested by Malawista and Sato (1969), the trophic tissue of Rhodnius may provide a rich supply of such materials. Thus, this system may serve as a model to help resolve some of the questions concerning the organization and function of microtubules.

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