PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, **147** 361-365 (1974)

Microtubules in Prolactin Cells of the Rat Anterior Pituitary Gland[1] (38342)

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Until a few years ago cytoplasmic microtubules were not generally recognized in many cells. This was due to the use of fixatives other than glutaraldehyde and to the common practice of keeping the fixing solutions cold. More recently, many investigators have demonstrated clear-cut microtubules in various tissues fixed in glutaraldehyde and postfixed in osmium tetroxide (1–6). Within the last year observations on microtubules in cells of the anterior pituitary gland have been reported by several investigators (7–9). These investigators have discussed a possible role of microtubules in cell secretion based on their morphological observations of pituitary glands incubated with agents known to alter microtubules such as colchicine, vincristine or vinblastine.

However, in our experience, the usual procedures for fixing anterior pituitary tissue do not reveal a great abundance of microtubules in the cells. Consequently, we have explored a variety of modifications of glutaraldehyde solutions in order to perfect a better method of preserving microtubules in the anterior pituitary cells of animals. This report illustrates the rather wide distribution of cytoplasmic microtubules in prolactin cells obtained from a series of lactating rats.

Materials and Methods. Mature female lactating rats (Sprague–Dawley) were used on the sixth day of lactation in this study. They were divided into the following four groups: (a) constant suckling, *i.e.*, litters were not removed from the mother, (b) litters removed 18 hr prior to sacrifice, (c) litters returned for 30 min following 18 hr separation and (d) litters returned for 60 min following 18 hr separation. Each group consisted of five animals. The rats were killed by decapitation and anterior pituitary glands were removed immediately, cut into small pieces and placed in the fixative. The fixative which gave best results consists of equal parts of the following two solutions: (A) 12% glutaraldehyde, 2×10^{-5} *M* magnesium sulfate and 2×10^{-3} *M* sucrose in 0.1 *M* cacodylate buffer (pH 7.0) and (B) 1 % osmium tetroxide in 0.1 *M* cacodylate buffer (pH 7.0). The tissues were changed several times with fresh solution during fixation at room temperature for 1 hr, then kept in an ice bath for an additional hr. They were then rinsed in two changes of ice cold distilled water over a 30 min period and put in a 0.5% aqueous solution of uranyl acetate overnight at 0°. Dehydration followed our usual method using graded steps of cold alcohol, followed by immersion in absolute acetone. Absolute alcohol and acetone were used at room temperature. The tissues were embedded in Spurr's plastic medium (10) and ultrathin sections were stained by lead citrate. For purposes of comparison, we also used Karnovsky's method (11) and primary osmium fixative (12).

Results. The mixture of glutaraldehyde and osmium which is described in this report gives the best preservation of microtubules we have seen in cells of the anterior pituitary gland. We have regularly observed microtubules in growth hormone cells and prolactin cells, and to a lesser extent in cells of other types, but in this study we paid particular attention to prolactin cells.

In the prolactin cells of lactating rats, many microtubules were seen but no distinct differences were observed in the number or localization of microtubules that could be correlated with the different groups of animals. Consequently, we wish only to illustrate the various sites in which microtubules can be seen in active

[1] Supported by USPHS Grant AM 12583.

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prolactin cells. In material from the constant lactating rats we observed clusters of microtubules in peripheral areas of cytoplasm (Fig. 1) as well as in the Golgi area (Fig. 2). The microtubules were often observed in very close association with secretory granules and mitochondria (Fig. 1). They were present also in close proximity to the nuclear envelope (Fig. 3) and were sometimes seen in the cytoplasm between parallel cisternae of the granular endoplasmic reticulum (Fig. 4). We have occasionally found microtubules in contact with the plasmalemma (Fig. 5).

Discussion. In the prolactin cells of the pituitary glands of lactating rats, cytoplasmic microtubules are quite numerous and they are widely distributed throughout all areas of the cell. With the same procedure microtubules have been observed in prolactin cells of nonlactating rats but it is our impression that they are less numerous. The microtubules were observed in all areas of cytoplasm of the prolactin cells although we saw no obvious differences in their number or distribution in relation to the different animal groups. We believe that quantititative morphometric procedures will be needed in order to determine if changes occur in relation to altered functional states. It was observed that the microtubules directly contact mitochondria, secretory granules and the cellular membrane; this is suggestive of their having a role in the movements of mitochondria or the migration of granules as has been proposed for various other kinds of cells (4, 13–17).

In the anterior pituitary cells, Pelletier and Bornstein (17) stated that microtubules were present in growth hormone cells incubated under normal conditions but that they disappeared 24 hr after the addition of 10^{-6} *M* colchicine. They considered that the microtubules may play a role in cytoplasmic mobility and granule movement. On the other hand, MacLeod *et al.* (9) reported that microtubules were not significantly involved in prolactin and growth hormone secretion. They based this conclusion on ultrastructural observations and radioimmunoassays of growth hormone and prolactin following incubation of pituitary glands with colchicine or vin-

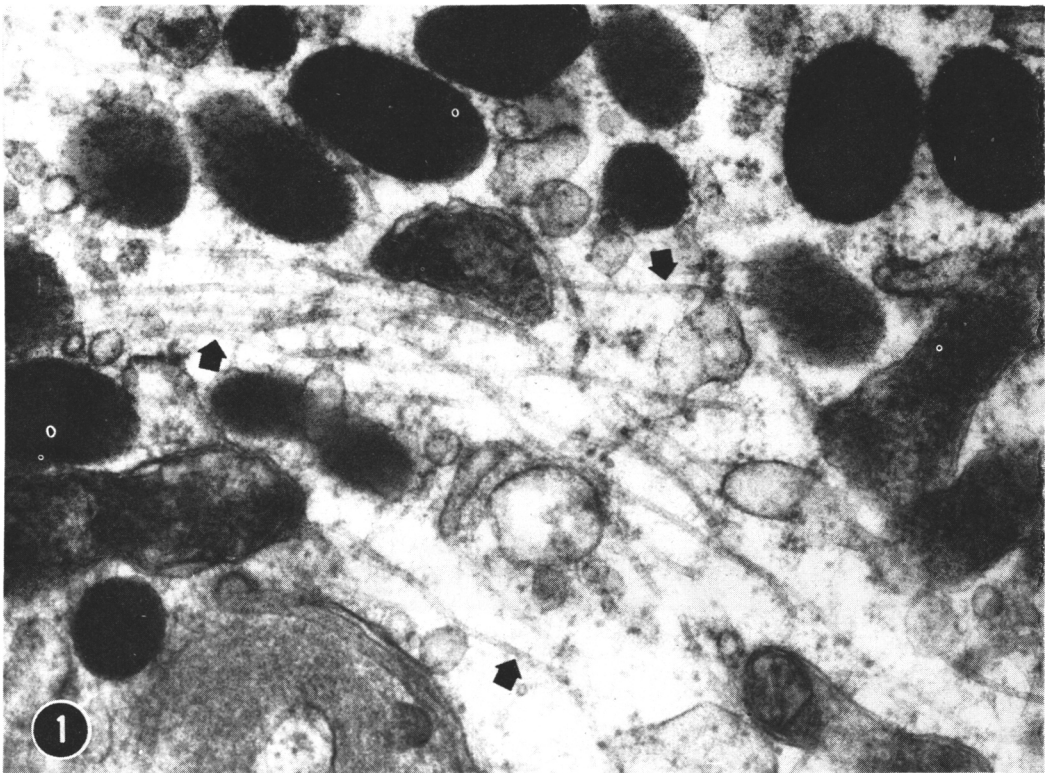


FIG. 1. Clusters of microtubules are present in peripheral areas of cytoplasm. (X 50,000)

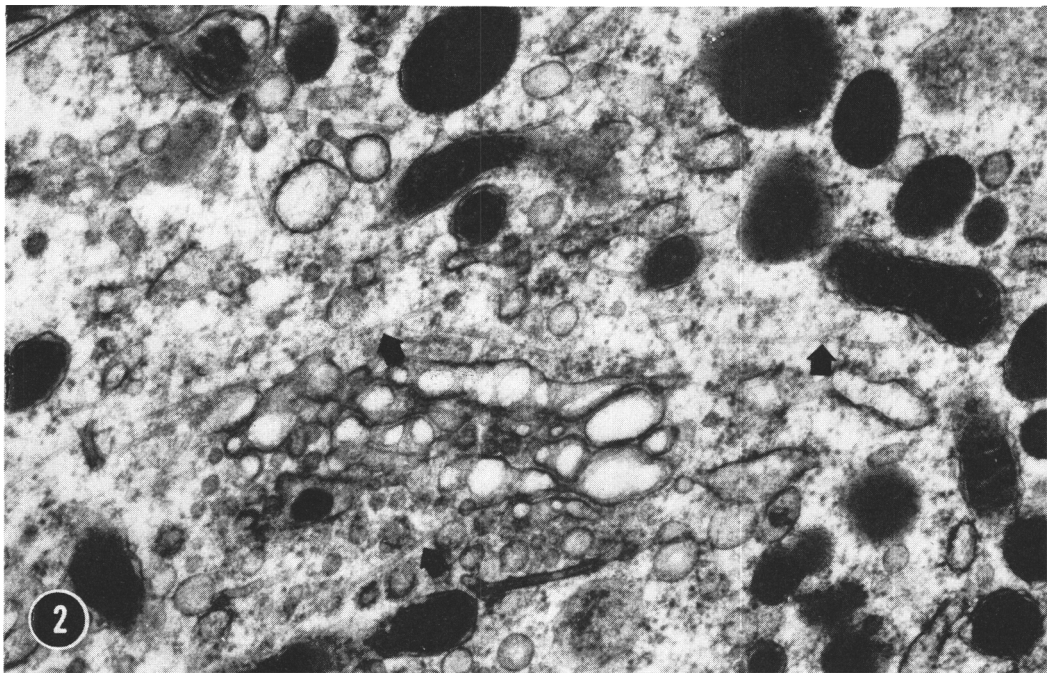


FIG. 2. Many microtubules may be observed in the Golgi area. (X 30,000)

cristine.

In our observations, the microtubules frequently appeared to form a reticulum although in some areas they were organized in more parallel arrays (Fig. 1). We believe that the fixation procedure which we have described gives a better preservation and retention of microtubules in pituitary cells than the various other fixatives which have been proposed.

Since it is well established that prolactin secre-

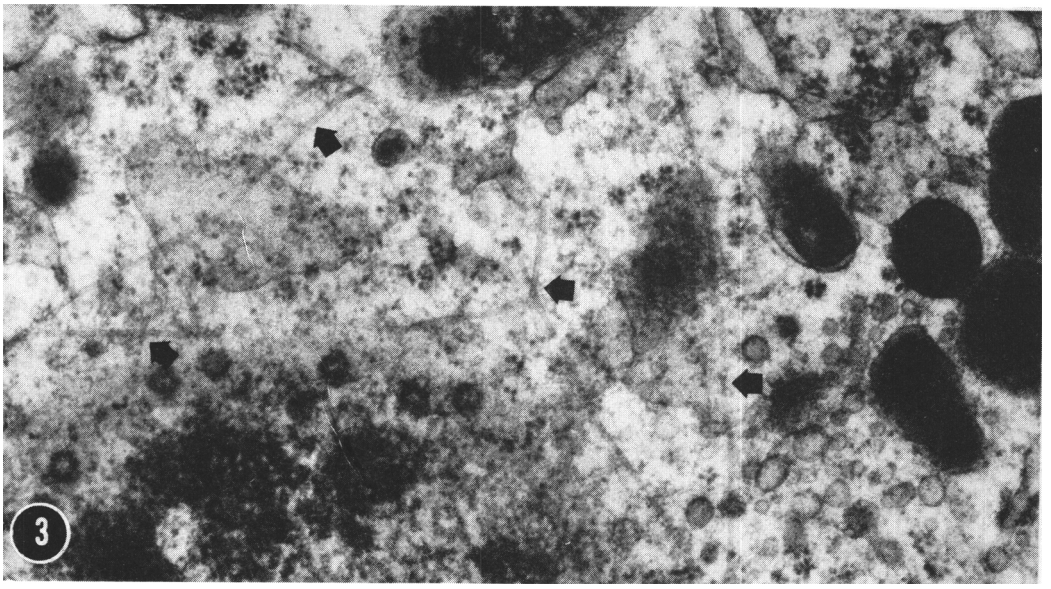
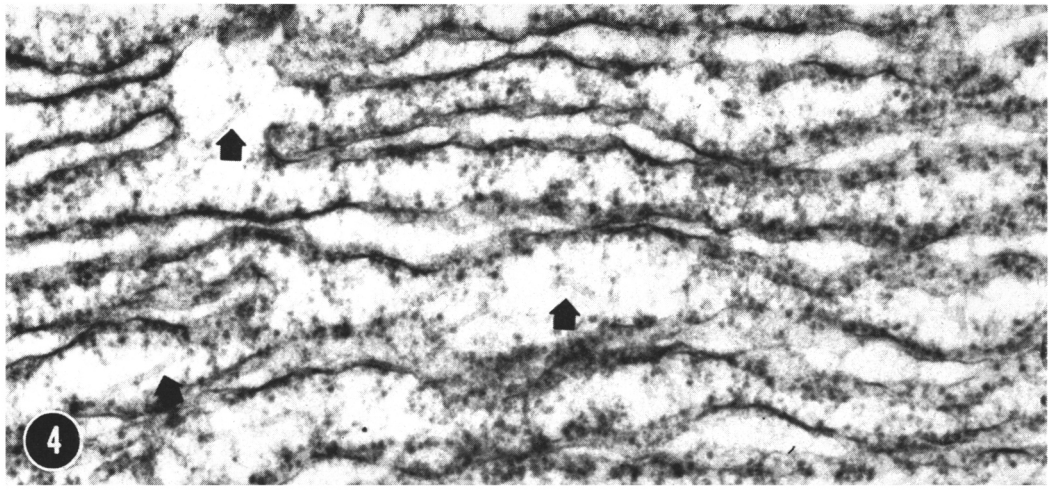


FIG. 3. Microtubules are present in close proximity to the nuclear envelope. (X 30,000)



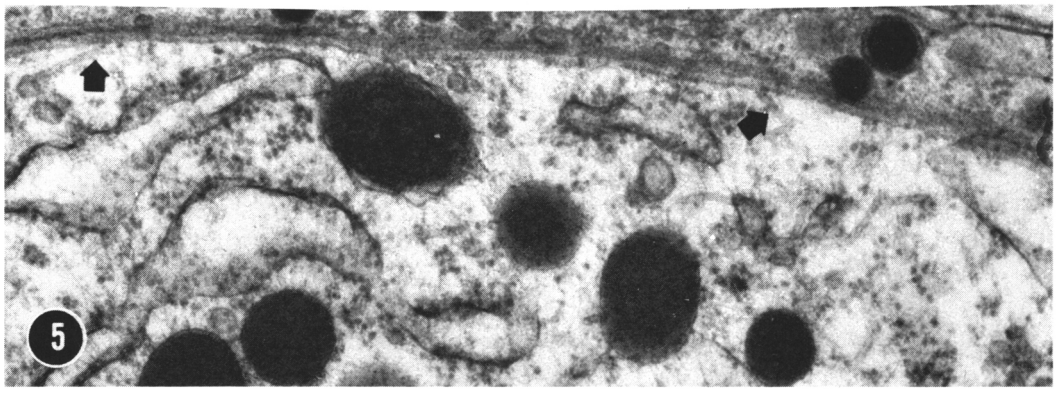


FIG. 5. Microtubules may be observed in contact with the plasmalemma. (X 50,000)

tion in the lactating rat can be modified by removal of the litter or by return of the litter to the mother (18, 19) we anticipate that this may be good material for further studies of microtubules in which quantitative methods are used.

Summary. An improved formula for a cacodylate-buffered glutaraldehyde and osmium tetroxide fixative which contains sucrose and magnesium sulfate is described; it provides excellent preservation and visualization of cytoplasmic microtubules in cells of the anterior pituitary gland. The extensive distribution of microtubules in prolactin cells of the rats killed during lactation is illustrated. They occur in close association with mitochondria, secretory granules, endoplasmic reticulum, Golgi lamellae, nuclear envelopes and plasma membranes. Although marked alterations in the number or distribution of the microtubules were not seen in relation to alterations in prolactin secretion, it is suggested that quantitative morphometric procedures may be necessary for a reliable evaluation of changes in microtubules.

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Received May 6, 1974. P.S.E.B.M., 1974, Vol. 147.