MICROTUBULES WITH 15 SUBUNITS IN COCKROACH EPIDERMAL CELLS

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Since Ledbetter and Porter (1964) described the 13 subunits which are visible in cross sections of negatively stained plant microtubules, subsequent observations have generally confirmed this number. By using Mizuhira's fixative composed of tannic acid and glutaraldehyde, it is easy to demonstrate the subunits of microtubules without optical reinforcement. Cytoplasmic microtubules and sperm axonemes, fixed with Mizuhira's fixative, similarly show 13 subunits (Mizuhira and Futaesaku, 1971, 1972; Futaesaku et al., 1972; Tilney et al., 1973).

This paper will describe a particular type of microtubule in insect epidermal cells fixed with the above fixative. The number of the subunits is found to be 15 in transverse sections.

MATERIALS AND METHODS

Small pieces of leg and wing muscles with the cuticle of cockroach *Blattella germanica* L. were fixed in 2.5% glutaraldehyde containing 2–4% tannic acid buffered with 0.1 M phosphate at pH 7.2 for several hours to overnight, as originally described by Mizuhira and Futaesaku (1971, 1972). After a brief washing with the buffer solution, the tissue blocks were postfixed in phosphate-buffered 1% osmium tetroxide for 1 h at 2°C, stained with 0.1% uranyl acetate in 90% ethanol for several minutes during dehydration in an ethanol series at low temperature, and embedded in Epon 812 through propylene oxide (Luft, 1961). Sections were cut on an MT-1 microtome (Ivan Sorvall, Newtown, Conn.) with a diamond knife, mounted on carbon-coated grids, stained with both uranyl acetate and lead citrate, and examined in a Hitachi HU-11A electron microscope at 100 kV. The magnification was calculated by using a grating. About 50 profiles of cross-sectioned microtubules recorded on photographic films were measured with a Nikon projection microscope (type 6C). When the microtubules were slightly elliptic in shape, each of the minor diameters was actually measured.

RESULTS

The epidermal cells of insects are situated between the muscle and cuticular layers, and contain many parallel microtubules in their cytoplasm (Fig. 1). The orientation of the microtubules and of the myofibrils is perpendicular to the cuticular layer (Auber, 1963; Caveney, 1969). When tannic acid penetrated into the cell, the microtubules appear to be stained "negatively[1]" (Figs. 2–5). The mean value and standard deviation of the outside diameter between outermost dense parts of the microtubules are 406 ± 14 Å. The inside diameter is 183 ± 16 Å. The wall is 111 ± 9 Å in thickness. The number of subunits is 15 (Figs. 2–4). In oblique sections, the subunits can be seen as discrete electron-lucent spheres (Figs. 2 and 3). Only one example with 13 subunits has been observed in the course of this study (Fig. 5). The outside and inside diameters of this microtubule are 320 and 140 Å, respectively.

When tannic acid failed to penetrate into the cytoplasm, the microtubules as well as other organelles showed "positively stained images" in which it is not possible to count the subunits (Figs. 1 and 6). In this case, the outside and inside diameters of the microtubules are 335 ± 11 and 181 ± 13 Å, respectively.

DISCUSSION

In earlier studies in which buffered osmium tetroxide alone was used as fixative, some tubular structures could be recognized in the cytoplasm, as in the caudal sheath of spermatids (Burgos and Fawcett, 1955; Nagano, 1962), the hydra (Slautterback, 1963), the Schwann cell of the shrimp (Hama, 1966), the nucleated erythrocytes (Fawcett and Witebsky, 1964), and as reported in the observations of Auber (1963) cited above. Auber (1963) has described the tubular structures in the epidermal cell of the Diptera as "tubular tonofilaments." These structures were subsequently characterized as microtubules (Porter, 1966; Fawcett, 1966). Microtubules of these kinds, as well as

[1] The term "staining" is used in this paper to refer to electron opacity effects resulting from treatments with glutaraldehyde, tannic acid, osmium tetroxide, uranyl acetate, and lead citrate. The term "negatively stained" is applied to images of microtubules in which the subunits stand out as discrete, electron-lucent spheres.

ciliary and flagellar filaments, appear to have greater stability than other microtubules preservable with glutaraldehyde followed by osmium tetroxide.

McIntosh and Porter (1967) have reported that during the development of the caudal sheath in the rooster spermatids, the microtubules increase in diameter from 240 to 350–400 Å; glutaraldehyde was used as prefixative in this study. When Heliozoa are subjected to low temperature, the microtubules increase in diameter from 220 to 340 Å (Tilney and Porter, 1967). Tyson and Bulger (1973) found that larger microtubules are induced by vinblastine, and suggest that C-shaped tubules are an intermediate form. Applying tannic acid, Tilney et al. (1973) have concluded that 13 subunits and their arrangement as protofilaments are universally constant with respect to both phylogeny and location, whether in a cilium or in the cytoplasm. Our results, however, demonstrate that there is a deviation in the number of subunits at least in epidermal cells of the cockroach. In this cell type, 15 is the dominant number and 13 is encountered rarely. No other number of subunits could be found.

The outside diameter of the microtubule with 13 subunits is obviously smaller than that of the microtubule with 15 subunits in the present study. Tilney and Porter (1967) suggested that the larger microtubular diameter, which appeared in Protozoa after treatment with low temperature, resulted from a change in the subunit arrangement caused by twisting. This explanation cannot apply to the formation of the large microtubules in cockroach epidermal cells, since each subunit could be identified fairly clearly.

Since microtubules in axons of the cockroach appear to be smaller in diameter than those in the epidermal cells, microtubules other than those in the epidermal cells may have 13 subunits.

In connection with the staining effect of tannic acid, the microtubules stained with tannic acid have a greater outside diameter than those without tannic acid staining, whereas the diameter of the center of low density is not so different whether the microtubules have been stained with tannic acid or not. Tannic acid may be bound to proteins by chelation to heavy metals (Futaesaku et al., 1972). Our results indicate that tannic acid conjugates mainly on the outer side of the subunits and penetrates to some extent between them, resulting in their visualization.

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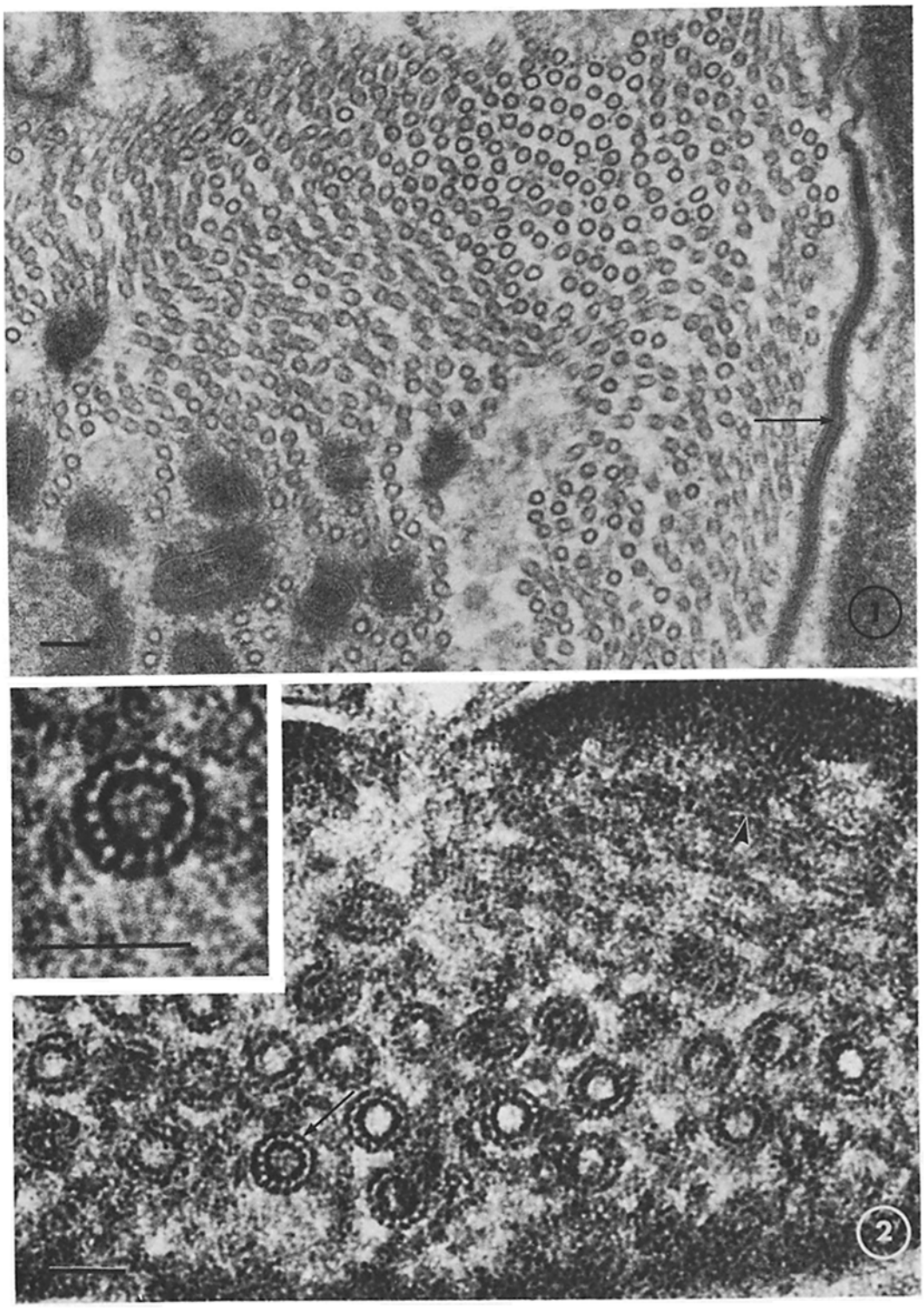

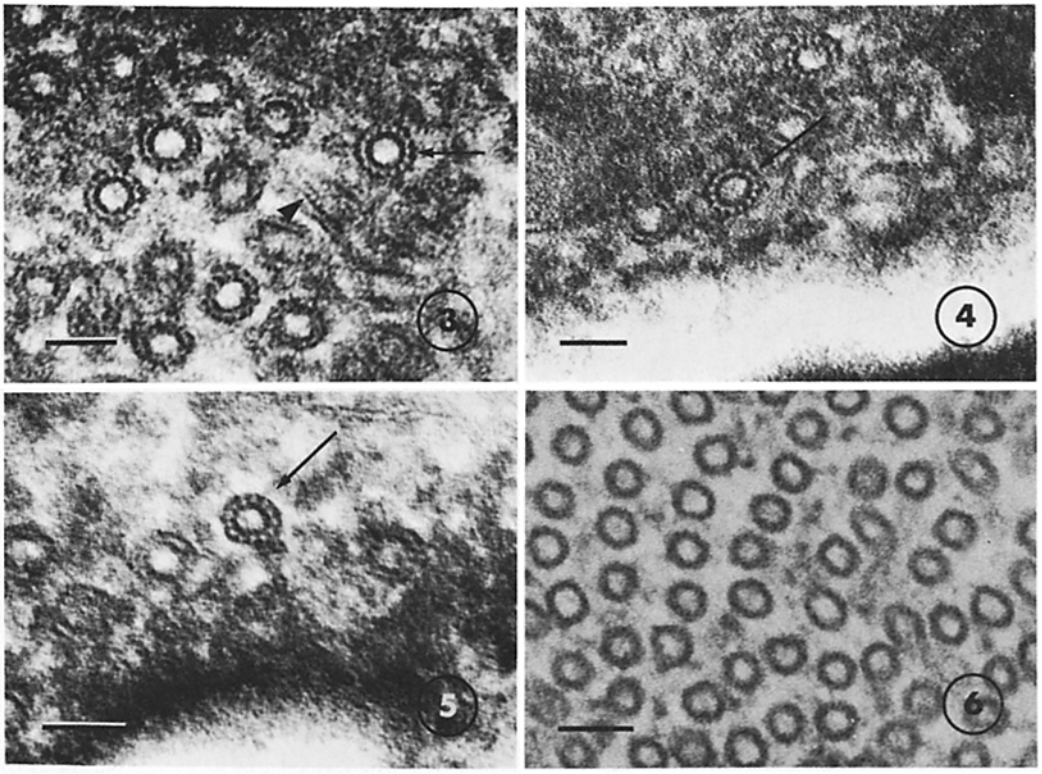

All electron micrographs presented here illustrate microtubules in the cockroach epidermal cell fixed with glutaraldehyde containing tannic acid, followed by osmium tetroxide. The microtubules were stained negatively except for Figs. 1 and 6, which show positively stained microtubules because of poor penetration of tannic acid into the cytoplasm. Bars equal 500 Å, unless otherwise indicated.

FIGURE 1 Epidermal cell with many microtubules. The arrow indicates the intercellular space with high density due to tannic acid. Scale, 0.5 μm. × 75,000.

FIGURES 2–4 Microtubules consist of 15 subunits (arrows). The mean outside diameter of microtubules is 406 Å and inside diameter is 183 Å. In Figs. 2 and 3, obliquely sectioned microtubules also show the subunits (arrowheads). The *inset* shows the microtubule in Fig. 2 at higher magnification. Fig. 2, × 230,000; *inset*, × 460,000; Fig. 3, × 190,000; Fig. 4, × 180,000.

FIGURE 5 Single exceptional microtubule (arrow) shows 13 subunits (320 Å outside diameter). × 220,000.

FIGURE 6 Higher magnification of the microtubules in Fig. 1. The mean of inside diameter (181 Å) is similar to that in Figs. 2–4, while the outside diameter (335 Å) is much smaller. × 160,000.

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