EFFECTS OF MITOTIC SPINDLE INHIBITORS ON NEUROTUBULES AND NEUROFILAMENTS IN ANTERIOR HORN CELLS

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An understanding of the chemistry of the microtubule and of its in vivo response to colchicine administration has been facilitated by the recent work of Taylor and his coworkers (1, 2). Robbins and Gonatas (3) have shown that colchicine causes the breakdown of mitotic spindle tubules of HeLa cells and a simultaneous increase in the apparent number of 90-A microfilaments in the cytoplasm. Similar changes have been observed by Padawer in the mast cell.[1] Finally, the homology between the spindle tubule and the neurotubule has been suggested (4).

After subarachnoid injection of colchicine, there is an accumulation of filaments in the neurons of the spinal cord anterior horn (5). Similar proliferation of neurofilaments is seen after subarachnoid administration of the mitotic spindle inhibitors vinblastine and podophyllotoxin. The experiments reported here show the reversibility of the effects of mitotic inhibitors on the neuron and suggest the possible identity of the subunits of the neurofilaments and neurotubules.

MATERIALS AND METHODS

18 rabbits of either sex and weighing from 3 to 4 kg were used in three series of experiments. In the first series 100 μg of colchicine in 100 μl of distilled water were injected into the subarachnoid space of the cisterna magna or of the lumbosacral spinal cord. In the second series 200 μg of vinblastine were diluted in 100 μl of distilled water and injected into the subarachnoid space at the cisterna magna. In the third series the animals were treated with 400 μl of a suspension of podophyllotoxin. The suspension was prepared by dissolving 1 mg of podophyllotoxin in two drops of 75% ethanol and diluting with distilled water to a final volume of 2 ml. Injection of the 200 μg was carried out as with vinblastine. There were six animals in each series, and two animals from each of the three groups were sacrificed 48 hr after injection. In the vinblastine and podophyllotoxin groups, all

[1] Padawer, J. Personal communication.

the remaining animals were sacrificed at 5 days. In the colchicine-treated group, two of the remaining animals were sacrificed at 5 days and the remainder at 2 wk.

At the end of the observation times, the animals were anesthetized with sodium barbital and perfused through the heart with 150 ml of paraformaldehyde followed by 5% chilled glutaraldehyde as described previously (5). After fixation, blocks were taken from the anterior horn of the lumbar or cervical cord and embedded in Araldite. Examination of specimens utilized the Siemens Elmiskop I electron microscope.

RESULTS

Clinical Observations

Within 6–8 hr after injection, all animals developed a slight weakness of the legs. After 24 hr, those animals receiving intracisternal injections showed quadriparesis whereas those receiving lumbosacral injections had paraparesis. Animals given intracisternal injections survived for a maximum of only 6 days owing to the development of bulbar involvement. Animals receiving lumbosacral injections were capable of long survival if given attentive nursing care.

Morphological Observations

In animals which were sacrificed 48 hr after injection of colchicine, vinblastine, or podophyllotoxin, numerous discrete bundles of orderly neurofilaments were observed in the perikarya of the anterior horn cells (Fig. 1). These filaments measured 90–100 A in diameter, were of indefinite length, and had frequent small side arms which may simply represent a protein flocculum (Fig. 2). They appeared essentially identical to normal neurofibrils. A few normal 220-A neurotubules were observed among the newly formed bundles of filaments (Fig. 3).

After 5 days the cytoplasm of the neurons in animals which had been treated with either vinblastine or podophyllotoxin showed a marked accumulation of 220-A tubules similar in appearance to normal neurotubules (Fig. 4). Sometimes the tubules were mixed with filaments (Fig. 5). The tubules were slightly more irregular in outline than normal, occasionally were continuous with vesicles, and were arranged in a somewhat irregular pattern. The tubule wall showed a profile with a single band in contrast to the triple-density unit membrane found in the smooth endoplasmic reticulum or other membranes. This observation was checked with through-focus series of micrographs. Mixed with these tubules were similar but less regular structures with walls constructed of unit membranes. The latter structures were probably not tubular but, rather, were flattened sacs. In some cells there were areas containing large numbers of tubules almost without filaments (Fig. 6).

The conversion to a tubular pattern was slower to develop in the colchicine-treated animals, the neurons of which showed a filamentous pattern 5 days after injection and a preponderance of tubules only after 2 wk.

DISCUSSION

The neuron appears to respond to mitotic spindle inhibitors by an initial loss of tubules and a proliferation of filaments which in turn are replaced by large numbers of tubules. It seems highly probable that there is an interconversion of the one structure into the other, and that therefore both structures are composed of the same basic material. On the basis of the morphological and chemical data which have been assembled concerning microtubules, a model can be hypothesized to account for these phenomena.

Recently, a 6S colchicine-binding subunit was isolated from the central pair of microtubules of the axoneme of the sperm tail (1), and a similar protein has been isolated from porcine brain[2] and purified. This subunit has a molecular weight of 120,000 (6). These molecular parameters are compatible with an ellipsoid approximately 45 × 90 A. Each subunit is a dimer composed of two similar protein molecules of 60,000 molecular weight (6). These monomers would correspond to the 45 A beads seen in the protofilaments of the microtubule (7). If the elliptical subunits were assembled side by side, they would form a 90 A ribbon which could appear to be circular on cross-section owing to twisting and overlap in the packing of the monomeric units (Fig. 7 *a* and *b*). Such a filament would have characteristics quite similar to those of the neurofilament. This filament could now be wound in a helix with a pitch of 10° so that a filament length equal to 13 elliptical subunits comprises a single turn as suggested by Shelanski (6) (Fig. 7 *c*). Such a structure would have an outside diameter of about 230 A, a lumen

[2] Weisenberg, R., G. G. Borisy, and E. W. Taylor. Data in preparation.

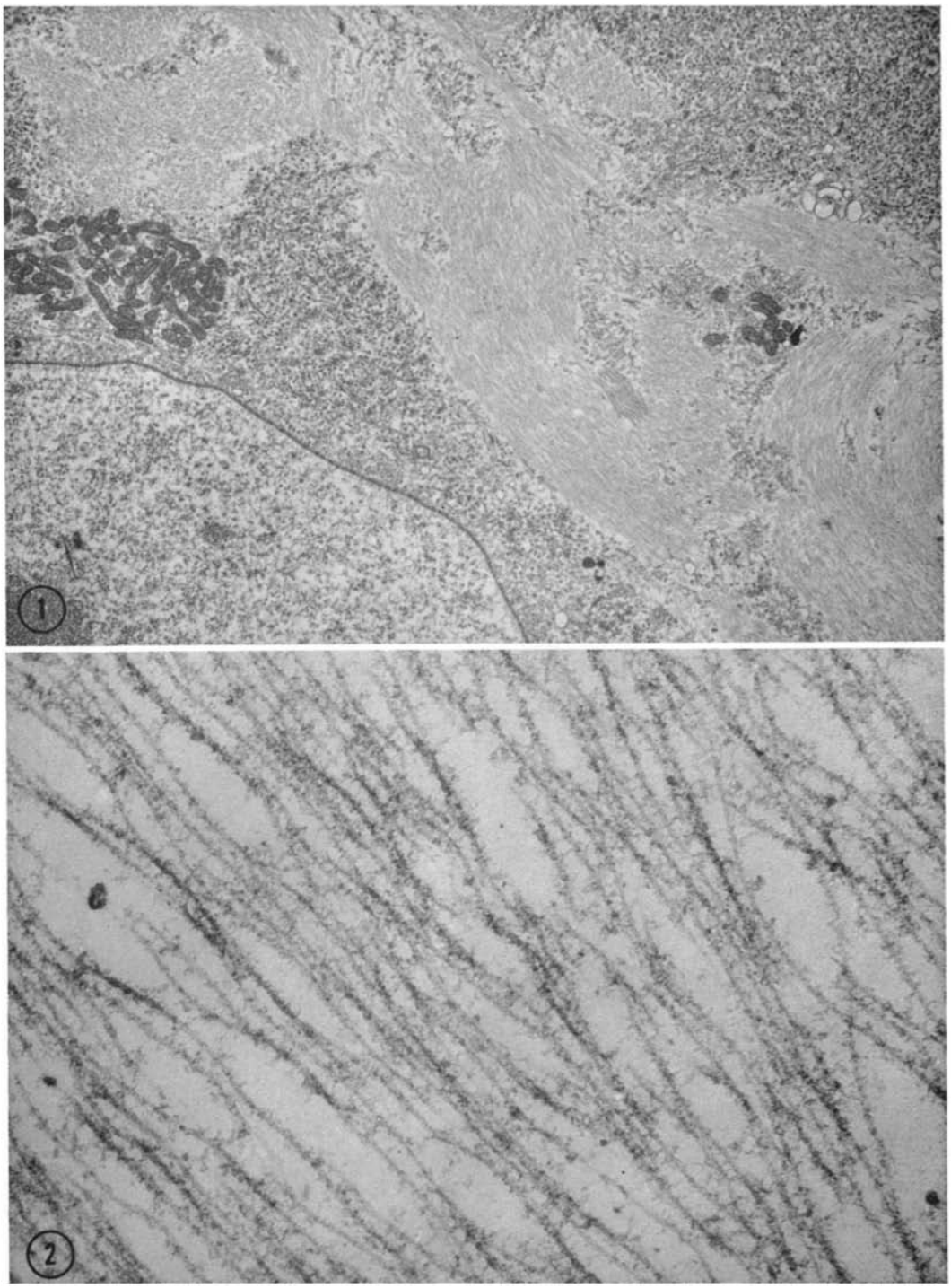

FIGURE 1 Spinal motor neuron of colchicine-treated rabbit, 48 hr after treatment, shows bundles of filaments in the cytoplasm, residual endoplasmic reticulum around the nucleus, and aggregated mitochondria. × 6,630.

FIGURE 2 Colchicine-induced neurofilaments with numerous side arms 48 hr after colchicine treatment. × 64,500.

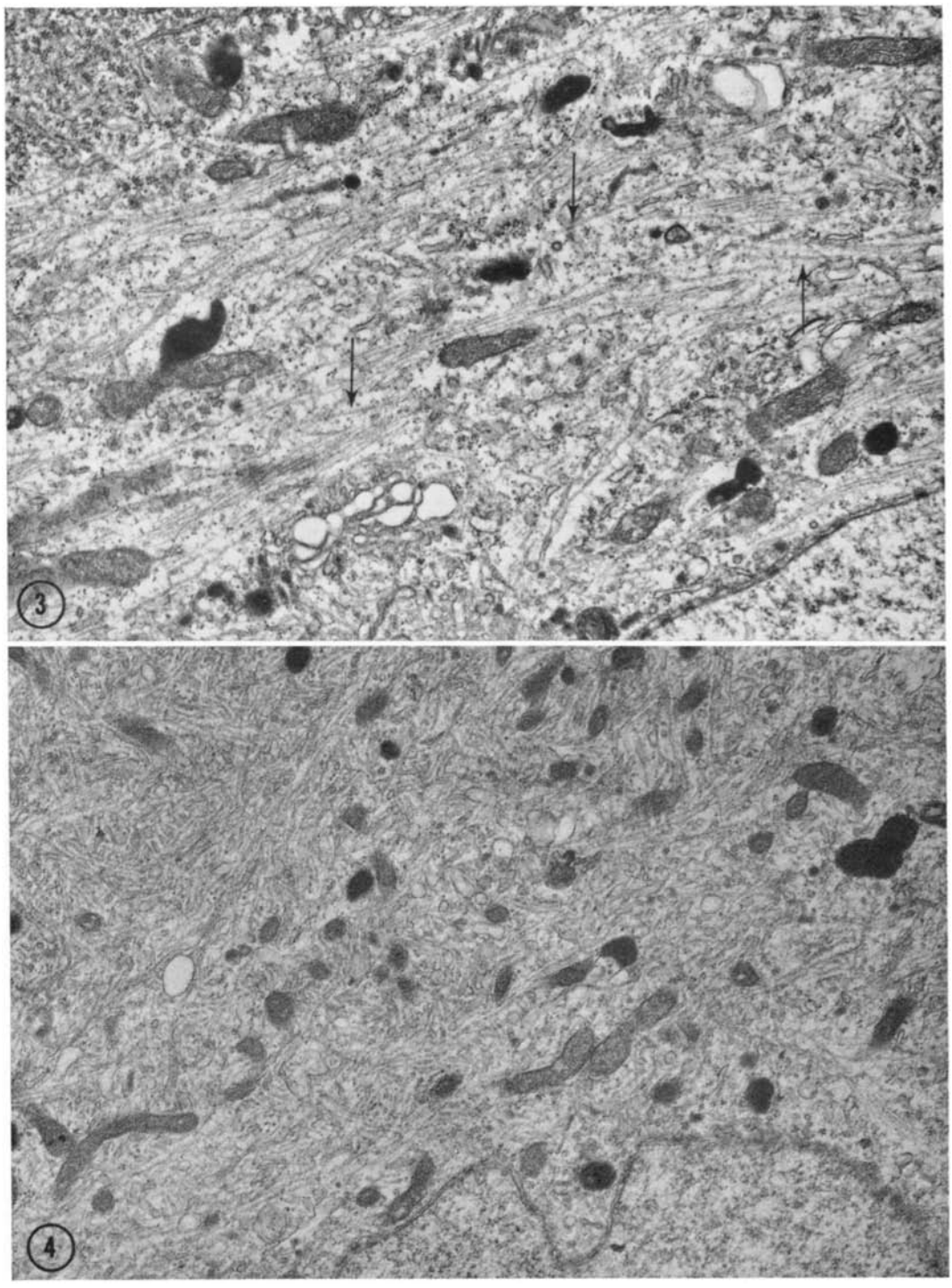

FIGURE 3 A few neurotubules (arrows) are present among the bundles of filaments in the acute stage (2 days) of podophyllotoxin treatment. × 21,500.

FIGURE 4 A chaotic array of neurotubules fills the soma 5 days after vinblastine treatment. × 21,000.

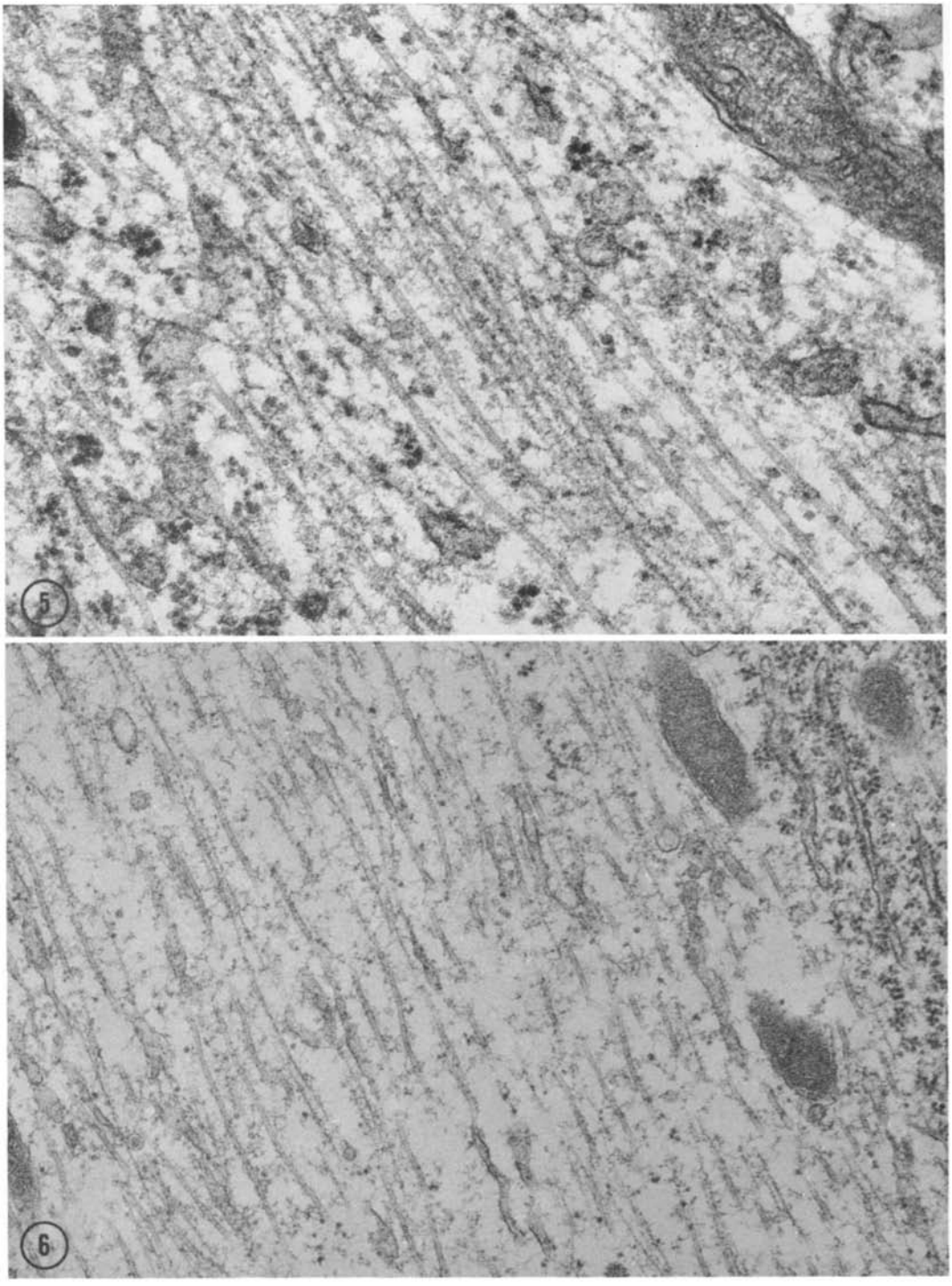

FIGURE 5 Numerous newly formed neurotubules mixed with filaments 15 days after colchicine treatment. × 55,500.

FIGURE 6 An area of neuronal cytoplasm contains many tubules almost free of filaments 21 days after colchicine treatment. × 33,000.

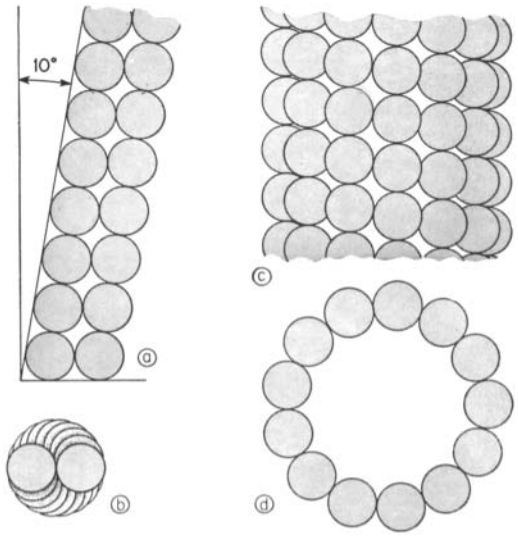

FIGURE 7 *a*, Diagram of the protofilament made up of paired subunits arranged as a ribbon. Each pair represents a 6S protein component. *b*, The ribbon as seen on-end with rotation of the subunits to create a circular cross-section. *c*, The protofilament is wound in a helix to form the tubule. *d*, The helix seen on-end made up of 13 molecules, each 45 A in diameter.

of 145 A, and would appear to be made up of 13 subunits on cross-section (Fig. 7 *d*). This concept would be in agreement with observations on the fine structure of the microtubule (8). Considerable variations in the diameter of microtubules have been reported in numerous cell types, but these would require only a change in the number of subunits per turn in the tubule. In some cells a linear arrangement of the protofilaments rather than a helix seems to be indicated. In the linear arrangement, side-to-side binding is probably of the same nature as that of the helix.

These inhibitors cause dissolution of most of the neurotubules but apparently do not interfere with the continued formation of neurofilaments. They might well block the coiling of the filament to form the tubule. Filaments are found in great excess in the affected soma, perhaps because of inadequate movement out of the perikaryon. Since microtubules are themselves very possibly responsible for axonal flow (9), their loss may also account for this piling of filaments into neurofibrillary tangles. With time, the spindle inhibitor is lost from the cell, and the neurofilaments coil up into large numbers of neurotubules.

This description of the mechanism of neurotubule formation is speculative, of course, but the evidence suggests a possible interconversion between the neurofilament and the neurotubuel and raises the strong possibility that one subunit protein is shared by the two structures. When the filamentous nature of these proteins is considered together with the mounting evidence for the actin-like properties of the microtubule protein (6, 10), one could imagine that the microtubules play a role in a contractile system which is responsible for axoplasmic flow.

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