

# MICROTUBULES AND FILAMENTS IN THE AXONS AND ASTROCYTES OF EARLY POSTNATAL RAT OPTIC NERVES

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## ABSTRACT

Changes in the population of microtubules and filaments within the cytoplasm of maturing axons and astrocytes have been studied during the early postnatal development of rat optic nerves. At birth, all of the axons are unmyelinated; most have a diameter of 0.2–0.3  $\mu$  and contain 4–10 microtubules. Neurofilaments do not occur with any frequency until about 5 days postnatal when they appear as individual groups, each containing 4–12. Subsequently, the neurofilaments of each group disperse so that they become more evenly distributed in mature axons. Developing astrocytes show similar but rather more dramatic changes. Most astrocytic processes contain only microtubules at birth, but during maturation filaments begin to appear in increasing numbers while microtubules become less common. This process continues until, in the mature fibrous astrocytes, filaments pack the cytoplasm and microtubules are rare. These observations suggest that the filaments within axons and astrocytes may be formed by the breakdown of microtubules.

Although the presence of canaliculi, or neurotubules, with a diameter of about 200 A was described in nervous tissue some 10 years ago (Palay, 1956), the occurrence of similar components, the microtubules, within other types of cells was not fully appreciated until glutaraldehyde was introduced as a fixative for electron microscopy (Sabatini et al., 1963). Since then, microtubules have been described in a large number of different cells, both animal and plant (see Anderson et al., 1966; Silveira and Porter, 1964; de-Thé, 1964; Sandborn, et al., 1965).

The purpose of the present communication is to draw attention to some of the changes that take place in the population of microtubules and filaments within the cytoplasm of maturing axons and astrocytes of the optic nerves of rats. It is suggested that the filaments within axons and astrocytes may be formed by the breakdown of microtubules.

## MATERIALS AND METHODS

The optic nerves were removed from postnatal rats fixed by perfusion through the heart with a solution of 4% formaldehyde and 0.5% glutaraldehyde in a phosphate buffer (Millonig, 1961) at pH 7.4 (Vaughn and Peters, 1966). Within half an hour of completing the perfusion, the optic nerves were removed and postfixed for 2 hr in a 1% solution of osmium tetroxide in phosphate buffer. The tissue was embedded in Araldite, and sections were double-stained on the grid with uranyl acetate and lead citrate.

## OBSERVATIONS AND DISCUSSION

In early postnatal optic nerves (Fig. 1) all of the axons lack myelin sheaths and are segregated into fascicles by the processes of the immature astrocytes that extend between them. At this time, the majority of axons are 0.2–0.3  $\mu$  in diameter and, although mitochondria, microtubules, and agranular reticulum are readily visible within the axoplasm, there are very few neurofilaments (Fig. 1).

Many microtubules with an external diameter of between 230 and 260 Å occur within these young axons. They run parallel to the length of the axons and for the most part are relatively straight, with no obvious dilatations. In transverse sections of axons (Figs. 2 and 3), the microtubules have a round cross-section in which a dark central dot may sometimes occur (Fig. 3), rather like the hub at the center of a wheel. Whether this central dot represents a rodlike core to the microtubules is not known because its corresponding profile has not yet been recognized in longitudinal sections.

The 60 Å thick wall of the microtubules has a globular appearance in cross-sections (Fig. 6, arrows; and Fig. 3). The outlines of the globules are rather indefinite, but about 10 or 12 may be distinguished in the walls of some of the tubules. The globules appear to be embedded in a matrix of a less dense material that sometimes forms an outer coating to the tubules and extends as whips into the surrounding intracellular space. Nothing has been seen of a trilaminar form to the walls of the microtubules (Sandborn, 1966).

In transverse sections of the optic nerve taken during the first few days of postnatal development, most of the axons are seen to contain 4–10 microtubules (Fig. 1). During the subsequent maturation, the number of microtubules increases somewhat (Fig. 2) as some of the axons become larger and the number with diameters greater than 0.3  $\mu$  increases. It is within these larger diameter axons that neurofilaments first begin to occur with any frequency at about 5 days postnatal. When they first appear within the axoplasm, the neurofilaments tend to occur as a single group,

often towards the periphery of the axon (Figs. 2, 4, and 5, *f*). Each group contains between 4 and 12 neurofilaments and, with time, the number of groups of neurofilaments within each axon increases (Fig. 6). Subsequently, the groups become dispersed, so that in the mature axons the neurofilaments are more evenly distributed throughout the axoplasm, although there may still be a tendency for them to be aggregated within areas (Fig. 7). The time of appearance of groups of neurofilaments and their subsequent dispersion varies throughout the population of axons, so that at 2 weeks after birth, for example, when some of the axons larger than 0.3  $\mu$  are enclosed within relatively well-developed myelin sheaths and contain many neurofilaments, other, small axons are still unmyelinated and lacking in neurofilaments.

Neurofilaments have an external diameter of 90–100 Å. They are relatively straight and, like the microtubules, run parallel to the length of the axons. In transverse sections the neurofilaments may also appear as tubes, having an electron-opaque wall about 30 Å thick that surrounds a light center (Figs. 5 and 6, *f*).

Somewhat similar but rather more dramatic changes occur in respect of the appearance of filaments, or fibrils, within the cytoplasm of maturing fibrous astrocytes. At birth, the astrocytes are stellate cells with a number of thin processes emanating both from the perikaryon and from a few broad, major processes. The thin processes of different cells (Fig. 1, *A*<sub>1</sub>–*A*<sub>3</sub>) extend towards each other and meet in such a way that they effect a fasciculation of the axons that are traversing the nerve at right angles to them (Vaughn and Peters, data in preparation).

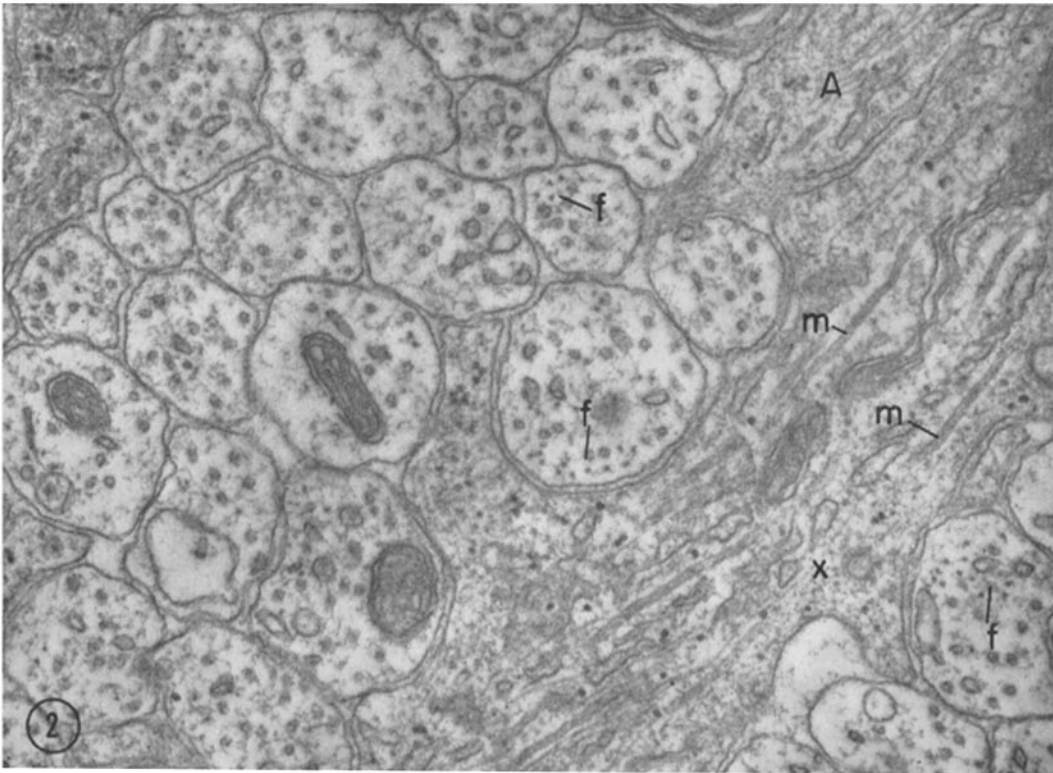
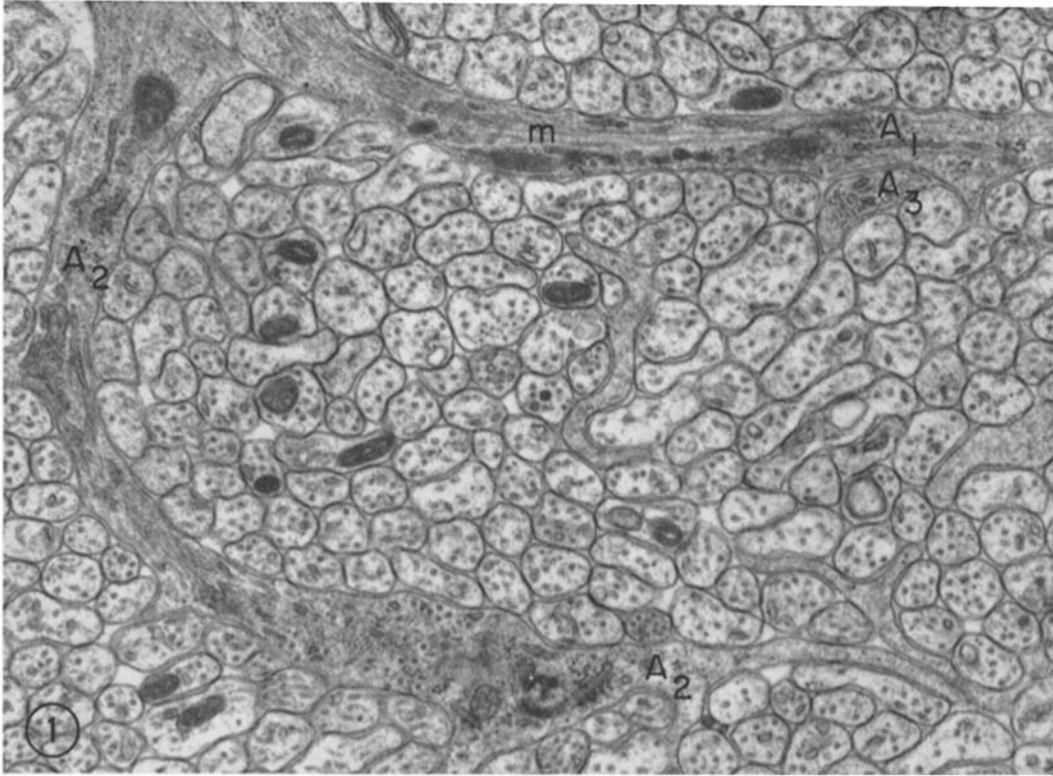
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#### Abbreviations

- A*, *A*<sub>1</sub>–*A*<sub>3</sub>, astrocytic processes
- f*, neurofilaments
- m*, microtubules in astrocytic cytoplasm
- N*, *N*<sub>1</sub>, axon
- P*, profile with hollow globular elements in wall
- P*<sub>*A*</sub>, astrocytic perikaryon
- z*, astrocytic filaments

FIGURE 1 Transverse section of a 3 day postnatal optic nerve, containing unmyelinated axons and a number of astrocytic processes. (*A*<sub>1</sub>–*A*<sub>3</sub>).  $\times 27,000$ .

FIGURE 2 Transverse section of a 9 day postnatal optic nerve, showing a longitudinally oriented astrocytic process (*A*) and a number of axons.  $\times 55,000$



Although a few of the astrocytic processes contain filaments at birth, most contain only microtubules. Like those of axons, the microtubules run parallel to the length of the processes (Figs. 1 and 2, *m*), are between 230 and 260 Å in diameter, and may sometimes be observed in cross-section to have a dense, central core and a globular periphery (Fig. 6, *m*). Groups of filaments are first apparent within a few of the more distal of the processes of the immature astrocytes (Fig. 1, *A*<sub>2</sub> and *A*<sub>3</sub>). With time, the filaments become more common and later are also found within the broader processes and perikarya. In the early phases of development, the filaments are intermixed with the microtubules (Fig. 1, *A*<sub>2</sub>; Figs. 2 and 6, *x*), but as the number of filaments increases, the number of microtubules decreases (Fig. 7) until, in mature fibrous astrocytes, microtubules are only rarely observed.

Like the axons, not all of the astrocytes mature at the same rate. Consequently, in the early phases of development, processes containing only microtubules are present alongside those from other cells that have either a mixture of filaments and microtubules, or filaments alone (Fig. 1). The filaments almost entirely fill the cytoplasm of mature fibrous astrocytes. They are about 80–90 Å in diameter and, like neurofilaments, may sometimes be observed in cross-section to have a dense wall surrounding a lighter center (Fig. 6, *x*).

It has been suggested, from studies on the microtubules in the developing lens rudiment (Byers and Porter, 1964) and in the axopods of a Heliozoan (Tilney and Porter, 1965), that one possible function of the microtubules is the support of an elongating extension of a cell. The developing axons and astrocytic processes are such extensions. The results of the present study suggest

that microtubules may also be the precursors of certain filamentous components of cells, in this instance the neurofilaments and astrocytic filaments. The main evidence for this interpretation is as follows:

1. During the maturation of astrocytes, the microtubules that are so prominent in the early stages of development begin to disappear from the cytoplasm at the same time as the concentration of filaments increases (Figs. 2 and 7).

2. The first neurofilaments that appear within the axons are in groups, each of which contains between 4 and 12 filaments (Figs. 4 and 5). Only later do these groups disperse and the filaments become more randomly distributed throughout the axoplasm.

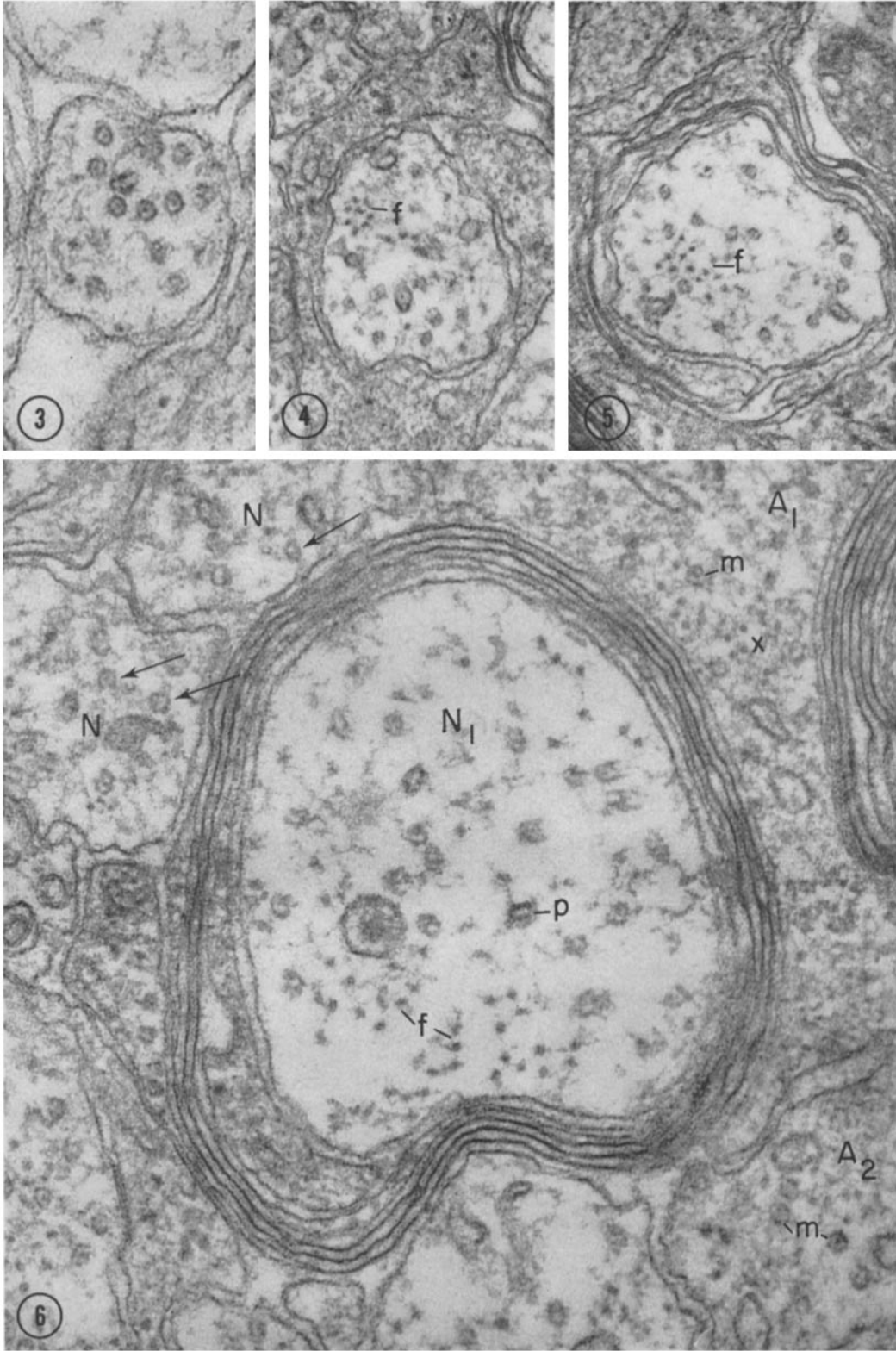
Although support for the concept of the formation of filamentous components has been sought in longitudinal sections of developing axons, where a search has been made for microtubules with frayed out ends and other possible intermediate stages in the formation of filaments, no direct evidence has been found. However, oval-shaped profiles whose walls appear to be composed of hollow globular elements, each with a diameter of about 100 Å (Fig. 6, *p*), have been observed very occasionally in transverse sections of axons. These profiles appear to be distinct from the thinner-walled microtubules and agranular reticulum, and could be interpreted as representing an intermediate stage in the formation of neurofilaments from microtubules. In this case, it must be postulated that the 100 Å hollow globular elements forming the walls of the profiles (Fig. 6, *p*) are derived from the 60 Å globules in the walls of the microtubules and that they represent the precursors of the neurofilaments. These precursors may then separate to form the com-

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FIGURE 3 Axon from a 9 day postnatal optic nerve showing microtubules with central cores.  $\times 135,000$ .

FIGURES 4 and 5 Transverse sections of axons from a 16 day postnatal optic nerve. The axons each contain a group of neurofilaments.  $\times 90,000$ .

FIGURE 6 Transverse section of a myelinated axon (*N*<sub>1</sub>) from a 16 day postnatal optic nerve. This axon contains neurofilaments (*f*) and a profile (*p*) with globules in the wall. Surrounding the myelinated axon are unmyelinated axons (*N*) with microtubules (arrows), and two astrocytic processes (*A*<sub>1</sub> and *A*<sub>2</sub>) with microtubules (*m*) and filaments (*x*).  $\times 130,000$ .



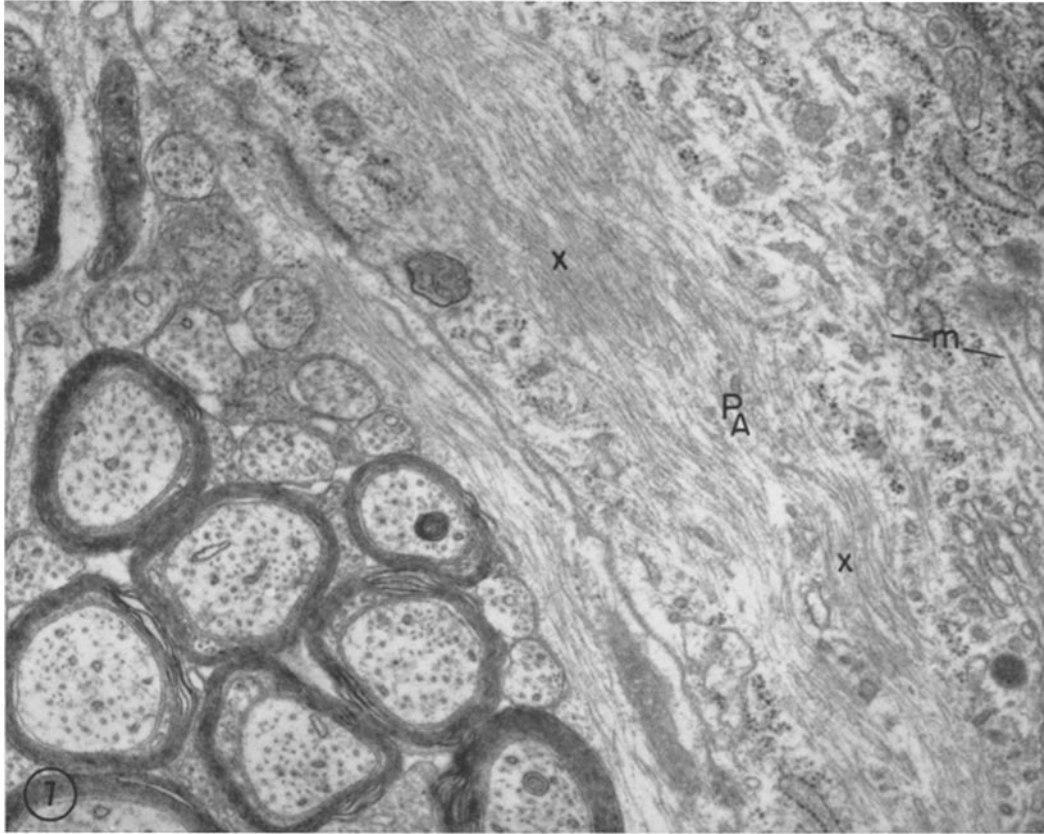


FIGURE 7 Transverse section of an astrocytic perikaryon ( $P_A$ ) containing many filaments ( $x$ ) and few microtubules ( $m$ ). On the left are both myelinated and unmyelinated axons. Note the content of neurofilaments in the former. 21 day postnatal optic nerve.  $\times 30,500$ .

compact groups of neurofilaments which are often observed in developing axons (Figs. 4 and 5).

The direct evidence that the walls of microtubules are composed of filaments comes from work in which microtubules have been mechanically disrupted. Thus, Pease (1963) reports that the microtubules in the flagella of rat sperm tails are composed of 10 longitudinally oriented filaments, and Barnicot (1966) suggests that there are likely to be between 9 and 12 filaments forming the microtubules from the spindles of mitotic cells. In sections of tissue, filamentous components do not appear to have been directly observed in the walls of microtubules oriented parallel to the plane of section, but globular units have been observed in the walls of microtubules in cross-section and these are thought to represent such filaments. In plant cells, for exam-

ple, Ledbetter and Porter (1963) observe 11–13 units, and Silveira and Porter (1964) report about 12 in the walls of microtubules from the spermatozooids of flatworms. In the present material, 10–12 units can sometimes be discerned, a number not exceeded by that of the neurofilaments in the earliest compact groups.

While other interpretations might be advanced to explain the present results, further support for the concept that certain cytoplasmic filaments may be formed from microtubules comes from the work of Auber (1962), on developing flight muscles of an insect. Auber observed that the cytoplasm around each developing myofibril contains microtubules, and from their disposition came to the conclusion that the microtubules give rise to the myofilaments.

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