Virchows Arch. Abt. B Zellpath. 12, 159—167 (1973)


# Retinal Alterations Induced by Intravitreous Colchicine*

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Received August 15/October 15, 1972

*Summary.* A single intravitreal injection of 20 μg of colchicine induces remarkable alterations in several retinal structures, which are accompanied by lost of pupilar reflex, deficit in visual behaviour and finally temporary blindness. The pigment cells revealed, after colchicine administration the presence of dense bodies, vacuolae and mitochondrial damage. Outer segments of photoreceptors presented fragmentation, lysis and final dissapearance of the laminar structure. Inner segments showed vacuolization and mitochondrial alteration. Some bipolar and ganglion neurons showed vacuolization and accumulation of filaments in the soma. The effects on the visual cells are related to a possible action on the formation and removal of the outer segments. The effects on the neurons are discussed in relation with the action of colchicine on other CNS neurons and with the blocking action of this drug on axoplasmic flow.

Colchicine, as well as other mitotic spindle inhibitors, produces striking structural alterations in CNS neurons. The induced changes consist mainly in an accumulation of filaments in the neuronal soma. This action has been atributed to blocking effects on the mechanism of microtubule formation (Shelansky and Taylor, 1968; Wisniewki *et al.*, 1968).

The neurons affected by colchicine also show other kind of alterations. Karlsson and Sjostrand (1969) reported that the drug, when injected intravitreally, interrupts the rapid axoplasmic flow in the ganglion cells axons. This effect has been related to the possible alteration of neurotubules which has been postulated as the cellular structures responsible for rapid axonal flow (Schmitt, 1969).

The aim of the present work was to study the effects on intravitreal colchicine on retinal structure. The main objective of the investigations performed was to search for morphological alterations in retinal neurons, which could be correlated with the changes induced by the drug in the axonal flow.

## Material and Methods

Twelve adult cats (2900–3300 g) were used. Nine animals were injected in both eyes with 20 μg of colchicine (Mann Research Laboratories) diluted in 40 μl of saline. A 15 × 5 BD needle directed towards the central part of the vitreous humor was used. Animals were perfused 2, 4, 6, 8, 10, and 30 days after the intravitreous injections with a solution containing glutaraldehide (4%) and formaldehide (6%) in a 0.1 M. phosphate buffer at a pH = 7.5. Pieces of both retinae were processed for light microscopy (hematoxiline-eosin stain) and for

* This work was supported by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina, and Fight for Sight, Inc. N.Y. (Grant in Aid No. G-340).

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electron microscopy (post-fixation in osmium tetroxide, dehidratation in a graded series of ethanol, stained with uranil acetate and lead citrate).

Three control cats were injected in both eyes with 40 μl of saline. At 4, 8, and 30 days after the injection, they were perfused and processed for light and electron microscopy. All the injections and perfusions were made under light barbiturate anesthesia.

Pupilar reflex and visual behavior (ability to distinguish stationary or moving objects) were tested dayly.

## Results

All animals injected with colchicine showed a progressive increase of pupilar diameter together with a decrement of pupilar reflex and visual behavior. These changes started the 3rd day after the injection and reached their maximum at the 5–6th day. During this period there was a permanent mydriasis, the pupilar reflex was absent and animals were behaviourally blind. The alterations persisted up to the 12–15th day post-injection, when the pupilar reflex and—to some extent—visual ability reappeared. At 20–25 days after the injections, animals showed quasi-normal pupilar reflex and visual behaviour. However, in several animals, pupilar diameter larger than normal was present at this time.

### *Light Microscope Observations*

Several morphological alterations were observed in the retinal structure associated with the mentioned changes in visual behaviour. On the 2nd day post-injection the optic microscopical study showed an increased thickness of the pigment epithelium, the rest of the retina presenting a normal aspect (Fig. 1 A). On the 4th day, the external segments of the visual cells lost their normal appareance: their boundaries were distinguished with difficulty and this layer was reduced in thickness (Fig. 1 B). The 8th day, the external segments disappeared and the pigment cells contacted with the remainder part of the visual cells (Fig. 1 C). The 30th day post-injection, the external segments redeveloped and presented a normal aspect. The pigment epithelium remained "vacuolized" (Fig. 2).

No alterations were observed in the rest of the retina.

### *Electron Microscope Observations*

The ultrastructural study showed progressive alterations in different retinal strata. The changes were slightly marked on the 2nd day after the injection, more pronounced at the 4–8th day and thereafter varying their duration according to the structure affected.

The cytoplasm of the pigment cells presented a great number of round-laminated bodies of dense appearance. The major alteration observed in these cells was the abundance of vacuolae which, in some cases, occupied a large extent of the cytoplasm. Vesicles and membranous debris could be found inside these vacuolae (Fig. 3). These alterations were observed even at the longest survival period studied (30 days).

Alterations of the visual cells were localized mainly in their external segments. On the 4th day post-injection, these structures were dilated showing disruption of the laminar structure. These changes were more marked on the 8th day: in

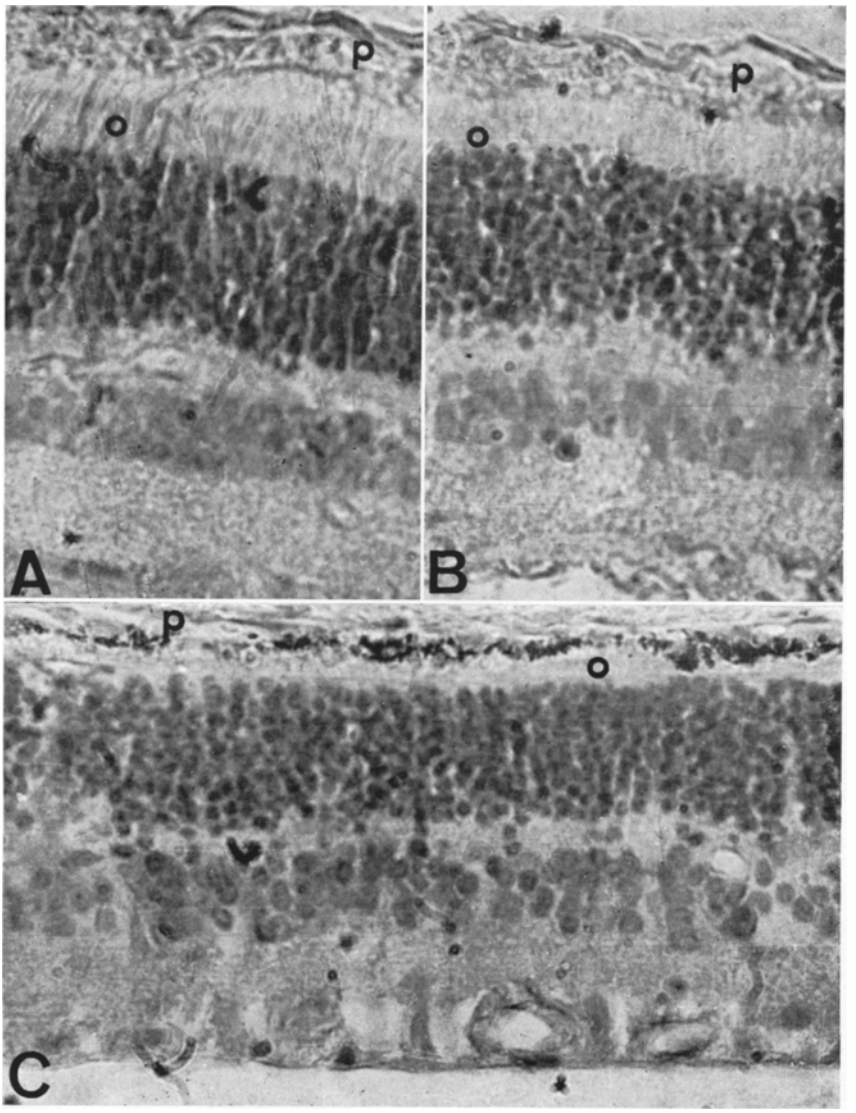


Fig. 1 A—C. Cat retina 2 (A), 4 (B), and 8 (C) days after intravitreous injection of colchicine. Note the increased thickness of the pigment epithelium (*p*) and the gradual decrease of the outer segments of the visual cells (*o*). Hematoxiline-eosine. Original magnification: ×450

some cases the outer segments were notably swollen containing only rests of their laminar structure. In other cases, they were retracted showing a dense appearence (Fig. 4 A). The inner segments showed less conspicuous changes which consisted in the presence of some vacuolae and alterations of the mitochondrial structure (swelling and disruption of cristae). Occassionally, fragments of the outer segments could be observed inside the inner segments (Fig. 4 B).

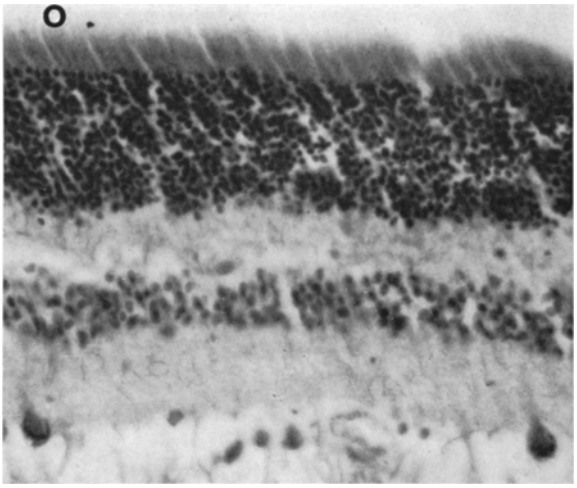


Fig. 2. Cat retina 30 days after intravitreous injection of colchicine. The outer segments (*o*) of the photoreceptors have recovered their normal aspect. Hematoxiline-eosine. Original amplification: ×450

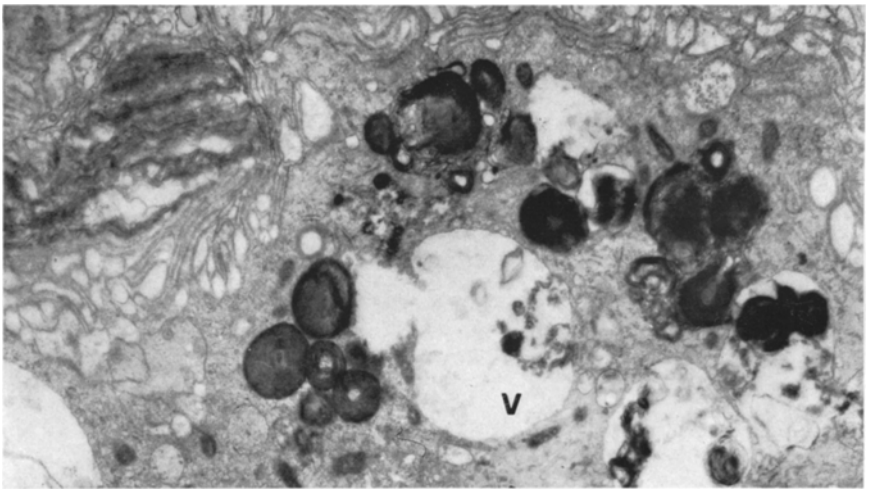


Fig. 3. A pigment cell citoplasm of the cat retina 8 days after intravitreous injection of colchicine. Note the abundance of round-laminated bodies and the presence of vacuoles (*v*) containing membranous fragments. ×8000

In those animals sacrificed 30 days after colchicine injection, the examination of the visual cells showed a normal and recovered structural organization of both inner and outer segments.

Effects of colchicine on the retinal neurons were irregular in its extent and varied for different animals. Some of the bipolar and ganglion neurons presented

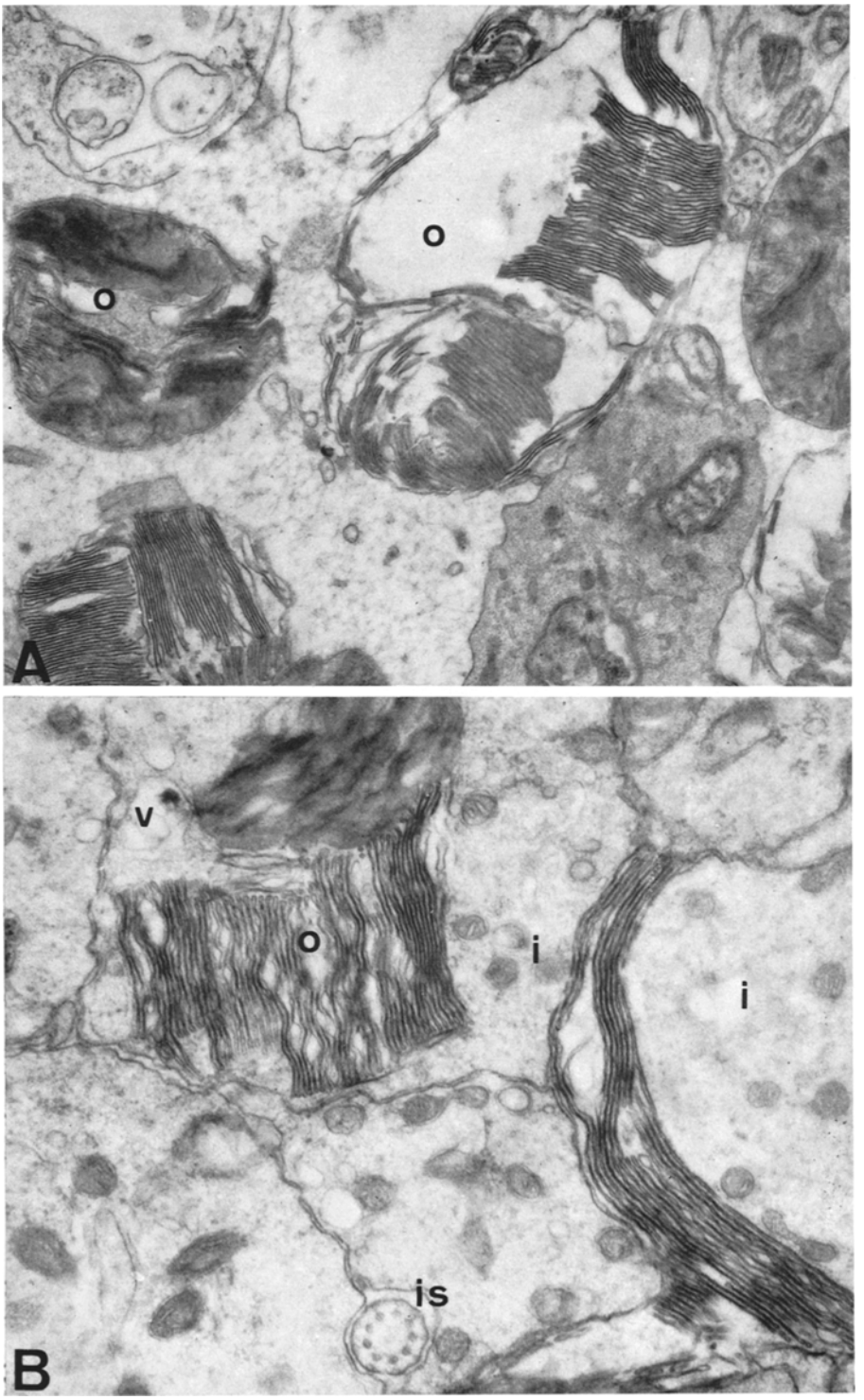


Fig. 4. A) Visual process 8 days after intravitreous injection of colchicine. The outer segments (*o*) are swollen and fragmented or retracted. ×18000. B) Inner segments (*i*) of visual cells 8 days after intravitreous injection ot colchicine. Note vacuolae (*v*) and rest of an outer segment inside the inner segment. ×20000

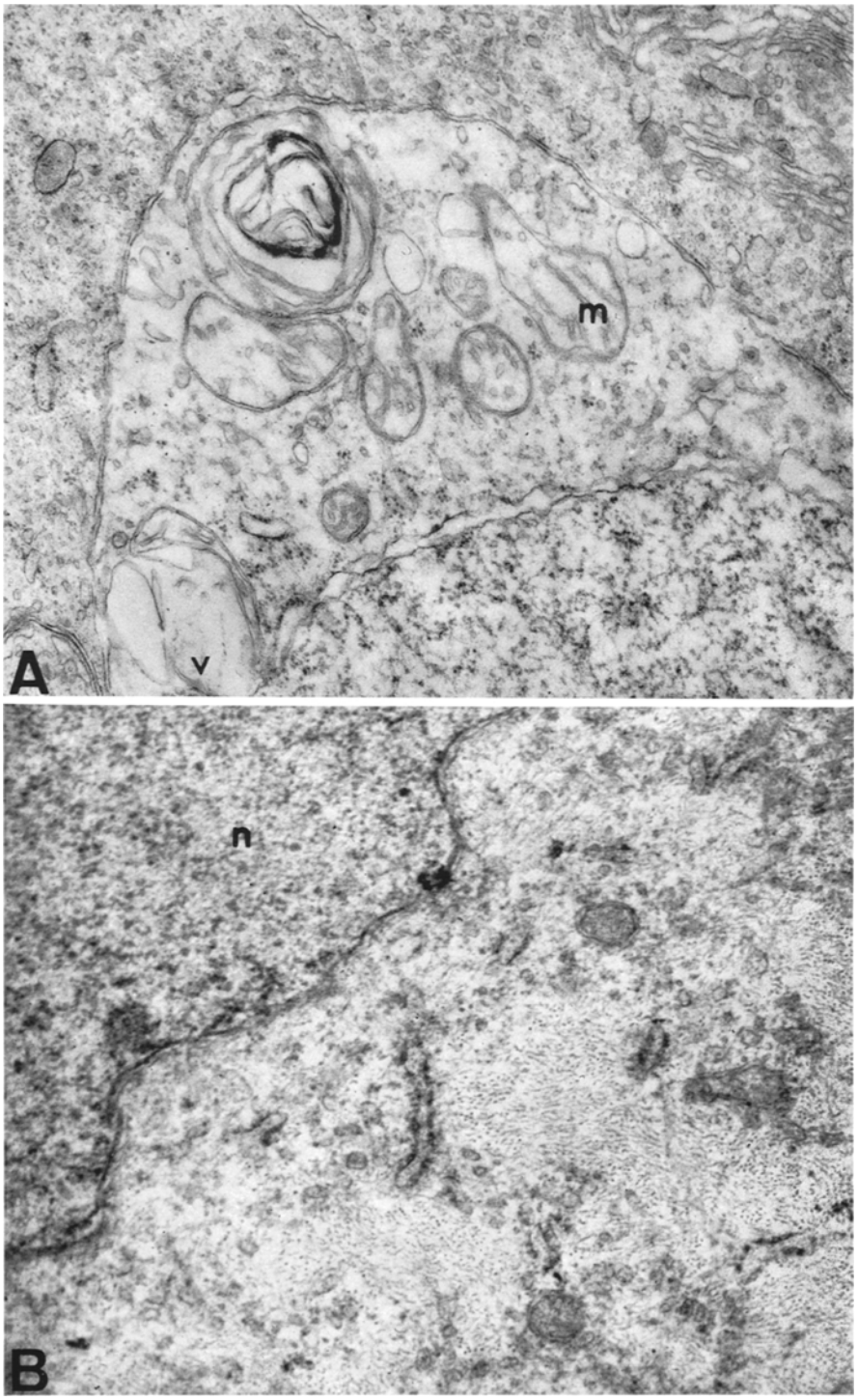


Fig. 5. A) A bipolar cell cytoplasm 8 days after intravitreal injection of colchicine. Note the presence of vacuolae (*v*) and distended mitochondriae (*m*). × 26000. B) Aspect of a ganglion cell cytoplasm 8 days after intravitreous injection of colchicine. An increased number of filaments (*f*) are seen dispersed in this portion of the cell. *m* mitochrondia; *n* nucleus. × 30000

alterations during the whole period studied. Bipolar neurons showed cytoplasmatic vacuolization (Fig. 5A) while the ganglion neurons showed an increase of filaments in their cytoplasm. The appearance of these filaments was similar to that of normal neurofilaments and were distributed in a diffuse array (Fig. 5B).

Neither behavioural nor anatomical changes were found in the control animals.

## Discussion

The main conclusion from the present investigation is that a single intravitreal injections of 20 μg of colchicine is sufficient to induce remarkable alterations in several retinal structures which are accompanied by visual deficit.

The rapid appearance of changes in the pupilar reflex and visual behaviour are easily explained on the basis of the early alterations found in the receptors. In fact, the temporal course of these alterations is similar to that of the visual impairment. However, it is also possible that early changes of the pigment, bipolar and ganglion cells could also play a role in determining the functional impairement found. This participation could explain the persistence of pupillar alterations in the long-term observations.

The mechanism of action of colchicine on the visual cells probably lies both in the process of formation of the outer segments (Young, 1967) and in the removal of these structures (Herron *et al.*, 1969). Although the drug does not seem to affect protein synthesis in the retina (Karlsson and Sjostrand, 1969), alterations observed in the inner segments suggest that the drug could affect the normal formation of the multilamellar structures of the outer segments. It could be hypothetized that colchicine affects the availability of enzymes in similar fashion as in peripheral nerves (Kreutzberg, 1969), affecting the high metabolic activity of photoreceptors. Alterations on the formation of outer segments would be similar to those produced by other factors that modify the metabolism of visual cells (Noell, 1958; Dawson *et al.*, 1971). Such a metabolic action of colchicine has been previously sustained by Ferguson (1952) in relation to the effect of this drug on isolated muscle.

The striking alterations of the pigment epithelium suggest that these cells also participate in the mechanism of visual cells alterations, at least during the earlier period of the process. The altered pigment cells could then induce changes in photoreceptors like those produced by sodium iodate (Noell, 1952) or diaminodiphenoxy alkanes (Ashton, 1957).

The most striking change induced by colchicine on the CNS neurons is the accumulation of filaments in the neuronal soma. This effect has been related to the binding of colchicine to the proteic subunit of neurotubules (Shelansky and Taylor, 1968), resulting in the blockage of neurotubule formation and the consequent accumulation of filaments (Wisniewski *et al.*, 1968). This alteration varies according to the type of cell affected: some neurons present an accumulation of tangled arrayed filaments, while others show no alterations or show a diffuse increase of filaments (Wisniewsky and Terry, 1967). According to our observations, retinal neurons show the second type of reaction. In relation with this it should be mentioned that Rodriguez Echandía *et al.* (1967) and Fernández *et al.* (1970) did not observe any change in neurotubules following colchicine treatment.

Karlsson and Sjostrand (1969) reported a blockage of the rapid flow of proteins in the axons of ganglion cells on the first day after intravitreous injection of colchicine. This blockage was atributed to the action of the drug on the neurotubular system. According to our observations, changes on ganglion cells neurotubules and filaments are scarce and are observed in a later period. This apparent discrepancy could be atributed to the different species used and to the dose employed in the present work. However, considering that the effect of colchicine varies in the different type of neurons, it is not unlikely that an interruption of the axoplasmic flow would occur in the ganglion cell fibers without a massive alteration of the microtubules and filaments in the neuronal soma.

According to the present results, the visual dificit and some of the retinal alterations induced by colchicine were reversible. In the peripheral nervous system colchicine provoked irreversible or transient changes depending on the dose injected: lower doses induced functional changes persisting only up to 15–20 days after (Angevine, 1957). Some of the effects induced by the drug in the C.N.S. seems to be also reversible. In fact, Wisniewsky *et al.* (1968) reported that the filamentous proliferation observed in the anterior horn cells reverted on the 20th day and related this critical period to the time during which the drug remains in the affected neurons. This factor, added to the time needed for the reconstitution of altered structures may explain the temporal course of the reversible retinal alterations induced by colchicine.

We acknowledge Prof. Dr. E. De Robertis for his continuing advice and criticism. We are grateful to Miss Emerita Jorge Vilela and A. J. Sáenz for their technical assistence.

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