probably assembled at the outer segment base into disc membranes[4,5]. The formation of new discs causes a scleral displacement of old discs which are engulfed and destroyed in the pigment epithelium, when they reach the tip of the rod outer segment[6,12]. Although the basic course of the phenomenon appears to be identical in all vertebrates already studied[5], the biosynthesis of rod discs seems to proceed more rapidly in the pigeon. A rod outer segment is completely renewed in 3 to 4 days which means that 9 to 12 discs are synthesized per hour. This is twice as rapid as in mice and rats[2], and 15 times more rapid than in frogs[4]. As this protein renewal includes continual regeneration of the rod visual pigment[6], a relationship between function and protein metabolism is suggested by this rapid membrane renewal rate in this highly organized retina.

The fundamental distinction between rod and cone protein renewal has also been observed in other species[5,13]. Further understanding of the renewal mechanism in the cone outer segments cannot be deduced from our data.

Résumé. Les segments externes des bâtonnets de la rétine du pigeon sont totalement renouvelés en 3–4 jours. La distinction fondamentale entre les méchanismes de renouvellement des protéines discales des bâtonnets et des cônes a été retrouvée dans cette espèce.

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Effect of Vincristine on the Ultrastructure of Rat Neurohypophysis

Microtubules have been considered to be involved in the movements of particulate components of the cytoplasm in a number of animal and cell models[1–4]. In nerve cells, where they are particularly abundant[5], it has been suggested that they contribute to axonal flow[6]. Supporting these concepts, there are recent studies demonstrating that the administration of colchicine, an agent that disrupts cellular microtubules, blocks the axonal transport of ceatylcholinesterase in sciatic nerve[7] and of labelled protein in the hypothalamo-neurohypophyseal system[8].

The present work was undertaken in order to study the effect on the ultrastructure of rat neurohypophysis induced by vincristine, which is known to precipitate microtubular protein in the form of crystalloid inclusions in many cell types[9].

Normal Wistar rats of both sexes, weighing between 250 and 350 g were used. After decapitation, the neural lobe of the hypophysis was quickly removed and incubated at 37°C in an oxygenated Locke's solution (NaCl 154 mM; KCl 5,6 mM; $CaCl_2$ 2.2 mM; $MgCl_2$ 1.0 mM; $NaHCO_3$ 6.0 mM; glucose 10 mM). Vincristine sulfate (Oncovin, Eli Lilly and Co. Indianapolis, Ind.) was added to the medium at $10^{-5}M$ for periods ranging from 15 to 180 min. The glands were subsequently fixed for electron microscopic studies in 5% phosphate buffered glutaraldehyde solution (pH 7.4). After postfixation in phosphate buffered OsO_4, the tissues were dehydrated in graded alcohol solutions and embedded in Epon 812. Thin sections were prepared with an LKB ultratome, treated with lead citrate and examined with a Philips EM 300 electron microscope.

The ultrastructure of rat neurohypophysis maintained in Locke's solution alone revealed a typical pattern even after 180 min of incubation. In particular the nerve fibers containing microtubules and neurosecretory granules, were well preserved (Figure 1).

One hour after incubation in the presence of vincristine, the axoplasm reveals crystalloid inclusions, the number of which increases with time of exposure, whereas the microtubules could be identified only rarely. At 180 min of

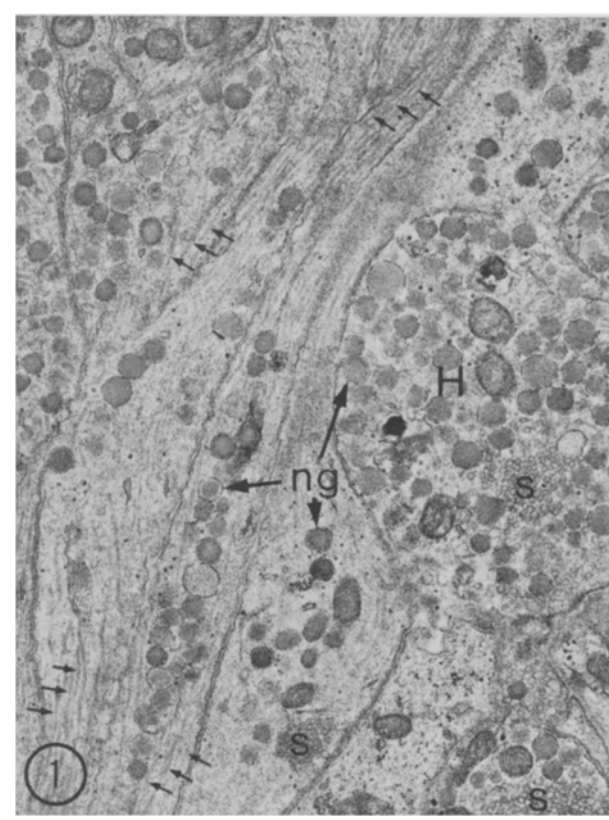

Fig. 1. Rat neurohypophysis incubated for 3 h in Locke's solution alone. Note the large number of microtubules (small arrows) in the nerve fibers. H, portion of a Herring body; ng, neurosecretory granules; s, synaptoid vesicles. ×17,500.

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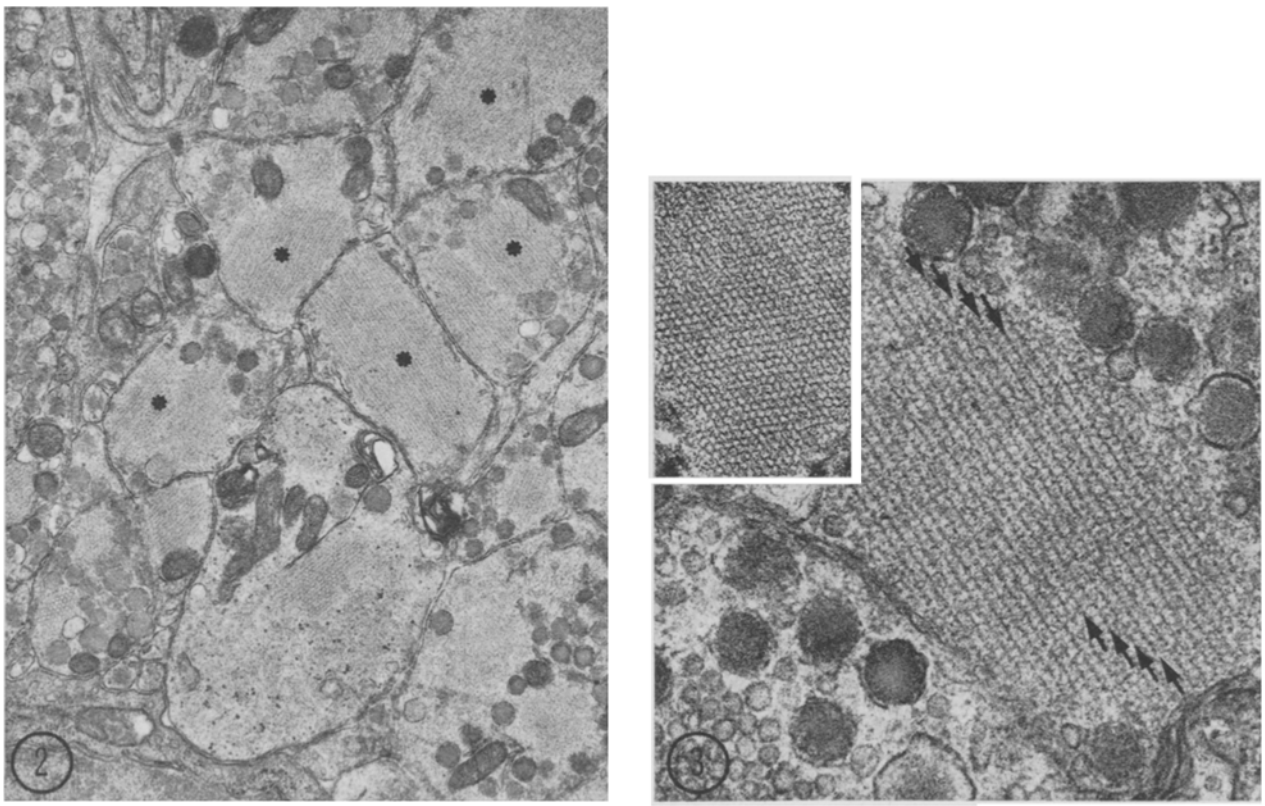

Fig. 2 and 3. Rat neurohypophysis incubated for 180 min in presence of vincristine. Fig. 2. Survey micrograph of several nerve fibers containing large crystalloid inclusions (*). × 14,000. Fig. 3. Part of a crystalloid inclusion in longitudinal section. Arrows indicate parallel arrays. When a crystalloid inclusion is sectioned horizontally (inset) a honeycombed pattern is exposed. ×48,000. Inset ×29,500.

ncubation, numerous fibers contain crystalloid inclusions which may occupy almost the entire surface section (Figure 2). The same inclusions are found in pituicytes, but less frequently. The inclusions were characteristically composed of bundles of closely packed tubules, appearing in longitudinal sections to be alligned in an orderly parallel fashion (Figure 3), while in cross sections they present a regularly repeating honeycomb pattern (Figure 3, inset).

The subcellular alterations in the axoplasm of rat neurohypophysis after incubation in the presence of vincristine resembled those described previously in other tissues[9]. Hence the morphology of crystalloid inclusions and the time course of their formation were identical. Except for microtubules, no other cytoplasmic organelles showed ultrastructural changes.

These results raise two questions, the first concerning the role of microtubules in axonal flow and the second concerning their role in hormone release at nerve terminals.

If one assumes that microtubules are involved in axonal flow[6], one would predict that this flow would be inhibited after exposure to vincristine. Consequently, the transport of any substance, including the neurosecretory granules, resulting from axonal flow from the hypothalamus to the neurohypophysis, would be reduced or even blocked somewhere along this pathway. Recently, NORSTRÖM et al.[8], using colchicine, another microtubular stabilizer, reported the inhibition of axonal transport of labelled proteins into the neurohypophysis.

Several other studies have suggested that microtubules participate in the process of hormone release, namely in endocrine pancreas[10,11], thyroid[12] and adrenal medulla[13]. Using substances such as colchicine, vinca alkaloids or heavy water, which interfere with function of microtubules, these authors have induced an inhibition of hormone release. In reference to the hypothalamo-neurohypophyseal system, one may ask whether the nerve fiber microtubules intervene in the process of vasopressin and oxytocin secretion by their action directly at nerve terminals, or indirectly by their effect on the axonal flow.

Résumé. Des hypophyses postérieures de rat ont été incubées en présence de vincristine, substance qui interfère avec le système microtubulaire et provoque la formation d'inclusions cristalloides caractéristiques. L'ultrastructure a révélé ces inclusions dans les fibres nerveuses et les pituicytes. La discussion porte sur les répercussions fonctionnelles que ces altérations morphologiques pourraient entraîner.

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