Protein Renewal in the Photoreceptor Outer Segments of the Pigeon Retina

The photoreceptor cells of vertebrate retinae are divided into several specialized compartments. The outer segment performs the light absorption and contains hundreds of piled membranous discs[1]. The inner segment is the site of the main synthetic and metabolic mechanisms. The synaptic terminal transmits the visual excitation to more central neurones of the retina.

The renewal of the disc membrane proteins, among them the photosensitive visual pigment, has been studied in several species, by autoradiographic[2–6], and by biochemical techniques[6]. This paper reports electron microscopic autoradiographic observations of this process in the rod and cone outer segments of the highly specialized pigeon retinae.

Four pigeons, *Columbia livia*, under light anesthesia were injected into the vitreous humor of the right eye, 100 µl of a solution containing 500 µC of ^{3}H-L-Leucine (S.A. 22 C/mMole), under standardized conditions described elsewhere[7]. They were sacrificed at 18 h, 3, 7 and 14 days after injection, respectively, by intracardiac perfusion of the different mixtures of paraformaldehyde and glutaraldehyde used by BRIGHTMANN and REESE[8]. After dehydration of the retinas in ethanol and embedding of the tissue blocks in araldite, autoradiographs for electron microscopy were prepared according to GRANBOULAN[9] and SALPETER and BACHMANN[10], using the Ilford L4 nuclear emulsion. After exposure for 6 weeks, the preparations were developed in microdol and fixed in sodium thiosulfate.

18 h after injection, 70% of the silver grains were contracted over a distance of 2 to 3 µm, at the base the rod outer segments (Figure 1). 3 days after injection, the rod outer segments were still heavily labelled, but most of the grains were now localized 3 to 6 µm from the tip and at about 18 to 21 µm from their base (Figure 2). 7 days after injection, the radioactivity was considerably less, and the few grains were scattered all over the rod outer segments. 14 days after injection no more grains were found.

In the cone outer segments, a different pattern was observed. 18 h and 3 days after injection, the radioactivity was weak and randomly distributed (Figure 1). 7 days after injection, as in the rod outer segments, few grains were still detectable; and none were visible 14 days after injection.

The radioactive proteins detected by silver grains are probably synthesized in the rod and cone inner segments, as already shown in other vertebrate retinae. In the rods, the newly synthesized proteins migrate in the inner segment, pass through the connecting cilium[11,12], and are

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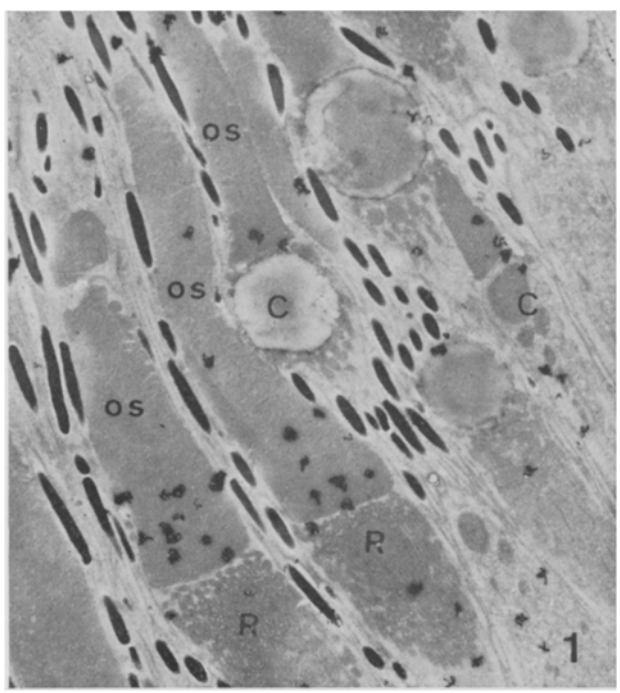

Fig. 1. Electron micrograph of photoreceptor cells 18 h after injection of ^{3}H Leucine. The autoradiographic grains are mainly concentrated over the base of the rod (R) outer segments (OS). ×5,600. R, rod; C, cone; OS, outer segment.

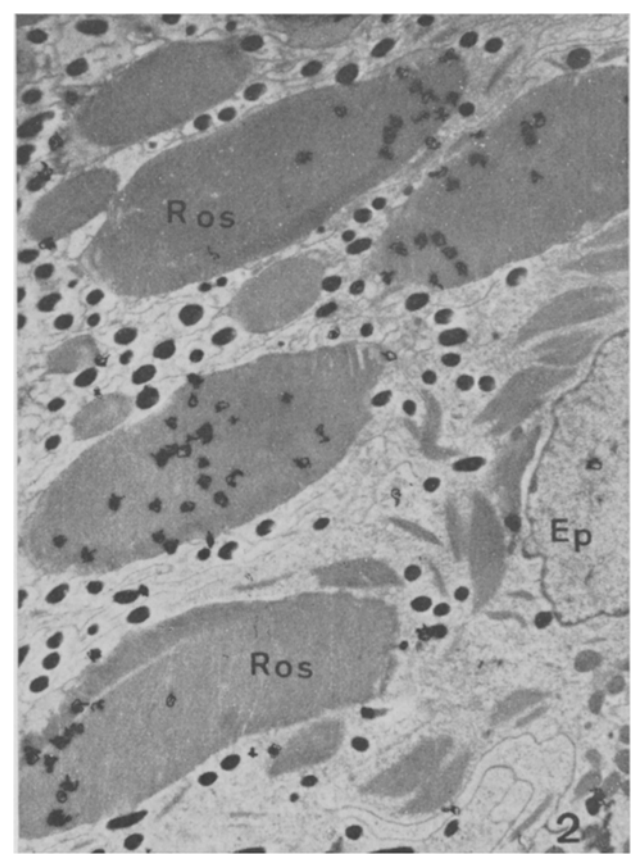

Fig. 2. Three days after injection, the grains are localized near the tip of the rod outer segment. Ep, pigment epithelial cells; ROS, rods outer segments. ×6,500.

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

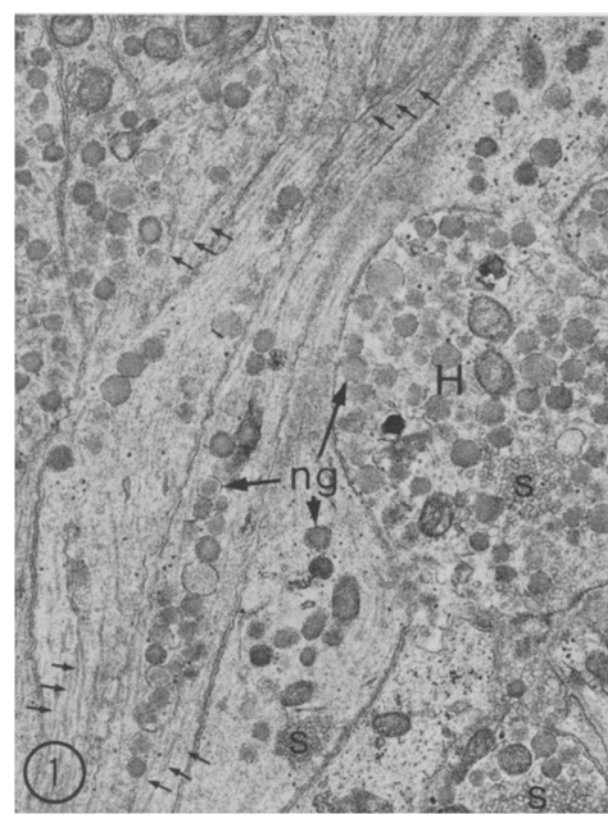

Fig. 1. Rat neurohypophysis incubated for 3 h in Locke's solution alone. Note the large number of microtubules (small arrows) in the nerve fibers. H, portion of a Herring body; ng, neurosecretory granules; s, synaptoid vesicles. ×17,500.

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