gland after hypophysectomy (Dempsey and Peterson[8]) and therefore suggest that the pituitary governs the structure and secretion of the pineal gland. Both recent ultrastructural and histochemical investigations show that the pineal is a secretory gland and belongs rather to the endocrine system than to the central nervous system (Cassano et al.[9]; Wolffe[10]; Arstila[11]). The secretory granules found in the rat pineal gland are supposed to be the site of synthesis or storage of melatonin, 5-hydroxytryptamine (5-HT) or other biogenic amines (De Iraldi and De Robertis[12]; De Martino et al.[13]). Chronic administration of reserpine leads to the almost complete disappearance of the dense granules. All the neurosecretory material in pineal gland is very much decreased in adult hypophysectomized rats. The mechanism by which the pituitary intervened in the maintenance of a normal structure and secretion of the pineal gland is not known, but probably it releases a specific polypeptide (tropic hormone?) for the pineal gland similar to that released for other endocrine glands and therefore the pineal gland should be considered as a pituitary-dependent gland.

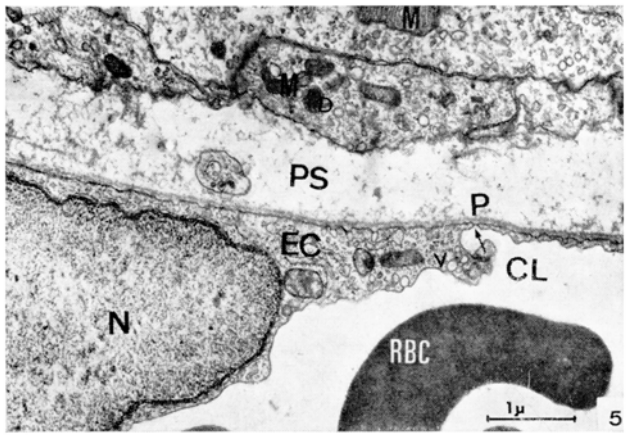

Fig. 5. Totally hypophysectomized rat pineal gland. Basal zone of an epiphyseal cell, showing an enlarged pericapillar space (PS), mitochondria (M), endothelial capillary membrane with many pores (P↑), endothelial cells (EC) with nucleus (N) and many small vesicles (V), red blood cell (RBC), D, small dense granule. Osmium tetroxide, Epon, lead citrate. × 22,000.

Résumé. Nous avons étudié les modifications de l'ultrastructure de la glande pinéale du rat après hypophysectomie et constaté: (a) une atrophie du réticulum endoplasmique, (b) réduction des ribosomes, (c) déplétion du matériel sécrétoire des granules, (d) altération des mitochondries, (e) réduction dans le volume des noyaux et des nucléoles et (f) un agrandissement de l'espace périvasculaire. Il est très probable que l'hypophyse antérieure est nécessaire pour maintenir la structure et la sécrétion de la glande pinéale, mais le mécanisme par quoi s'exerce ce contrôle reste encore inconnu.

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Various Types of Amine-Storing Vesicles in Peripheral Adrenergic Nerve Terminals

The sympathetic postganglionic nerve terminals contain vesicles of at least 2 different types: (a) small vesicles measuring about 500 Å in diameter which are either empty or contain a dense core (types II and III of Grillo and Palay[1]). As shown in previous studies[2,3], empty and dense core vesicles represent, however, most probably a homogenous population differing in the degree of amine filling only. (b) Large vesicles measuring 700–1200 Å (mean 900 Å) in diameter regularly containing a dense core (type I of Grillo and Palay[1]).

It is generally accepted that the dense cores of the small vesicles represent noradrenaline (NA)[4–7], but it is not known whether the large dense core (LDC) vesicles also store NA. After treatment with reserpine all small vesicles become empty, but the osmiophilic content of the large vesicles persists. This was taken as evidence by various authors that the LDC vesicles do not store NA[5–7]. It was found, however, that after incubation of various tissues in NA containing solutions[2] or after treatment of animals with 5-hydroxydopamine (5-HODA)[3], the dense cores of the large vesicles became more osmiophilic and often somewhat larger[8]. These observations might indicate that the LDC vesicles are capable of storing amines, at least under these experimental conditions. It was the purpose of this investigation to bring more direct cytochemical evidence that the LDC vesicles of sympathetic postganglionic nerve terminals in the iris and vas deferens of the cat do contain under normal condition biogenous amines.

The iris and vas deferens of controls and of animals pretreated with reserpine (2 mg/kg i.p. 20 h before the experiment) or α-methylmetatyrosine (MMT) (200 mg/kg i.p. 20 and 4 h previously) were removed under anaesthesia with Nembutal® and fixed for 2–4 h in 3% phosphate buffered glutaraldehyde. Half of the fixed tissue was stored overnight in a phosphate-sucrose solution and then overfixed with OsO_4. The remainder of the tissue was treated overnight in a potassium dichromate solution at pH 4.1[9], then dehydrated and embedded in the usual

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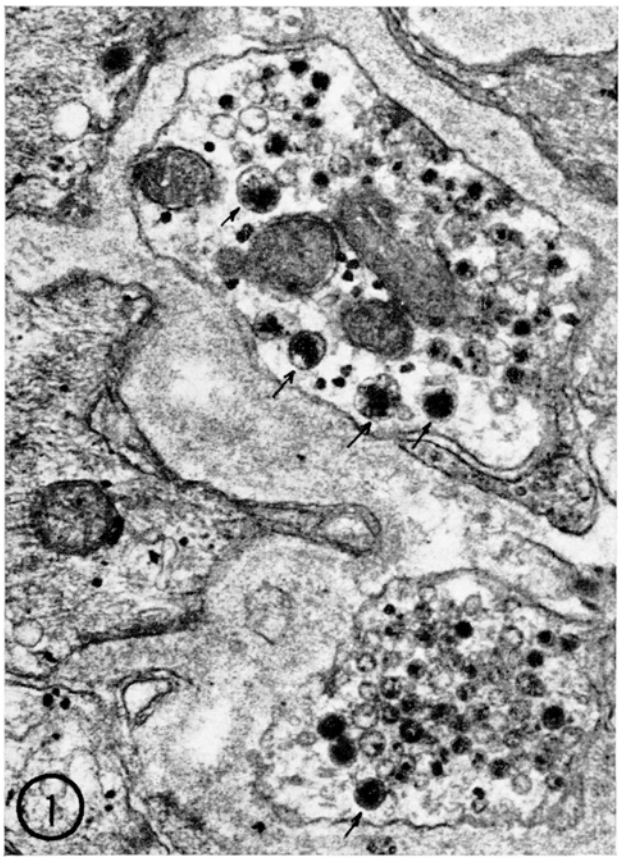

Fig. 1. Iris of cat, glutaraldehyde-OsO_4 fixation, uranyl acetate and lead citrate contrasted. Besides small vesicles the adrenergic nerve terminals contain several large dense core vesicles (→). × 50,000.

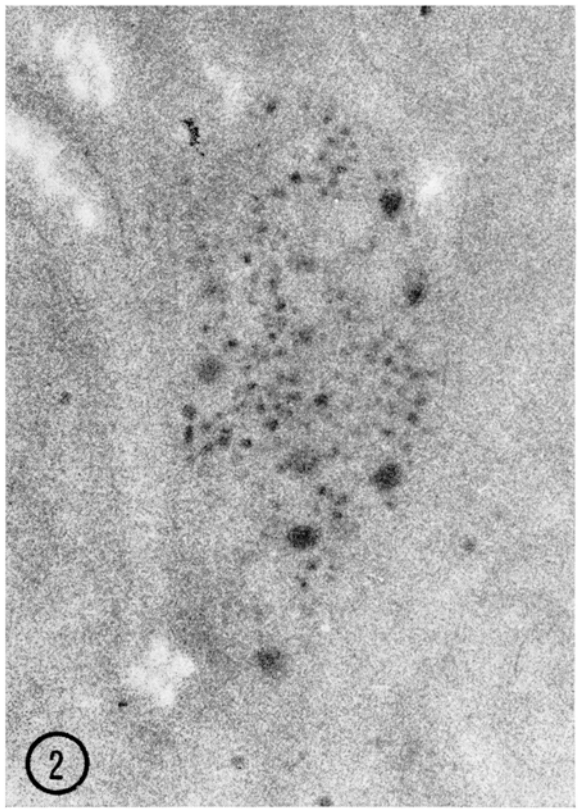

Fig. 2. Iris of cat, glutaraldehyde fixation, dichromate treated without OsO_4 fixation and without ultrathin section contrasting. In the poorly contrasted background a cluster of large and small electron denser centres are the only visible structures. This cluster represents an adrenergic nerve terminal. × 50,000.

way without any OsO_4 treatment together with glutaraldedyde-OsO_4 fixed tissues. The ultrathin sections were either left uncontrasted or contrasted with lead citrate alone or with uranyl acetate followed by lead citrate.

The adrenergic nerve terminals in both iris and vas deferens of non-treated cats fixed in glutaraldehyde and OsO_4 contained the different classical types of vesicles. Although the LDC vesicles represented only a small proportion of the total number of vesicles present in most nerve terminals, some nerve terminals were found where the LDC vesicles were relatively numerous (Figure 1). Their diameters varied between 700 and 1300 Å and their dense cores filled a large part of the vesicle. The contrast of the dense core varied from grey to black. After pretreatment with reserpine or MMT all small vesicles were empty, but no marked differences in either the number, size or intensity of contrast could be detected in the LDC vesicles, which is in line with the observations of others[5-7]. Consequently, the LDC vesicles contain an osmiophilic material which is resistant to reserpine and MMT and

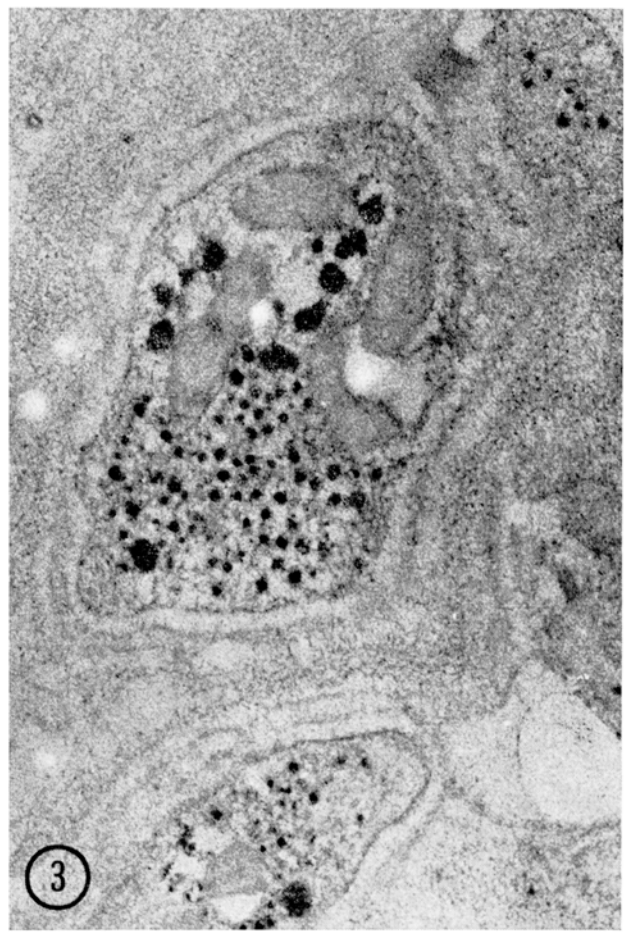

Fig. 3. Iris of cat, glutaraldehyde fixation, dichromate treated without OsO_4 fixation, lead citrate contrasted. The adrenergic nerve terminal is recognizable. The highly contrasted large and small dense centres represent the dense cores of large and small vesicles. × 50,000.

which is most probably not related to biogenous amines.

The ultrathin sections of iris and vas deferens from control animals, which were fixed in glutaraldehyde and potassium dichromate without OsO_4 treatment and which were not further contrasted by ionic stains gave very poor overall contrast. At small magnifications only the melanine rich pigment granules of various cells of the iris appeared strongly contrasted by the dichromate treatment. However, at higher magnifications numerous centres of electron density clustered together were found in the poorly contrasted zone adjacent to the pigment-rich myoepithelial cell layer (Figure 2). It is in this region of the iris that most of the adrenergic nerve terminals occur. In similar ultrathin sections with additional lead contrasting the general morphology became recognizable, and it was evident that these clusters of electron density represented the dense core vesicles of adrenergic nerve terminals (Figure 3). The vesicles could only be recognized by their dense cores since the membranes were poorly stained by this technique. It was apparent that at least 2 different types of electron dense centres were present. By their size and number they could easily be related to the dense cores of the small and the LDC vesicles (compare Figure 1 with Figure 2 and Figure 3). The results were superimposable in the vas deferens.

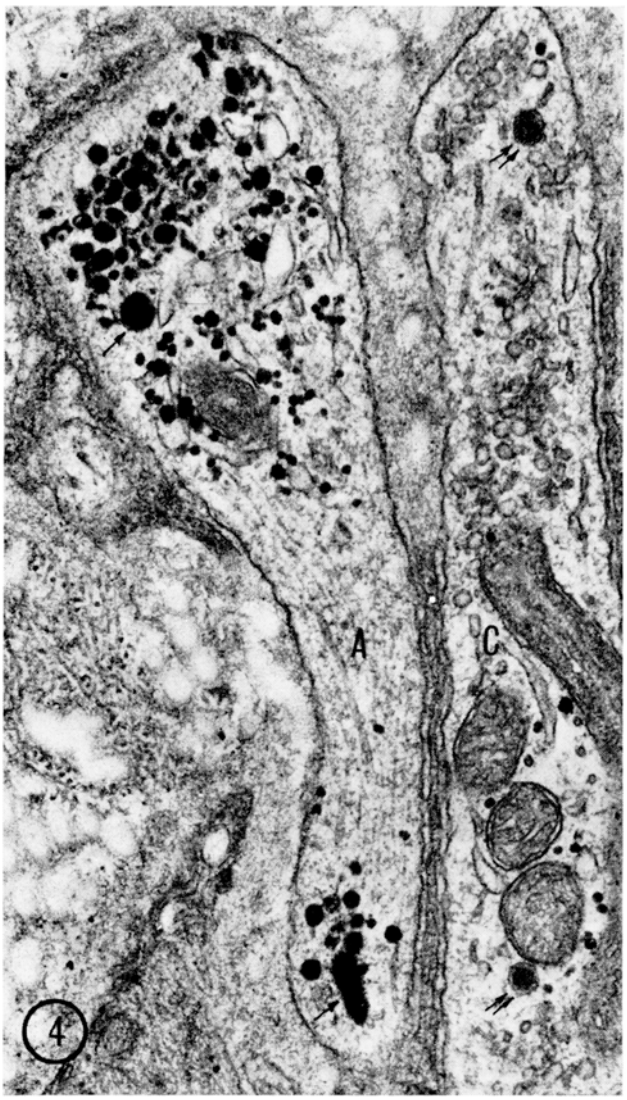

Fig. 4. Iris of cat after treatment with 5-HODA[3]. Glutaraldehyde-OsO_4 fixation, uranyl acetate and lead citrate contrasted. The small vesicles of the adrenergic nerve (A) contain strongly osmiophilic material, whereas the small vesicles of the cholinergic nerve (B) remain empty. The LDC vesicle (→) of the adrenergic nerve appear much stronger contrasted than the LDC vesicle (⇉) of the cholinergic nerve. × 50,000.

According to WOOD[10] the dichromate cytochemical technique is specific for NA, dopamine and 5-hydroxytryptamine. In our material it was confirmed that this technique is indeed highly specific for biogenous amines with the sole exception of melanine pigment granules of the iris. This was further demonstrated in the iris and vas deferens of cats treated with reserpine or MMT, where similar clusters of small and larger electron dense centres were no longer detectable. The melanine granules of the iris, however, did not change their contrast by any of the above treatments.

From these results we believe that at least some of the LDC vesicles of the adrenergic terminals do store biogenous amines in the normal condition. It remains to be elucidated whether it is NA or a related amine.

In glutaraldehyde-OsO_4 fixed tissues, cholinergic nerve terminals contain small empty vesicles as well as LDC vesicles. During these investigations we were not able to detect in glutaraldehyde-dichromate treated sections any large dense electron accumulations which were not accompanied by the smaller ones, which would indicate that the cholinergic LDC vesicles in contrast to the adrenergic LDC vesicles do not contain biogenous amines. These results are in agreement with, and extend earlier findings[2,3,8], where it was noted that after incubation of tissue slices in NA containing solutions or treatment of animals with 5-HODA, only the LDC vesicles of adrenergic nerve terminals increased their density, whereas the LDC vesicles of cholinergic nerve terminals remained unchanged (Figure 4). Consequently, the LDC vesicles of the autonomous nerve terminals contain at least 2 different classes of material both of which contribute to their osmiophily, but only one of them being a biogenous amine.

In summary, combined cytochemical and pharmacological investigations on the iris and vas deferens of cats have brought forth strong evidence that in adrenergic nerve terminals biogenous amines are not only stored in the small but also in the large dense core vesicles (type I of GRILLO and PALAY[1]). No amine could be detected in the corresponding vesicles of the cholinergic nerve terminals.

Résumé. La combinaison de techniques cytochimiques et pharmacologiques a permis de démontrer que les grandes vésicules à contenu dense des terminaisons nerveuses sympathiques postganglionnaires de l'iris et du canal de chat renferment une substance analogue ou identique à celle renfermée dans les petites vésicules à contenu dense, c'est-a-dire la noradrénaline. Les grandes vésicules à contenu dense des terminaisons nerveuses parasympathiques ne renferment pas cette amine biogène.

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