

have a dense 'cap' around all or part of their surface. In others, this densification includes progressively greater quantities of the droplet matrix producing as a terminal configuration a dense wrinkled particle. The dense wrinkled particles are found only in the gut lumen, particularly in the posterior regions where they are often the predominant component of the gut contents. Furthermore, there is morphological evidence for uptake by the epithelium of entire droplets, but only of those showing little or no evidence of degradation. A droplet in the apparent initial stage of engulfment can be seen in Figure 5.

The dense wrinkled material is entirely removed when thin sections are incubated for 1½ h in saturated picric acid in 95% alcohol. Incubation in alcohol alone has no effect. This solubility is characteristic of hematin which has been previously identified in the gut of *S. mansoni*⁵. It is postulated that the dense material is hematin and that the lipid-like material is some intermediate of hemoglobin degradation. Close examination of micrographs from the anterior gut lumen does, in fact, show an apparent merging of the hemoglobin matrix with that of the lipid-like material. It may be that 2 types of hemoglobin digestion exist – an extracellular type in which hematin is the end product and an intracellular type in which an intermediate is phagocytosed and broken down to simple end products. It may be speculated that extracellular digestive enzymes, perhaps produced by the oesophagus, initiate the digestion of hemoglobin. The digestive process may then be completed after uptake of the droplet by the epithelium.

Other than the apparent uptake of the lipid-like droplets and the existence of micropinocytotic caveolae there is no obvious sign of phagocytotic activity. The edges of the gut lamellae are often observed inserted into depressions in the matrix of the numerous pieces of hemoglobin found in the lumen. This would indicate that the gut

lamellae may perform a digestive function as well as possibly serving to increase the area of the absorptive surface.

The gut lamellae react strongly when tested for the presence of acid phosphatase activity (Figure 4). The reaction is confined to the inner core of the lamellae and acid phosphatases may be localized on the inner portion of the plasma membrane. A similar condition also exists on the tegument surface of *S. mansoni* where acid phosphatase activity is restricted to the inner side of the surface plasma membrane^{4,6}.

Résumé. L'épithélium de l'intestin de *Schistosoma mansoni* est un syncyte de structure pareille dans les 2 sexes. Des lamelles hérissent la surface et de nombreuses invaginations basales en fentes rayent l'épithélium de la paroi basale plasmique. On constate l'existence d'une digestion extracellulaire, ainsi que l'évidence morphologique d'une utilisation des gouttes lipides entières et des micropinosyncytes. Des procès cytoplasmiques du parenchyme s'attachent par des complexions jonctionnelles («junctional complexes») à la base de l'épithélium de l'intestin et mettent en connection les 2 systèmes. La localisation de l'activité de la phosphatase acide dans les lamelles de la paroi intestinale est précisée.

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Department of Zoology, Queen's University of Belfast (Northern Ireland), 4 October 1967.

⁵ D. W. HALTON, Parasitology 57, 639 (1967).

⁶ Acknowledgment. This research was carried out while the author was in receipt of a Wellcome Trust Overseas Studentship.

Ultrastructure of the Pineal Gland after Hypophysectomy

Experimental investigations show that the anterior pituitary controls the structure and function of the thyroid gland, adrenal cortex and gonads; the part played by the anterior pituitary in the control of the parathyroid or insular pancreas is a matter of dispute and its control of the pineal gland is as yet unknown.

Although it has been demonstrated that the pineal is an endocrine gland secreting polypeptide hormones (LERNER, CASE and TAKAHASHY¹; FARRELL and ISAAC²) very little is known about the factors controlling its secretory activity.

In a previous report (MILCU and LUPULESCU³) the presence of degenerative cellular and nuclear changes in the pineal gland of hypophysectomized rats observed by means of the light microscope were described.

The present paper deals with the ultrastructural changes of the pineal gland after hypophysectomy in rats.

Material and Methods. The experiments were carried out on adult male rats, which were divided in 2 groups. The first was formed of totally hypophysectomized adult male rats, weighing 260–280 g; the second one of control adult rats. The rats were sacrificed by ether anaesthesia at intervals of 7, 15, and 30 days after hypophysectomy and the pineal gland was removed. For study by the electron microscope, the pineal glands were collected immediately and fixed in PALADE's fixative⁴, then postfixed in an

aqueous uranyl-acetate solution, and embedded in Vestopal-W⁵, or in Epon-812⁶. The ultrathin sections were cut in the LKB-ultratome, and then stained with lead citrate⁷ and examined under a JEM-7C or Hitachi-11-electron microscope.

Results. Electron microscope observations showed clustered, oval, parenchymal cells (pinealocytes) in the pineal gland of the control rats. The fine structure of the epiphyseal cells was variable in appearance. The nuclei were oval or round and in the cytoplasm, as in other endocrine glands, the endoplasmic reticulum was made up of numerous pleomorphic vesicles; within the cytoplasmic matrix the ribosomes were free or disposed in rosettes or clusters. A higher magnification revealed 2 distinct types

¹ A. LERNER, I. CASE and Y. TAKAHASHY, J. biol. Chem. 235, 1992 (1960).

² G. FARRELL and W. McISAAC, Archs. Biochem. Biophys. 94, 543 (1961).

³ ST. MILCU and A. LUPULESCU, Commun. Neurol. Endocr. 7, 11 (1957).

⁴ G. PALADE, J. exp. Med. 95, 285 (1952).

⁵ A. RYTER and E. KELLENBERGER, J. ultrastruct. Res. 2, 200 (1958).

⁶ J. LUFT, J. biophys. biochem. Cytol. 9, 409 (1961).

⁷ E. REYNOLDS, J. Cell Biol. 17, 208 (1963).

of secretory granules; the first large granules of irregular shape contained an homogeneous material of low electron density, and the second, slightly smaller granules (Figure 1) of medium electron density and contained several grains in their centers. The second type of granules were oval and measured approximately $0.7\text{--}1.2\ \mu$ in diameter, and were delimited by a single, visible membrane. Numerous elongated, rod-like or oval mitochondria, grooved by cristae could also be seen. Among the parenchymal cells, sinusoidal capillaries and perivascular spaces could be observed; these capillaries were delimited by fenestrated endothelium (Figure 2).

Electron microscopic studies of the pineal glands of hypophysectomized rats revealed significant ultrastructural changes in the cytoplasm and nuclei of the pinealocytes, e.g. atrophy of the endoplasmic reticulum, smaller and collapsed ergastoplasmic vesicles, and a decrease of secretory material. Thus, both types of secretory granules appeared shrivelled and were reduced in number and size (Figure 3). The mitochondria were smaller, with a modified ultrastructural pattern. Golgi apparatus was seldom observed near the nuclei which have an irregular envelope

divided into several compartments by deep invaginations of the nuclear (Figure 4) membrane. The volume of nuclei and also of nucleoli were reduced. The perivascular spaces were evident, much enlarged with endothelial cells and the lumen contained red blood cells (Figure 5).

Discussion. From our electron microscope studies it is evident that after hypophysectomy, intense and progressive ultrastructural changes take place in the rat pineal gland, e.g. atrophy of the endoplasmic reticulum, depletion of secretory material from both types of granules, reduction in volume of the nuclei and also of nucleoli with many infoldings and indentations of nuclear membrane, and shrivelling of the mitochondria with stunted cristae pattern.

These fine structural changes of the pineal cells are similar to those observed by other authors in the thyroid

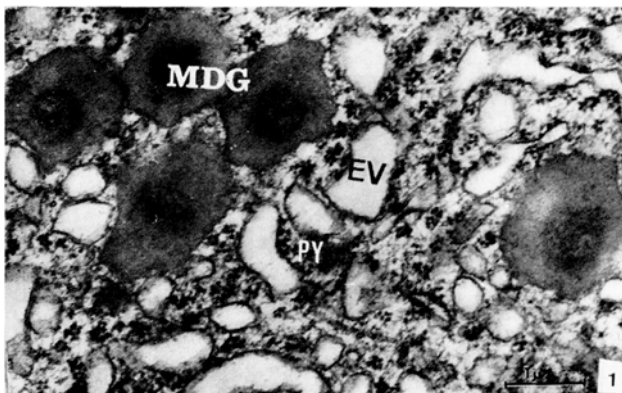


Fig. 1. Control rat pineal gland. A cytoplasmic zone of an epiphyseal cell, showing several granules of medium electron density (MDG) with an irregular outline; ergastoplasmic vesicles (EV), some containing a substance of the fine texture, many free ribosomes or polysomes (PY). Osmium tetroxide, Vestopla-W, lead citrate. $\times 22,000$.

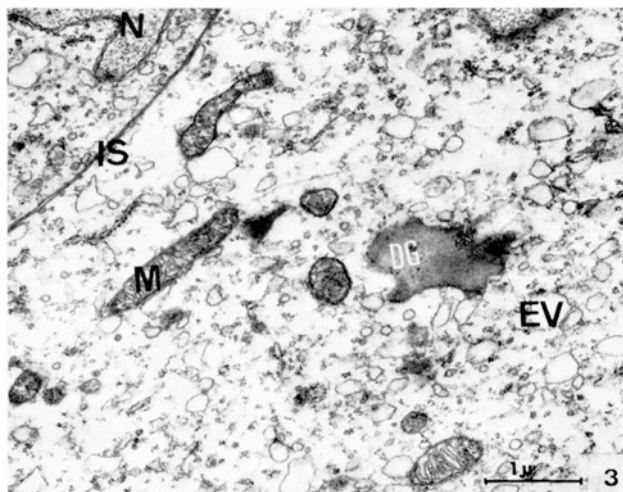


Fig. 3. Totally hypophysectomized rat pineal gland. A cytoplasmic zone of an epiphyseal cell showing atrophy of ergastoplasmic vesicles (EV), smaller mitochondria (M) and decrease of secretory granules (DG); IS, intercellular space and nucleus (N). Osmium tetroxide, Epon, lead citrate. $\times 22,000$.

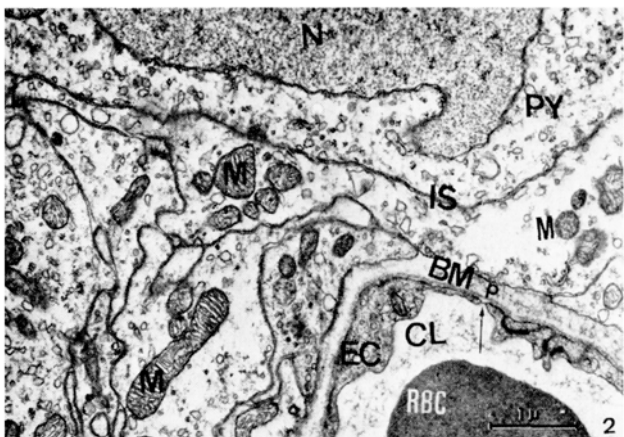


Fig. 2. Control rat pineal gland. Basal zone of an epiphyseal cell. N, nucleus; several mitochondria of various shapes (M); free ribosomes or polysomes (PY); basement membrane (BM), endothelial cell (EC), capillary lumen (CL), pores (p) and red blood cells (RBC). IS, intercellular space. Osmium tetroxide, Epon, lead citrate. $\times 22,000$.

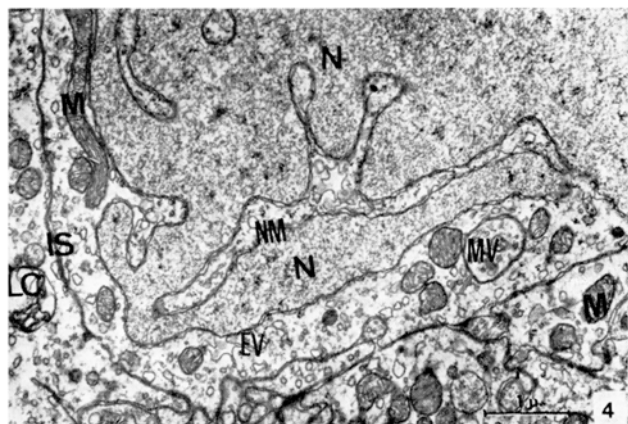


Fig. 4. Totally hypophysectomized rat pineal gland. Epiphyseal cells separated by evident intercellular space (IS). Nucleus (N) with many infoldings of nuclear membrane (NM) giving a cog-like pattern; several mitochondria (M) with blurred mitochondrial cristae; atrophic endoplasmic reticulum (EV); LC, lamellar concentric formations. Osmium tetroxide, Vestopla-W, lead citrate. $\times 22,000$.

gland after hypophysectomy (DEMPSEY and PETERSON⁸) 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; WOLFFE¹⁰; ARSTILA¹¹). 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 ROBERTIS¹²; 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.

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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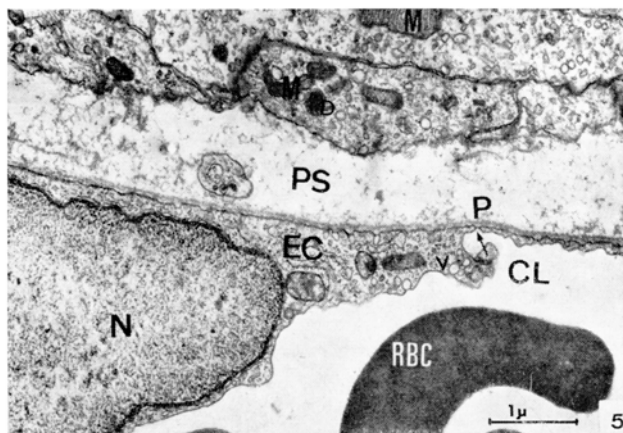


Fig. 5. Totally hypophysectomized rat pineal gland. Basal zone of an epiphyseal cell, showing an enlarged pericapillary space (PS), mitochondria (M), endothelial capillary membrane with many pores (P), endothelial cells (EC) with nucleus (N) and many small vesicles (V), red blood cell (RBC), D, small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

⁸ E. DEMPSEY and R. PETERSON, *Endocrinology* 56, 46 (1955).

⁹ C. CASSANO, A. TORSOLI, A. PERUZY and C. DE MARTINO, *Folia endocr.* 14, 755 (1961).

¹⁰ E. WOLFFE, in *Structure and Function of the Epiphysis Cerebri* (Eds. J. A. KAPPERS and P. SCHADE, *Progr. in Brain Res.*, Elsevier, Amsterdam 1965), vol. 10, p. 332.

¹¹ A. ARSTILA, *Neuroendocrinology* 2 (suppl.), 1 (1967).

¹² A. P. DE IRALDI and E. DE ROBERTIS, *Experientia* 17, 122 (1961).

¹³ C. DE MARTINO, G. TONIETTI and L. ACCINNI, *Experientia* 20, 556 (1964).

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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 PALAY¹). 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 PALAY¹).

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 solutions² or after treatment of animals with 5-hydroxydopamine (5-HODA)³, the dense cores of the large vesicles became more osmiophilic and often somewhat larger⁸. 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 OsO₄. The remainder of the tissue was treated overnight in a potassium dichromate solution at pH 4.1⁹, then dehydrated and embedded in the usual

¹ M. A. GRILLO and S. L. PALAY, 5th Int. Congr. Electron Microscopy (Academic Press, New York 1962), vol. 2, p. U-1.

² J. P. TRANZER and H. THOENEN, *Experientia* 23, 123 (1967).

³ J. P. TRANZER and H. THOENEN, *Experientia* 23, 743 (1967).

⁴ D. E. WOLFE, L. T. POTTER, K. C. RICHARDSON and J. AXELROD, *Science* 138, 440 (1962).

⁵ T. HÖKFELT, *Experientia* 22, 56 (1966).

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⁸ J. P. TRANZER and H. THOENEN, unpublished observations.

⁹ J. G. WOOD and R. J. BARNETT, *J. Histochem. Cytochem.* 12, 197 (1964).