

and, accordingly, the arcs can span the same length. The arcs are clearly part of the peripheral contour, and the granule is the central osmiophilic granule of polyhedral-globular units as seen in oblique view. When the intraperiod line is single and continuous, the myelin lamella is constituted by 2 rows of globules only. Finally, the intraperiod line can consist of a sequence of osmiophilic granules set at a distance of about 90–120 Å one from the other, each appearing to be, more or less clearly, the center of a polyhedral-globular unit seen in face-on view.

When the myelin lamellae show splits, they are usually found to occur at the level of a continuous intraperiod line, giving the impression that the bonds between the granules of the major dense line and the adjacent clear globules are firmer than the bonds between rows of clear globules.

Since the described findings agree with those obtained on frog<sup>2</sup>, rat and guinea-pig<sup>16</sup> brain cortex, they seem to justify the conclusion that membranes of amphibian and mammalian central and peripheral nervous system, fixed, dehydrated and embedded in various ways, consist essentially of osmiophobic globules and osmiophilic granules, and to suggest the hypothesis of the arrangement of these globules and granules into polyhedral-globular units. This hypothesis is attractive in that it furnishes an explanation of the presence of osmiophilic granules in the lamella of myelin on the basis of a particular orientation of polyhedral-globular units with respect to the section plane.

It is difficult to say what such electron microscopic appearance may mean in terms of real structure of the biological membrane. When very high magnifications are employed, one must take into consideration how much the embedding process may contribute to the structural organization of the cellular constituents, as well as the effects of fixation and dehydration on the tissue as sources of artifacts, even if, as in our case, one is dealing with results consistently obtained with 3 different em-

bedding media. With this problem in mind, we began to study the effects upon myelin ultrastructure of different chemical and physical treatments applied before fixation. In short segments (about 2 mm) of isolated frog sciatic nerves kept immersed in 0.1% trypsin in Ringer for 16 h at room temperature, the myelin showed areas of structural disorganization in which the lamellae appeared disrupted and substituted by agglomerates of osmiophobic globules irregularly interspersed with osmiophilic granules (Figure 6). These preliminary results seem to indicate that, in the experimental conditions to which the frog nerve fibers were subjected, not only the disruption of myelin lamellae but also the scission of the hypothesized polyhedral-globular units into subunits were obtained<sup>16</sup>.

**Zusammenfassung.** Das Myelin peripherer Nerven von Amphibien und Säugetieren scheint, mit Hochleistungselektronenmikroskop untersucht, aus einer regelmässigen Anordnung von osmiophilen Granula und osmiophoben Globula zu bestehen. Solche Untereinheiten scheinen häufig polyedrische Gebilde zu formen, in welchen zentrale Granula jeweils von 6 osmiophoben Globula umgeben sind. Es ist beim Froschischiadicus möglich, die Struktur des Myelins zu zerstören und die Granula von den Globula durch Behandlung mit Trypsin zu trennen.

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<sup>15</sup> Unpublished results.

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### Role of the Nuclear Membrane in Smooth Endoplasmic Reticulum Formation in White Rat Pinealocytes

Although both granular and agranular endoplasmic reticulum have been described in rat pinealocytes<sup>1</sup>, the agranular one is much more abundant<sup>1</sup>. The presence of a well-developed agranular reticulum is also a characteristic of pinealocytes in other species<sup>2</sup>. This structure is present mainly in the form of vesicles, the amount of which may vary in different physiological or experimental situations<sup>3</sup>. The present work was undertaken in order to obtain further information on the origin and nature of this cytoplasmic component.

**Materials and methods.** Adult white rats of both sexes of about 200 g were used. Their pineal glands were dissected under ether anesthesia and immediately fixed in Palade's fixative<sup>4</sup> (1% osmium tetroxide in a 7.4 veronalacetate buffer at 0°C) and embedded in buthyl-metacrylate. Thin sections were cut with a Porter-Bloom microtome, and examined with a Philips EM 100 electron microscope.

**Results.** A well-developed smooth endoplasmic reticulum was observed in pinealocyte cytoplasm. This was mainly formed by characteristic cisternae and small vesicles with a diameter varying between 250 and 1500 Å (Figure 1).

Nuclear membrane showed the typical double-layered constitution; its pores did not appear to be a simple membrane discontinuity since they appeared closed by a thin diaphragm to which a mass of electron dense material had joined (Figure 2). Some ribosomes were observed in close contact with the external surface of the outer layer (Figure 1). Blebs of this outer layer were frequently seen; they varied in size from one zone to another (Figures 1 and 3). At the site where blebs

<sup>1</sup> A. MILOFSKY, Anat. Rec. 127, 435 (1957).

<sup>2</sup> E. ANDERSON, J. Ultrastruct. Res., Suppl. 8, 1 (1965).

<sup>3</sup> V. SAHLEAUN and R. HOLBAN, Studii Cerc. Endocr. 13, Suppl. 5, 617 (1962).

<sup>4</sup> G. E. PALADE, J. exp. Med. 95, 285 (1952).

appeared, few or no adherent ribosomes were present. Many smooth membrane vesicles of different sizes (Figures 2 and 3) were often observed in close relation to the blebs. Typical multivesicular bodies were occasion-

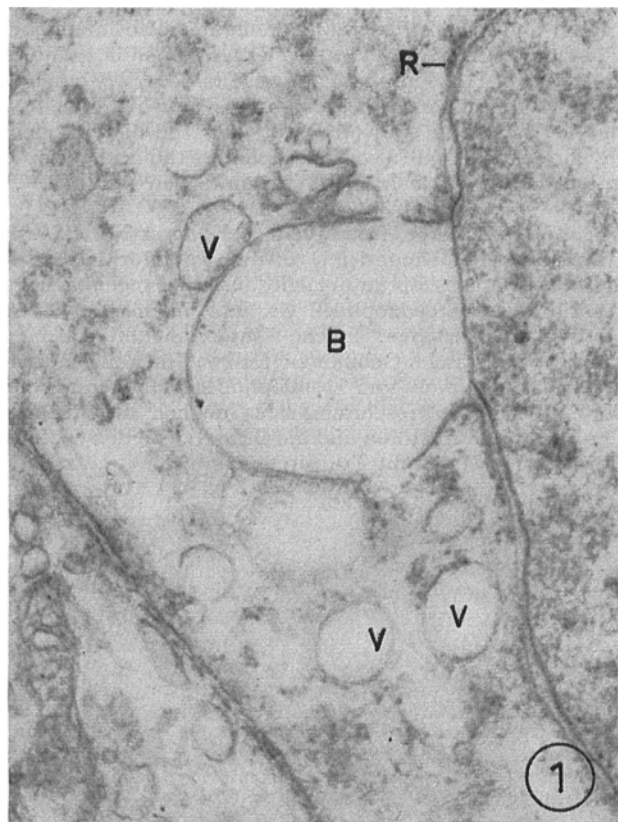


Fig. 1. Small vesicles of the endoplasmic reticulum (V), and a bleb (B) of the outer nuclear membrane. The nuclear envelope presents ribosomes adhered to the external surface (R).  $\times 32,000$ .

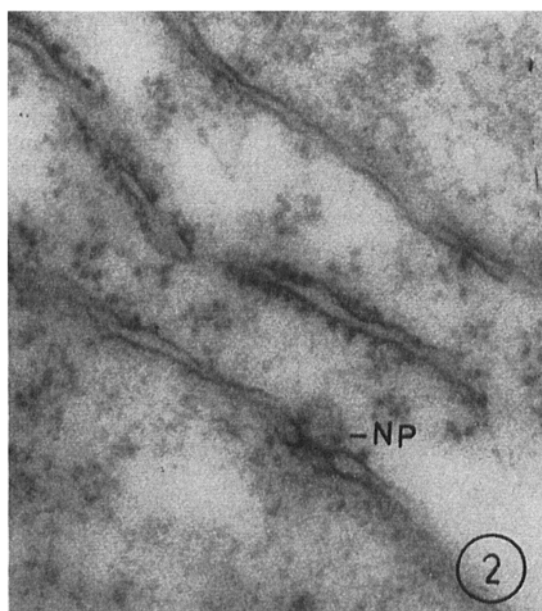


Fig. 2. Nuclear pore (NP) showing a thin diaphragm and adherent dense material.  $\times 79,000$ .

ally seen in the vicinity of the nuclear membrane. Granular endoplasmic reticulum was also present but it was very scarce.

**Discussion.** Although no definitive conclusion has yet been reached in relation to pineal gland function, a great amount of evidence tends to indicate that it may have a secretory activity in relation to sexual functions. The presence in pinealocytes of a fair amount of agranular endoplasmic reticulum together with the results obtained by SAHLEAUN and HOLBAN<sup>3</sup> who observed variations in the number of agranular vesicles after castration and after sex hormones administration suggests that this cytoplasmic structure may be related with secretory function of pinealocytes.

The participation of nuclear membrane in the formation of endoplasmic reticulum has been suggested by several authors<sup>2,5-9</sup>. Blebbing of the outer layer of the nuclear membrane has been described in other cell types by several authors<sup>10,5-9</sup>. It has also been described in the bovine pineal cells by ANDERSON<sup>2</sup>. The presence of varying amounts of agranular vesicles in close relation to nuclear blebs observed by us tends to add support to the view that agranular reticulum is formed at the nuclear membrane.

The presence of 'diaphragms' obturating nuclear membrane pores has been described before<sup>2</sup>. Its meaning remains as yet obscure.

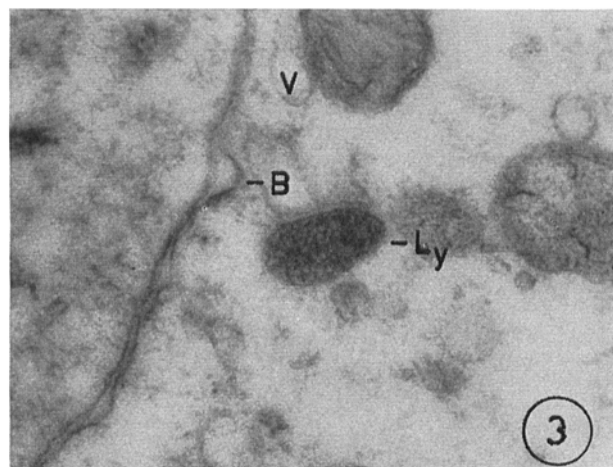


Fig. 3. Scattered vesicles (V) and a lysosome-like particle (Ly).  $\times 52,500$ .

**Résumé.** La glande pinéale du rat blanc est étudiée ici au microscope électronique. L'association observée entre les vésicules agranulaires et les «blebs» de la membrane nucléaire est un argument en faveur de la théorie selon laquelle l'origine du reticulum endoplasmique est à rechercher dans la membrane nucléaire.

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<sup>5</sup> E. ESSNER and A. B. NOVIKOFF, *J. biophys. biochem. Cytol.* 15, 289 (1962).

<sup>6</sup> R. G. KESSEL, *J. biophys. biochem. Cytol.* 19, 391 (1963).

<sup>7</sup> S. W. HSU, *Z. Zellforsch. mikrosk. Anat.* 58, 17 (1962).

<sup>8</sup> R. HADEK and H. SWIFT, *J. biophys. biochem. Cytol.* 13, 445 (1962).

<sup>9</sup> W. H. CLARK, *J. biophys. biochem. Cytol.* 7, 345 (1960).

<sup>10</sup> E. ANDERSON and E. CHOMYN, *Anat. Rec.* 148, 254 (1964).