tioned[2,3]. To this should be added that EKSTRÖM and LENNINGER[8] have shown that there is choline acetyltransferase in the ductal tissues. The cholinergic nerves probably induce contraction of the muscle layer. The perfusion studies indicated that nerve-induced luminal narrowing may impede flow but it is not known whether this occurs under physiological conditions. It is thought unlikely that under normal conditions a big back pressure against secretion would be induced by the muscle contractions, but this may occur under pathological influences. The function of the muscle layer is obscure. In vitro the muscle exhibits rhythmical activity which was considered to be myogenic[3]. If such activity occurs in vivo it may have a milking effect on the contents. But what of nerve induced contractions? These possibly arise in response to reflex stimuli and the effect may be to impart a greater rigidity to the duct. Furthermore a strong contraction may help to hinder a sudden rise of pressure in the main duct from passing out to the parenchyma, for it seems likely that the smaller branch ducts will be occluded to some extent by a strong contraction of the muscle layer.

Zusammenfassung. In der Wandung des *Ductus pancreaticus major* der Katze wird eine dünne Schicht von Muskelfaserzügen beschrieben. Histochemisch wird festgestellt, dass es sich bei fehlender adrenergischer um cholinerge Innervation dieser Muskelzellen handelt.

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Zinc Iodide-Osmium Tetroxide Reactive Substances in the Matrix of Granulated Vesicles in the Rat Pineal Nerves

In different works[1-3] it has been demonstrated that the mixture of zinc iodide-osmium tetroxide (ZIO) stains different types of synaptic vesicles. In the granulated vesicles of the pineal nerves, this technique reveals two components[4]: the central core and the matrix, which comprises the space between the vesicle membrane and the core. The matrix is more intensively stained than the core.

Studies made in our laboratory[5] demonstrated that ZIO reaction is temperature and time dependent. An increasing number of synaptic vesicles and other subcellular components appear positive when the temperature and time are increased. Granulated vesicles of different monoaminergic nerves do not react in the same way at a given schedule of fixation. Thus, when ZIO is used at 4 °C for 2 h (ZIO 4–2), the matrix of the granulated vesicles of the pineal nerves appears electron dense, whereas that of the granulated vesicles of the vas deferens nerves remains electron lucent[2]. If the fixation is done at 20 °C for 15 h (ZIO 20–15), the matrix of the granulated vesicles of the vas deferens nerves is also deeply stained[6]. The granulated vesicles of the pineal nerves have the same appearance with ZIO 20–15 as with ZIO 4–2. However the findings related to the vas deferens nerves suggested us that ZIO 4–2 and ZIO 20–15 could reveal different components of the matrix of granulated vesicles of the pineal nerves.

This posibility was explored by fixing with ZIO 4–2 and ZIO 20–15 pineal glands of rats treated with reserpine or *p*-chlorophenylalanine (*p*-CPA). Reserpine was administered i.p., at the dose of 10 mg/kg and the animals killed 4 h after the injection. Rats treated with *p*-CPA were given 2 × 300 mg/kg i.p. separated by a 48-h interval. They were killed 24 h after the last injection. Control rats received equivalent volumes of saline in both treatments.

ZIO 4–2 (Figure 1) reaction in the control pineal nerves of the rat has already been described[2]. Most of the vesicles show a heavily stained matrix. In a small proportion of vesicles the matrix is unstained and in them a dense core can be distinguished. With ZIO 20–15 (Figure 2) the appearance of synaptic vesicles is similar to that obtained after ZIO 4–2, but there is a greater reaction in the mitochondria and the cytoplasm.

In the glands treated with reserpine, the matrix and the core of small granulated vesicles become negative with ZIO 4–2, as shown previously[2]. With ZIO 20–15 (Figure 4) the matrix of a small number of vesicles remains but most of the vesicles are depleted. The membrane of the vesicles unstained is barely visible. As previously described[7] *p*-CPA depletes ZIO 4–2 reactive substances in the matrix and partially in the core (Figure 5). On the contrary, substances reacting with ZIO 20–15 (Figure 6) are not depleted by *p*-CPA and the aspect of nerve terminals is similar to that of the controls.

The finding here described confirms that *p*-CPA depletes ZIO 4–2 reactive material in the matrix of the granulated vesicles of the pineal gland of the rat and demonstrates that this drug does not affect ZIO 20–15 reactive material. On the contrary reserpine depletes both of them.

It is interesting that, while reserpine depletes catechol and indoleamines, *p*-CPA specifically inhibits the synthesis of serotonin in the pineal nerves[8,9]. ZIO 4–2 reactive material is also depleted by tyramine and oxypertine. It has been demonstrated with a histochemical approach that tyramine also depletes serotonin in the pineal

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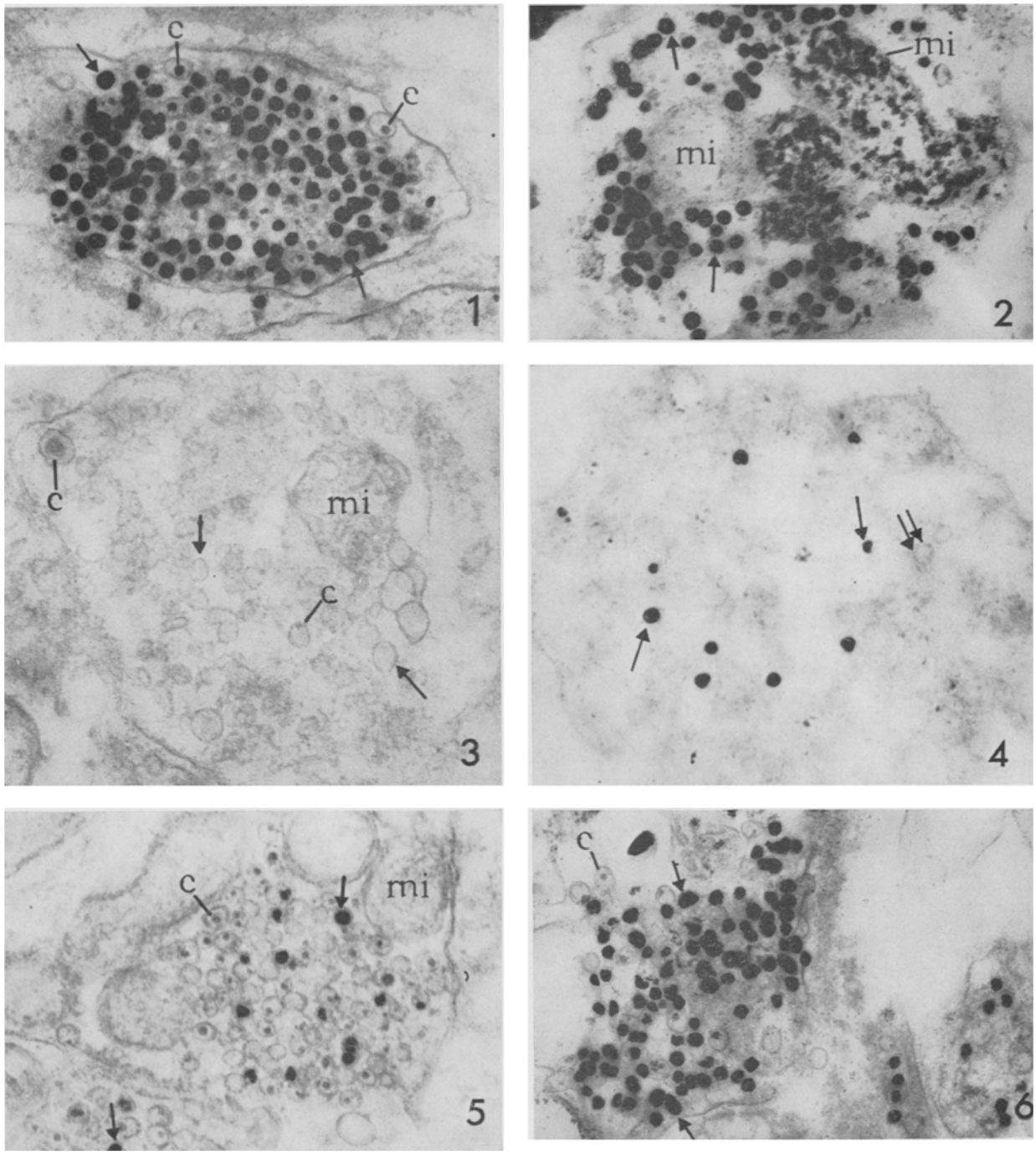

Fig. 1 and 2. Nerve endings from normal pineal glands. 1. Gland fixed with zinc iodide-osmium tetroxide at 4 °C for 2 h (ZIO 4–2), ×60,000. 2. Gland fixed with zinc iodide-osmium tetroxide at 20 °C for 15 h (ZIO 20–15). ×60,000. In both cases the matrix is deeply stained in most of the small vesicles (↑). A paler core can be distinguished (c) in the vesicles with unstained matrix.

Fig. 3 and 4. Nerve endings from pineal glands treated with reserpine. 3. Gland fixed with ZIO 4–2. The matrix reaction has disappeared in the small vesicles (↑). A core can be distinguished in a few vesicles (c). ×60,000. 4. Gland fixed with ZIO 20–15. A matrix reaction is evident in a small proportion of vesicles (↑). Most of the vesicles are emptied and their membrane is barely visible (↑↑). ×60,000.

Fig. 5 and 6. Nerve endings from pineal glands treated with *p*-chlorophenylalanine. 5. Gland fixed with ZIO 4–2. The matrix reaction remains only in a few of the vesicles (↑). The cores reduced in size and electron density (c) are visible in most of them being the matrix unstained. ×60,000. 6. Gland fixed with ZIO 20–15. The matrix is deeply stained in most of the vesicles (↑). The visible cores are similar to those of the Figure 5. mi, mitochondria.

nerves[10]. The action of oxypertine on serotonin stores is accepted by some authors[11]. A previous work of our laboratory[10] indicates that ZIO 4–2 reactive material does not correspond to noradrenaline in the pineal nerves of the rat.

With these considerations in mind, the hypothesis may be formulated that in the matrix of the granulated vesicles of the pineal nerves of the rat, ZIO may reveal different substances according to the schedule used and that they are related in some way with the different transmitter substances stored in these vesicles. The action of *p*-CPA here described would indicate that ZIO 4–2 reactive material is related with serotonin. The nature of this material is uncertain at the present time. The presence of serotonin demonstrated in the core with the histochemical technique of WOOD[2] could not be revealed in the matrix. If this does not preclude the presence of serotonin in the matrix, it strongly suggests that ZIO 4–2 reactive material correspond to some other component of the vesicle also affected by *p*-CPA. This possibility has been discussed elsewhere[7].

Resumen. Se demuestra que la reserpina depleciona los componentes de las vesículas sinápticas de los nervios pineales de la rata revelables por la mezcla de yoduro de zinc-tetróxido de osmio a 4 °C durante dos horas y a 20 °C durante 15 h mientras que la *p*-CPA sólo depleciona los componentes revelables a 4 °C durante 2 h.

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[14] We are grateful to Miss M. LÓPEZ for her skillful technical assistance.

Marquage intense de la couche des «wedge granules» dans les oocytes en prématuration de la Caille japonaise (*Coturnix coturnix japonica*) après injection de L-leucine-^{3}H

Dans la cicatricule d'oocytes de la pigeonne en période finale de grand accroissement, BARTELMEZ[1] a décrit la présence d'une couche cunéiforme («wedge granules layer»). VAN DURME[2] a retrouvé cette couche dans les oocytes de plusieurs espèces d'oiseaux. Elle est constituée de granules vitellins plus intensément colorables que les granules vitellins environnants du disque germinatif. A son bord interne (vers la vésicule germinative) la couche cunéiforme déverse dans un lit de granules extrêmement fins également très colorables («polar granules» de BARTELMEZ[1]) qui se glissent en dessous du pôle profond de la vésicule germinative.

Matériel et méthode. Des cailles japonaises en période de ponte régulière, maintenues à une température constante de 22 °C avec illumination continue, ont été utilisées. Les animaux ont été soumis à une injection par voie i.p. de 1 mCi de L-leucine-4, 5-^{3}H (1 mCi/ml, 58 Ci/m*M*, Amersham Radiochemical Centre); 3 jours après cette injection, les cailles ont été tuées par décapitation. Après ouverture de l'abdomen, les plus grands oocytes intrafolliculaires pédonculés sont prélevés et fixés dans l'alcool acétique (3:1 vol) pendant 4 h. La cicatricule est marquée au charbon de bois, car, après fixation, l'emplacement de la vésicule germinative devient presque invisible. Pendant leur passage dans les alcools le disque germinatif de ces oocytes est séparé de la plus grande partie du jaune sous-jacent. Après enrobage à la paraffine la cicatricule des oocytes est débitée en coupes sériées de 7 µm d'épaisseur, paralèles à l'axe qui relie le pôle nucléaire à l'antipôle. Les coupes sont ensuite émulsionnées par l'émulsion nucléaire L 4 de Ilford (England). Après une exposition de 1 mois et le développement de l'émulsion, les coupes sont colorées à l'hématoxyline ferrique de Groat et contrecolorées à l'éosine. Des coupes d'oocytes de cailles n'ayant reçu aucune injection de précurseur radioactif ont subi un traitement autoradiographique identique (coupes témoin).

Résultats. Sur les autoradiographies des plus grands oocytes on constate un marquage intense au niveau de la couche des «wedge granules. L'incorporation est si intense qu'elle est facilement visible au microscope avec les objectifs les plus faibles (Figure 1). On voit nettement que le marquage se prolonge jusque dans la profondeur de la masse vitelline sous-jacente. Les bords de la couche marquée sont recourbés vers la colonne vitelline reliant le latebra de PURKINJE[3] au noyau de PANDER[4].

Cette dernière structure, entièrement circonscrite par la couche fortement marquée, se présente ainsi exactement comme la coiffe d'un champignon. A l'encontre de sa couche envelopante, intensément marquée, le noyau de Pander présente peu ou pas d'incorporation (Figure 2).

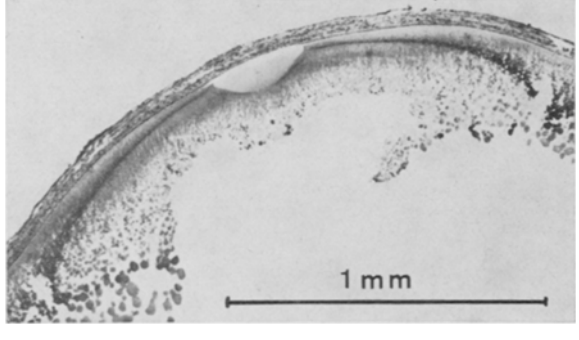

Fig. 1. Autoradiographie d'une coupe d'oocyte (diamètre 14 mm) de caille, 3 jours après une injection i.p. de L-leucine-^{3}H. Malgré le grossissement très faible la couche fortement marquée est facilement visible.

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