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Short Communication

On the Presence of Conspicuous Electron Dense Bodies in the Pinealocytes of the Pig*

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Summary. The pinealocytes of the pig contain a large number of aldehyde-fuchsin positive granules. In order to determine their nature an ultrastructural study was carried out. Numerous bodies having a maximal diameter of about 1,600 nm were found. These elements showed a great variety of internal structure, ranging from a "homogeneous" content and amorphous dense aggregates to lamellate bodies. Although only a few of them displayed a positive reaction for acid phosphatase, their morphological appearance strongly suggests that they belong to the lysosomal system.

Key words: Pineal gland – Pig – Lysosomes.

Granules showing diverse morphological features have been described in the pinealocytes of various animals by many authors. Moreover, the pineal of the pig is unique in displaying cells with abundant aldehyde-fuchsin positive granules which have the appearance of secretory material (Owman, 1964). This fact encouraged us to search in this gland for ultrastructural features clarifying the nature of these stainable structures.

Materials and Methods

Six normal adult pigs of both sexes were obtained from a slaughterhouse. For ultrastructural studies, the material was fixed for 3 h in a phosphate buffered p-formaldehyde-glutaraldehyde solution (Karnovsky,

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1965), postfixed for 1 h in 1 % phosphate buffered osmium tetroxide and embedded in Araldite. Thin sections were stained with uranyl acetate and lead citrate.

For enzymatic studies, the tissues were fixed for 1 h in 2 % glutaraldehyde in 0.1 M cacodylate buffer pH 7.4 with 34 g/l sucrose. Detection of acid phosphatase (AcPhase) activity was performed following Meda (1978), according to the methods of Gomori (1956) and Barka and Anderson (1962). Incubations were done at 37° C for 2 h. Control batches were incubated in a substrate-free medium. After incubation, the material was washed in the corresponding buffer and postfixed for 1 h in 1 % osmium tetroxide in 0.1 M cacodylate buffer pH 7.4. The tissues were then processed for electron microscopy as described above. For light microscopy the pineals were fixed in Bouin's fluid, embedded in paraffin and stained with paraldehyde-fuchsin, according to Gabe (1953).

Results and Discussion

Most of the pinealocytes contain numerous dense bodies of different sizes, having a maximal diameter of about 1,600 nm, scattered within the cytoplasm (Fig. 1 a). These inclusions possess a wide variety of internal structure, ranging from a "homogeneous" content and amorphous dense aggregates to lamellated bodies. All of them are limited by a single unit membrane.

The study of AcPhase activity in the pinealocytes revealed that most of these dense bodies do not contain reactive material. However, this technique permitted to distinguish some organelles which can be interpreted as lysosomes. These contain round dense inclusions with fine granular material that clearly reacts with the AcPhase method (Fig. 1 b). Some of them include only dense reaction product, but others contain also unreactive substance and an AcPhase positive peripheral zone (Fig. 1 c). Control specimens did not show any deposit revealing AcPhase activity. Aldehyde-fuchsin showed granules similar to those already described by Owman (1964) in the pig.

In the pinealocytes of the monkey, similar inclusions as those described in the present paper, although very scarce in number, were assumed to be of secretory nature by Wartenberg (1968). Ariëns Kappers (1969), discussing the observations of Wartenberg, suggested that additional evidence should be gathered to rule out that such inclusions are lysosomes. For this reason we investigated the presence of AcPhase in these structures. It is well known that this enzyme universally characterizes the lysosomes (de Duve and Wattiaux, 1966).

In the present material, the AcPhase method revealed structures that may be interpreted as primary lysosomes. Probable secondary lysosomes were also found, i. e., membrane-bounded bodies containing both AcPhase and unreactive material in various stages of degradation. Granulolysis or crinophagy occurs in both normal (Meda, 1978) and stimulated endocrine cells, and seems to eliminate an oversupply of secretory material (Smith and Farquhar, 1966). The present observations demonstrate that granulolysis occurs also in normal pig pinealocytes.

Although in our material AcPhase activity could be demonstrated only in a small number of bodies, there is a close morphological resemblance among the whole population of these inclusions, suggesting that all of them belong to the lysosomal system. Furthermore, three main steps in the lysosomal cycle are presently accepted, not all of them characterized by the presence of AcPhase activity (De Duve and Wattiaux, 1966; Meda, 1978). The question remains why the pinealocytes of the pig show such a large number of lysosomes, as compared with other species studied thus far.

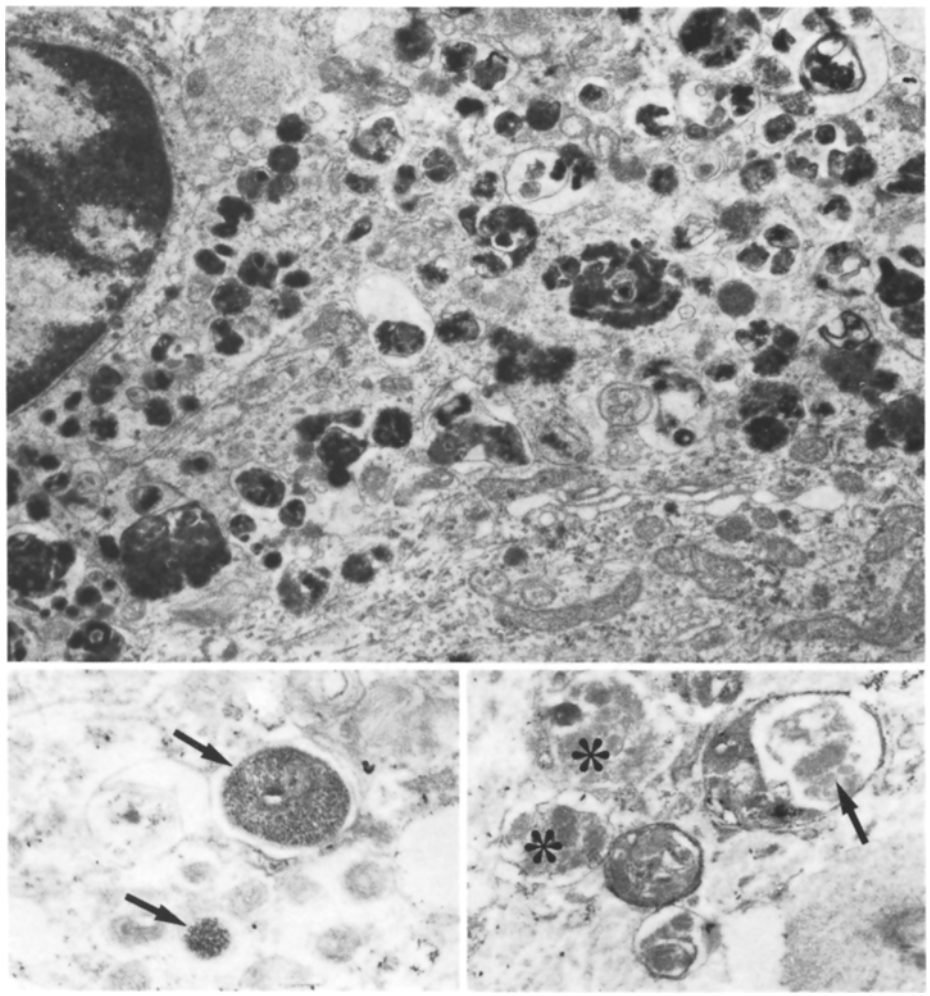

Fig. 1. a Pig pinealocyte displaying numerous dense, heterogeneous inclusions within cytoplasm. ×10,000. **b** AcPhase technique. Rounded bodies (*arrows*) with positive fine granular material, in cytoplasm of pinealocyte. ×20,000. **c** AcPhase technique. Cytoplasmic inclusions in pinealocyte containing unreactive substance (*arrow*). Reaction product restricted to bounding membrane. Note nonreactive heterogeneous dense inclusions (*asterisks*). ×20,000

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