Occurrence of Adrenaline and Noradrenaline Cells in the Adrenal Gland of the Gentoo Penguin (*Pygoscelis papua*)

The earlier work of HILLARP and HÖKFELT[1] supported the idea that in the adrenal medulla there are 2 separate strains of adrenochromafin cells: adrenaline and noradrenaline storing cells. Numerous studies in which different techniques were used[1-5] showed the occurrence of these cell types in the adrenal glands of various species. In relation to birds, this was first reported by ERANKÖ[6]. In some species the cellular strains were shown to be anatomically separate[7].

The problem of the distribution of these types of cells in relation to the cortical tissue still remains open. Therefore, in order to establish the cellular patterns of the glands in their corresponding taxonomic hierarchy, it is interesting to know the nature and distribution of the adrenaline and noradrenaline containing cells in adrenal glands of highly different species. With this idea in mind, a study on the nature of the chromaffin cells of the adrenal gland of the Gentoo penguin (*Pygoscelis papua*) was performed during the author's mission to the Antarctic Penninsula. The animals were caught in their natural habitat, brought to the station and the glands were removed under nembutal anesthesia. The adrenal glands in this specie are situated between the gonads and the renal veins. They are included in the connective tissue of the ovaric stroma in the female and separated from the gonads in the male. As in other birds the glands show columns of 'cortical' cells and islets of chromaffin tissue, both surrounded by an endothelial lining. The chromaffin islets are irregular in shape and size and are sometimes represented by isolated chromaffin cells inserted between the 'cortical' cells.

In order to elucidate the amine content of the cells, glutaraldehyde-silver reaction technique[5,8] was applied. With this technique the noradrenaline storing cells become glutaraldehyde silver positive and the adrenaline storing cells glutaraldehyde silver negative. When this technique was applied to this specie, most cells were shown to belong to the noradrenaline storing type.

With light microscopy the noradrenaline cells appeared dark brown, having regular silver-reduced precipitate, and with a negative image of the nucleus. The adrenaline storing cells were identified by the refringent contour of the islets (Figure 1). The autonomic nerves next to the gland showed the presence of some silver positive cells (Figure 2). These cells can be clearly distinguished from the isolated sympatethic neurons lying along the nerve, which do not react with the silver ammoniacal solution.

Electron-microscopy studies with material fixed in glutaraldehyde and post-fixed in osmium textroxide, both in Millonig buffer[9], allowed the distinction of 2 types of cells. The first type is more abundant and has granules of

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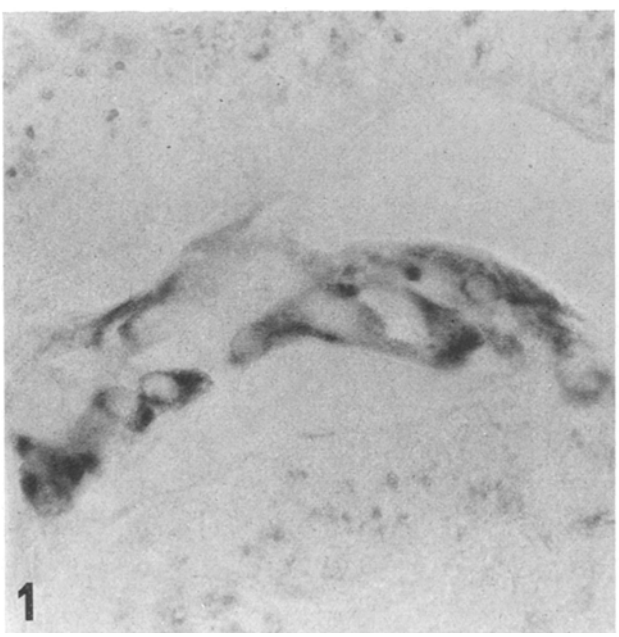

Fig. 1. Chromaffin islet treated with glutaraldehyde-silver reaction. Noradrenaline storing cells appear in dark. Some negative cells may be seen in the center of the islet. × 640.

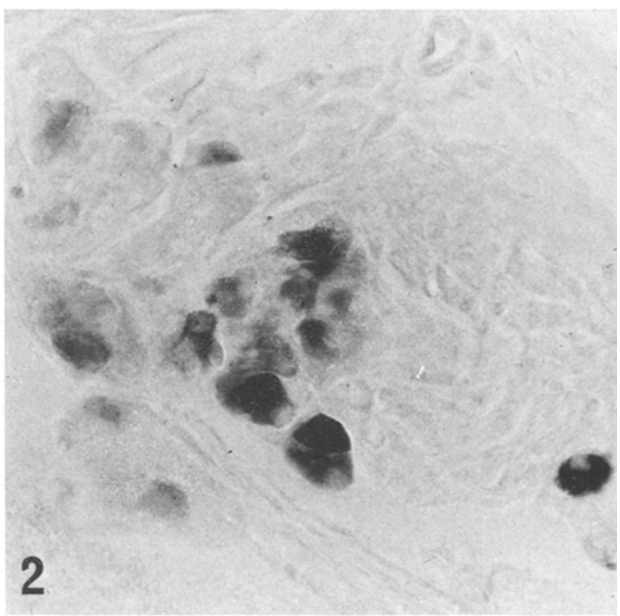

Fig. 2. An autonomous nerve showing small silver positive cells. × 640.

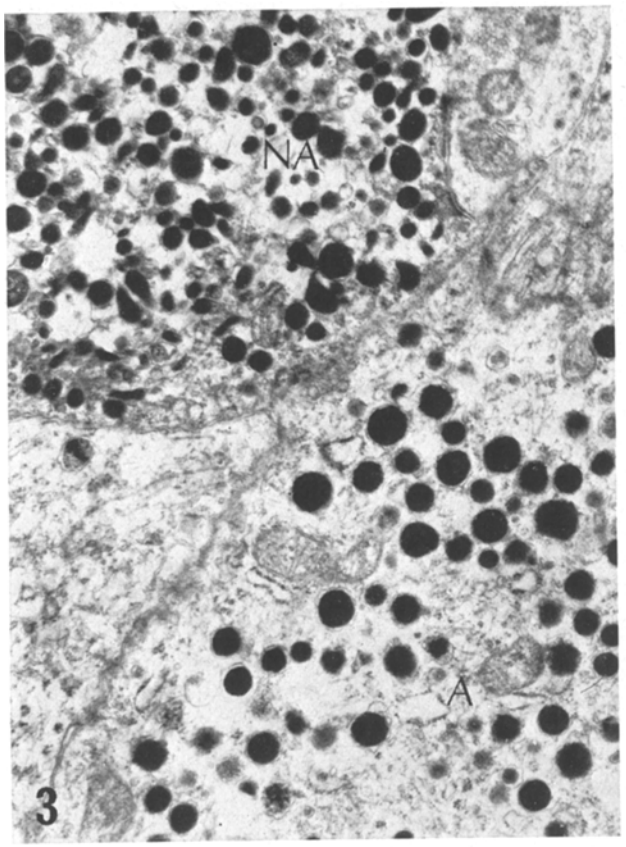

Fig. 3. Electron micrograph showing the granular morphology of the noradrenaline storing type (NA), above, and the adrenaline storing type (A), below. × 15,000.

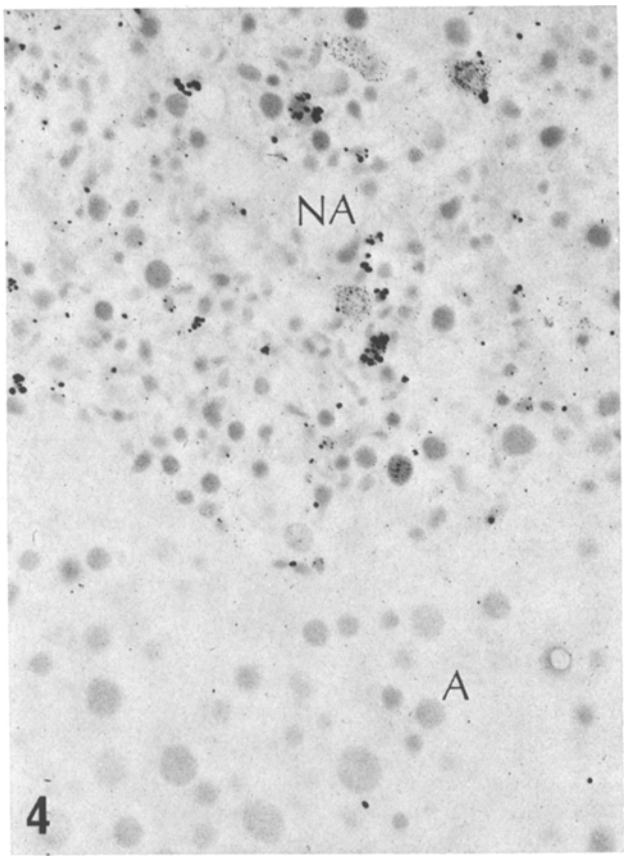

Fig. 4. Electron micrograph of a similar field of the preceding figure showing abundant silver precipitate in the granules of the noradrenaline storing type (NA). × 15,000.

very irregular size, ranging from 800 to 5000 Å. They also have high electron density. The membrane is closely attached to the granular material and the granules are of very different forms: spherical, rods and comma shaped, with predominance of the 2 latter types (Figure 3 above). With the glutaraldehyde silver technique these cells showed reduced silver particles inside the granular material (Figure 4, above). The morphological and histochemical picture of these cells agrees with previous descriptions of the noradrenaline storing type[3–5,7]. The second type of cells was characterized by the presence of regular, spherical granules smaller than those of the preceding type (Figure 3, below). The vesicular membranes were generally separated from the granules by a lumen wider than 100 Å. The granules were homogenous but some of them showed a dense central part and a particulate peripheral part (Figure 5). These cells do not show any positive glutaraldehyde-silver reaction (Figure 4, below) and their characteristics agree with those of adrenaline storing cells[3–5,7]. Although almost no granules of an adrenaline storing cell reacted when silver ammoniacal solutions were applied, in some cells we could find isolated granules which precipitate silver particles. It has been suggested that this positive reaction could be due to the presence of adrenaline precursors in these places[8].

The relative content of adrenaline and noradrenaline is highly variable in different species. Adrenaline predomi-

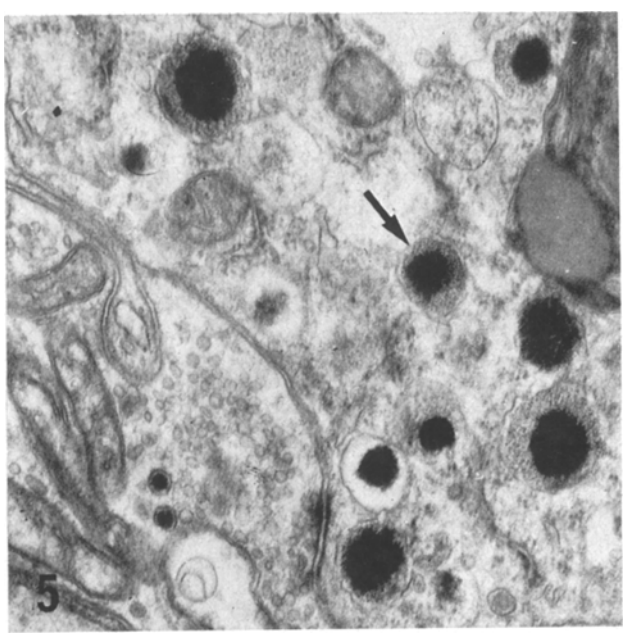

Fig. 5. Granules of an adrenaline storing cell showing a particulate peripheral part (arrow). Nerve terminal on the left. × 22,500.

nates in some, while in others noradrenaline is present in higher content. SHEPHERD and WEST[10] proposed the hypothesis that the corticoadrenal tissue and its hormones bear an importance in determining the type of the predominant amine in the adrenal gland. Since then many controversial works on the subject have been published. In spite of the particular position of the *Pygoscelis papua* in the systematic classification, the relationship between its cortical tissue and the adrenaline and noradrenaline containing cells is similar to that shown by all the birds studied up to now[6,11,12,13].

Resumen. Se investigó con microscopía óptica y electrónica y aplicando la técnica de glutaraldehido-plata[5,8] la distribución y características de las células que contienen adrenalina y noradrenalina en el pinguino Papúa (*Pygosceles papua*).

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Effect of Hypophysectomy or Hysterectomy on the Luteal 20α-Hydroxysteroid Dehydrogenase in Pregnant Rats

The ovarian level of 20α-hydroxysteroid dehydrogenase (20α-HSD) seems to represent, in the rat, one of the principal regulatory mechanisms in the catabolism of progesterone[1]. This enzyme is found only in corpora lutea (CL)[2]; it appears at a definite time in the newly-formed CL, i.e. after 2–3 days in the oestrous CL (late diestrus) and 20 days after mating in the pregnancy CL, i.e. shortly before parturition[3,4].

Some experimental data make it possible to assume that the appearance of this enzyme is under hypophysal control[1,5,6]. The secretion of prolactin, especially, seems to inhibit its onset in the CL[1,7].

In 1938, ASTWOOD[8] demonstrated in the rat that the activity of the CL in maintaining pregnancy in its early period and pseudopregnancy, is under hypophysal control. According to LINDNER and SHELESNYAK[9] this activity manifests itself in a high progesterone/20α-hydroxyprogesterone ratio in the blood.

One can thus presume that in early pregnancy, as in pseudopregnancy, the absence of luteal 20α-HSD activity is maintained by the hypophysal incretion. In middle and late pregnancy, when ova are already implanted, it can be assumed that placental incretion is responsible for maintaining the CL 20α-HSD negative.

Experiments evaluating the 20α-HSD activity in pregnancy or pseudopregnancy CL of hypophysectomized or hysterectomized rats have been performed in order to test these possibilities.

Materials and methods. Female albino rats, 250–300 g body weight, with controlled regular oestrous cycles were used. Pseudopregnancy was obtained by mating the female rats with vasectomized males. The day of cohabitation is assumed as day 0 of pseudopregnancy. Pregnancy was induced by a 24 h cohabitation of proestrous females with males of proven fertility and confirmed by the presence of sperms in the vagina the morning after (day 1 of pregnancy).

Hypophysectomy was performed by transpharyngeal route and confirmed by histological examination of the 'sella turcica' on the day of sacrifice. Hysterectomy was performed by transabdominal route. The different experimental conditions and the number of animals are shown in the Tables.

At sacrifice, the ovaries of the animals were dissected out, quickly frozen by CO_2 and then processed according to the methods previously described[4] for the detection of the 3β-hydroxysteroid (3β-HSD) and 20α-HSD activities. The reaction for 3β-HSD was used to identify all the CL.

Results. (1) Pregnancy. The data summarized in Table I show that when the hypophysectomy is performed before the 11th day of pregnancy, all the CL display a strong enzymatic 20α-HSD activity 3 days after the operation. On the other hand, when the hypophysectomy is performed after the 13th day of pregnancy, the pregnancy

Table I. Effect of hypophysectomy on corpora lutea of pregnant rats

Day of operation	Day of sacrifice	No. of animals	No. of animals with 20α-HSD negative CL
1	4	4	0
3	6	4	0
7	10	5	0
11	14	10	8
13	16	8	8
15	18	3	3

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