

ten. Hinsichtlich der Topik DNS synthetisierender Zellen in den einzelnen Zonen der Niere besteht dabei zwischen der physiologischen Zellerneuerung bei Kontrollen und der kompensatorischen Regeneration nach kN und kI nur ein quantitativer Unterschied.

Summary. By continuous infusion of ^3H -thymidine, autoradiographic studies were performed to compare cellular proliferation in the compensatory regenerating kidney of rats after contralateral nephrectomy or contralateral temporal (1 h) ischaemia with physiological cell renewal in controls. Under each experimental condition,

the highest labelling index is observed within the stratum labyrinthicum and then a gradual decrease occurs from there to the stratum subcorticale, zona intermedia and basalis. As to the topic of DNA synthesizing cells in the different zones of the kidney, there are only quantitative differences between physiological cell renewal of the controls and compensatory regeneration following contralateral nephrectomy and contralateral ischaemia.

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87 Würzburg (Deutschland), 6. Dezember 1967.*

Catecholamines and Granular Vesicles in Adrenergic Axons of the Developing Pineal Body of the Rat¹

The pineal body of the rat is innervated exclusively by sympathetic post-ganglionic fibers from the superior cervical ganglion². These fibers contain agranular vesicles, and granular vesicles of 2 types, large and small³⁻⁶. There is now considerable evidence that the small granular vesicles (SGV) contain norepinephrine (NE)⁵⁻⁸. In this report the development of the sympathetic innervation of the pineal body was studied by correlating histochemical fluorescence with electron microscopic observations. Special attention was given to the sequence of appearance of the 2 types of granular vesicles in the developing axons and its bearing on the intra-axonal distribution of catecholamines.

For fluorescence histochemistry, pineals from Holtzman rats of both sexes were treated according to the method of FALCK and HILLARP⁹ (cf. FALCK and OWMAN¹⁰).

The ages and the number of specimens per group were: 19-day-old fetuses, 2; 2 h post-partum, 3; 22-24 h, 4; 2-day, 5; 3-day, 5; 6-7 day, 6; 10-11 day, 3; 21 day, 4.

In 2-hour-old animals, bright green nerves were observed in the capsule, especially at the apex and dorsal side of the gland below the vena cerebri magna (Figure 1a). At 24 h a few isolated nerve bundles penetrated the pineal, and at 2 days typical green varicose terminals were seen around vessels or among the pineal cells in some areas of the gland (Figure 1b). Their number increased and at 21 days was similar to that observed in the adult gland⁹. The green fluorescence observed in the nerves of the developing pineal body indicates the presence of a catecholamine, most probably NE^{9,10}.

To investigate binding sites for NE in young adrenergic nerves, sympathetic fibers of the rat pineal body were studied by electron microscopy. The animals were the following ages: 2-24 h, 15; 2-3 days, 9; 5-8 days, 9; 15 days, 3; 21 days, 3. The pineal bodies were fixed by immersion or perfusion in 3% glutaraldehyde in 0.1M phosphate buffer adjusted to pH 7.3. The specimens were embedded in epoxy resin, sectioned, double stained and studied with a Hitachi HU 11A electron microscope.

On the day of birth most of the nerve bundles studied were in the capsule and there is no doubt that they correspond to those seen in the fluorescence microscope. The axons in these bundles contained neurotubules, neurofilaments, multivesicular bodies, glycogen particles, abundant smooth endoplasmic reticulum, mitochondria, agranular and granular vesicles. Granular vesicles, however, were only of the large type (Figure 2a) with a mean diameter of 820 Å. At day 2 a few SGV were seen in axons of small and large nerve bundles situated inside the gland or in its capsule (Figure 2b). The number of

these vesicles was considerably larger at day 7, and, at 2 weeks, clusters of more than 100 granular vesicles were occasionally seen (Figure 2c). The absence of the SGV from adrenergic axons of the pineal body during the first 24 h after birth does not seem to be due to inadequate fixation since glutaraldehyde, followed by osmium tetroxide, is an excellent fixative for the demonstration of granular vesicles in the rat pineal body⁴.

These data show that the large granular vesicle (LGV) precedes the SGV during development. Apparently the 'granules' recently described by WECHSLER and SCHMEKEL¹¹ in sympathicoblasts of the chick embryo are LGV indicating that those vesicles appear very early in development. The function of the LGV is unknown. Their presence in adrenergic fibers, even before contacts with effector cells are established, indicates that their function might not be related to neuroeffector transmission. On the other hand, the SGV appear only after the nerves have penetrated and branched inside the pineal gland and

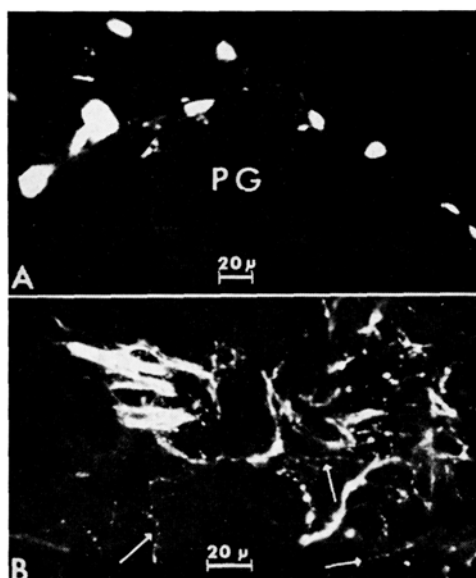


Fig. 1. Fluorescence photomicrographs of rat pineal bodies treated according to the method of FALCK and HILLARP. (A) 2 h of age. Cross section of the pineal gland (PG) showing large nerve in the capsule displaying intense green fluorescence. No nerve is seen inside the gland at this age. (B) 2 days of age. Nerve terminals with typical varicosities (arrows) inside the gland at its dorso-apical portion.

have established the first contacts with the effector cells. About this time the first typical varicose terminals were seen by fluorescence microscopy.

Since the SGV is the NE storage particle in adrenergic fibers of the adult^{5-8,12}, its absence from these fibers on the day of birth poses the problem of where the intra-axonal NE is localized in adrenergic fibers of fetal and early postnatal animals. To study this, nerves of the newborn were examined after pharmacological depletion of NE. Reserpine (8 animals, 5 mg/kg, i.p., Serpasil, Ciba) given to rats immediately after birth completely abolished fluorescence of the nerves in animals sacrificed 15–20 h after the injection. However, this treatment did not cause any modification of the ultrastructural characteristics of the nerve fibers in 3 animals that were examined. No apparent change was observed in the number of the LGV, and their dense cores were not modified. A similar result was obtained in 2 animals injected with metaraminol (4 mg/kg, i.p., Aramine, Merck) and sacrificed 6 h later. These data agree with those obtained in adult animals^{3,5-8} and indicate that the NE of fetal and early postnatal adrenergic axons is not contained in the LGV. Since no

SGV were found, it is believed that this NE was not bound to granular vesicles. The possibility that some NE is contained in agranular vesicles cannot be ruled out. However, since these vesicles are not abundant it is likely that most NE found in fetal and early new-born sympathetic fibers exists unbound or loosely bound in an extravascular compartment.

One of the accepted functions of the SGV is to bind and store NE, thereby protecting it from enzymatic destruction by intra-axonal monoamine oxidase (MAO)¹³. The coexistence of MAO with the large extra vesicular pool of NE that presumably exists in immature fibers would require that this NE be protected from the enzyme by a different mechanism. One such mechanism that has been suggested for adrenergic nerves of adult animals is binding to phospholipids in intra-axonal membrane systems¹⁴. A more likely possibility is that NE is really 'free' in the axoplasm while MAO is absent or occurs in a very small amount. The appearance and increase in the amount of bound (vesicular) NE coincides with the appearance and increase in intra-axonal MAO activity. This view is supported by the small amount of MAO present in immature animals: the enzyme is present in fetal and new-born rats in activities less than 20% of that observed in the adult¹⁵. In the rat pineal body significant MAO activity was detected only after about 1 week of age^{16,17}.

Resumen. Inmediatamente después del nacimiento, las fibras adrenérgicas del cuerpo pineal del ratón, contienen solamente vesículas granulosas del tipo grande. Con dos días, comienzan a aparecer las vesículas granulosas pequeñas; que son las que contienen nor-epinefrina. Se piensa que antes de ésta edad, la nor-epinefrina se localizaría principalmente en un compartimiento extra-vesicular.

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Fig. 2. Electron micrographs of adrenergic axons in pineal bodies of rats fixed with 3% glutaraldehyde followed by OsO_4 . (A) 24 h of age. This axon contains a large group of LGV which is the only type that occurs at this age. (B) 2 days of age. The arrows indicate typical SVG which were observed for the first time at this age. (C) 15 days of age. Axon dilation containing a large number of SGV, few agranular vesicles and one LGV (arrow).

¹ This material was presented at the meetings of the American Association of Anatomists, Kansas City, Missouri, 1967.

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¹⁷ We thank Dr. J. C. HAMPTON for his guidance in electron microscopy. This work was supported by a fellowship from the Rockefeller Foundation (A. B. M. MACHADO) and grant No. NIH 5 SO 1 FR 0531106.

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