BRIEF NOTES

THE DEMONSTRATION OF A SECRETORY MATERIAL AND CYCLE IN THE PARENCHYMAL CELLS OF THE MAMMALIAN PINEAL ORGAN

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THE function of the pineal organ in man and other mammals is still an enigma, although gradually accumulating evidence suggests a relation to antigonadotrophic activity [3]. The state of uncertainty regarding the functional significance of the pineal organ is at least partly due to the lack of evidence clearly demonstrating secretory activity within the pineal. Previously described pineal parenchymal cytoplasmic granulations and staining reactions are not clearly indicative of secretory activity and a cytological secretory cycle has not been successfully demonstrated [1]. A cytological technique for showing the specific activity of pineal parenchymal cells should aid greatly in analyses of the factors regulating the pineal and in the standardization of pineal preparations used in experimental therapy.

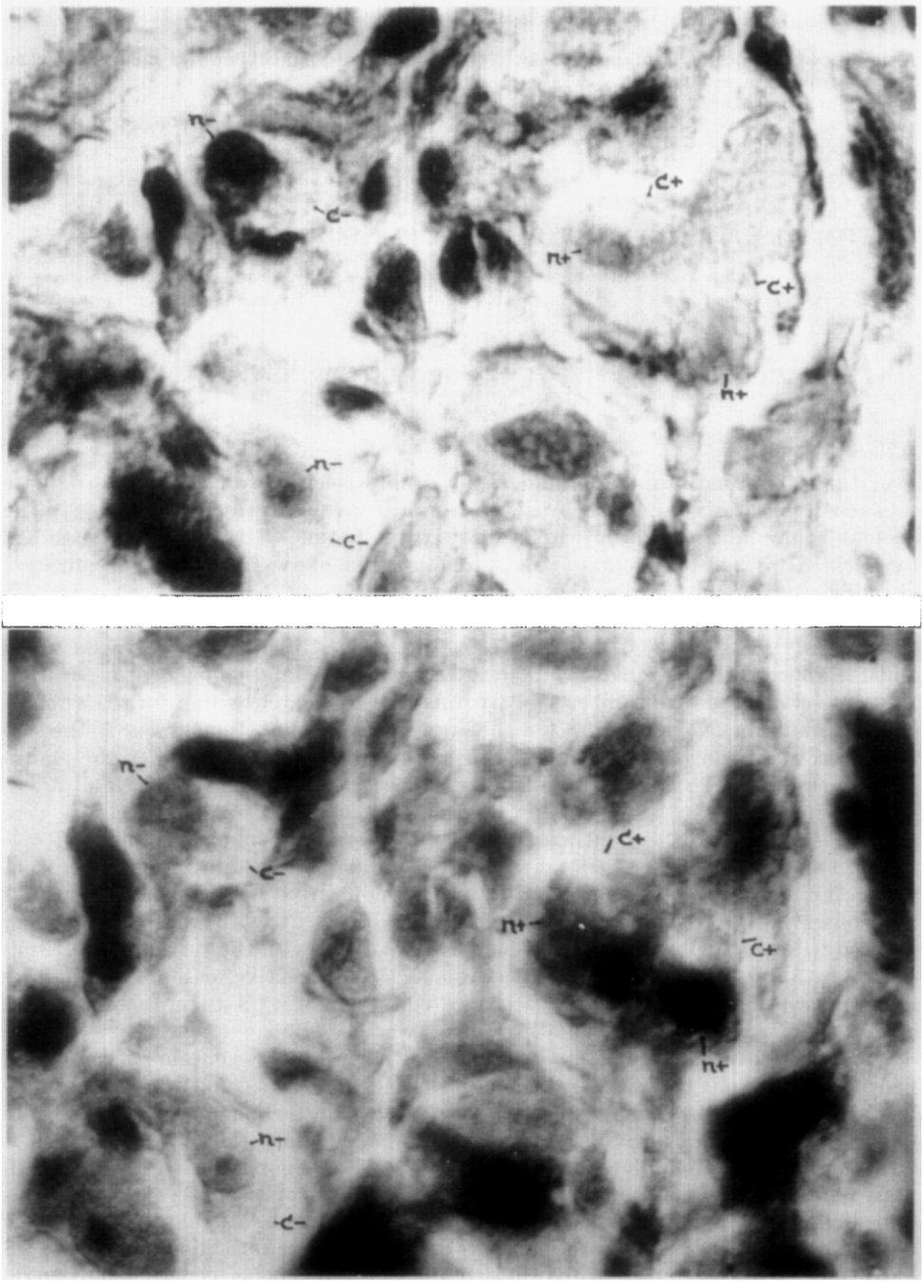
It was discovered in the study reported herein that the parenchymal cells of rat (Sprague-Dawley strain) pineals fixed for 16–24 hours in 10 per cent formalin buffered to neutrality with monobasic and dibasic sodium phosphate display an intensive and specific staining reaction with the chrome alum hematoxylin and phloxine technique of Gomori [2] for staining pancreatic islets. After fixation the pineals were washed in distilled water for 24 hours, dehydrated through ethanol solutions, cleared in benzene and embedded in paraffin. Sections seven microns in thickness were then stained according to Gomori's technique [2]. It was found that initial fixation in Bouin's fluid is unsatisfactory and that the step entailing refixation of the deparaffinized sections in Bouin's fluid for 24 hours is necessary for successful results with the pineal. In order to differentiate the cytoplasmic material of the pineal chromophils from the other phloxinophilic structures such as the erythrocytes, the following procedure was added after the phloxine (National Aniline Div., dye content 83 per cent, C.I. No. 778, Certification No. NPh-14) staining, acidification and washing steps in the Gomori procedure: (1) stain for about 1 minute in a solution containing 2 g orange G (National Aniline Div., dye content 93 per cent, C.I. No. 27, Certification No. NO-14), 0.5 g aniline blue (National Aniline Div., C.I. No. 707, Certification No. NK-8) and 1.0 g of phosphomolybdic acid per 100 cc. distilled water; (2) wash rapidly

in distilled water and 95 per cent ethanol; (3) dehydrate in two changes of absolute ethanol; and (4) clear in xylene and mount in clarite or HSR. After this procedure, the erythrocytes are colored red and the pineal chromophils are orange or yellow.

Chromophilic and chromophobic parenchymal cells and intermediates are numerous in all rat pineals examined. In the chromophobes, the cytoplasm is clear, the nucleus stained with hematoxylin and the nucleolus stained centrally with phloxine or orange G. In the chromophils the entire cell including the nucleus is stained with phloxine following the chrome alum hematoxylin and phloxine procedure. After staining with orange G and aniline blue the red phloxine-staining of the chromophils is reduced or replaced by a yellow to dark orange staining by orange G. Chromophils in which only the nucleus or only the nucleus and immediately adjacent cytoplasm are stained with phloxine or orange G are common. Chromophobes show a continuous series from small cells with little apparent cytoplasm and small darkly stained nuclei to large cells with a large area of nearly clear cytoplasm around large pale nuclei with large nucleoli. From chromophobes of the latter type, transitional forms are seen to chromophils. The nucleolus enlarges and lies next to the nuclear membrane. Orange areas in the immediately adjacent part of the cytoplasm then appear. Increased amounts of the specifically staining material gradually fill the chromophils, at first, particularly around the nucleus, so that the nucleus is no longer stained with hematoxylin but becomes a blurred dark orange area. The cytoplasm of chromophils, unlike that of chromophobes is usually concentrated in the protoplasmic processes of the cells. The processes, containing intensely stained material, frequently extend to the outer edges of capillaries, which are abundant in the pineal. Occasionally a group of chromophils is found arranged around an irregular area of intensely staining material. Heavily stained chromophils show a non-granular cytoplasm containing scattered, clear vacuoles. The same is true of the material found along capillaries and in clusters of secretory chromophils. Cells that may represent a post-secretory phase of activity have little apparent cytoplasm and a small granular nucleus which still stains with phloxine or orange G. Differential parenchymal cell counts in sixteen rats of both sexes and different ages showed the following ranges in percentages: chromophobes, 41–90 per cent; presecretory chromophils, less than 1–12 per cent; secretory chromophils, less than 1–14 per cent; post-secretory chromophils, 6–41 per cent. It should be noted that while fibroblasts and other cells in the connective tissue can be distinguished from the pineal parenchymal cells in the slides, neuroglial cells, which are much less numerous than the parenchymal cells, are included with the chromophobes since they are not always distinguishable.

The pineal parenchymal cells in the rat, thus, appear to take part in a secretory process. The effects of experimental procedures and the results of histochemical tests on the pineal chromophils will be presented later.

Figs. 1 and 2. Photomicrographs of a small area in a pineal (adult male rat) stained according to the procedure here described. Fig. 1 (top) was taken without the use of filters; Fig. 2 (bottom) was taken with a violet filter at the light source in order to demonstrate the yellow and orange cytoplasmic material of the chromophiles. The nuclear (*n*) and cell (*c*) membranes of two examples each of chromophiles (+) and chromophobes (—) are labeled. Note the presence of intermediate cell types, and the reduction in nuclear size and the obscuring of the nucleus in chromophiles.


n-
C-
C+
n+
C+
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AUTORADIOGRAPHIC EVIDENCE OF RADIOSULPHATE UPTAKE BY THYROID GLAND OF RAT

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BY means of apposition autoradiography it has been shown that radiosulphate injected into rats is taken up by several organs including thyroid gland [8]. The present communication deals with radiosulphate uptake of thyroid gland studied by electron-track autoradiography, a technique which allows an accurate localization of radioactive compounds in the tissue structures.

Albino rats, weighing approximately 200 g, were given a single subcutaneous injection of $Na_2\ ^{35}SO_4$ (1.5 μC/g body wt.). 124 hrs. after the injection, rats were killed and the thyroid glands removed, fixed in Carnoy's fluid and embedded in paraffin. Thyroid sections, deparaffinized and hydrated, were coated with Ilford G5 photographic emulsion in "gel form" to a thickness of about 40μ. In some cases the sections were thoroughly washed in water (1 hr.) before being covered with the photographic emulsion. The plates were left for 24 hrs. after the emulsion set and dried, before being processed (temperature development method) [5]. The sections were then stained through the emulsion layer by the Unna-Pappenheim method [7].

Fig. 1 shows that most of ^{35}S taken up by thyroid gland is localized in the colloid, whilst the epithelial cells exhibit a very weak radioactivity. The same picture has been observed in sections which had been washed for 1 hr. with water before being covered with the photographic emulsion. A comparison with the surrounding tissues (thyroideal and tracheal cartilage, perioesophageal connective, oesophageal epithelium, muscles) showed that the cartilages contain more ^{35}S than the thyroideal colloid. The radioactivity of the other tissues was negligible.

The question then arises as to the chemical nature of ^{35}S-containing compound which is present in the thyroideal colloid. The fact that this compound is unsoluble in water and in all the solvents used in the histological technique seems to indicate that ^{35}S is contained in an organic non-lipidic compound. It is likely that ^{35}S is incorporated into thyroglobulin which is the only component of the colloid still present under these conditions. On the other hand, thyroglobulin is known to contain sulphurated amino acids. However, it has been shown in rats that liver, cartilage and hairs [2, 12] are unable to incorporate radiosulphate into thioaminoacids. ^{35}S has been found only incorporated into sulphurated mucopolysaccharides in several