

STUDIES ON CYTOCHEMISTRY OF HORMONE ACTION

PART XV. THE EFFECT OF ADRENOCORTICOTROPHIC HORMONE (ACTH) ON ALKALINE PHOSPHATASE ACTIVITY IN THE PARS INTERMEDIA OF THE CAT'S HYPOPHYSIS

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INTRODUCTION

Karkun *et al.* (1954) reported a marked hypertrophy of the basophile cells of the pars intermedia of cats treated with ACTH. They also observed cytological changes in these elements which clearly resembled the hyaline changes of Crooke (1935). On the basis of these findings they suggested that the basophiles were probably stimulated by ACTH to secrete excess amounts of melanophore hormone. The data presented hereunder subscribe additional evidence in support of this concept.

The presence of alkaline phosphatase has been demonstrated in the pars distalis of the guinea-pig (Abôlins, 1948) and the cat (Romieu *et al.*, 1951). But no attention appears to have been paid to the localization of the enzyme in the pars intermedia. We, therefore, take this opportunity of reporting the distribution of alkaline phosphatase in the pars intermedia of the cat's hypophysis.

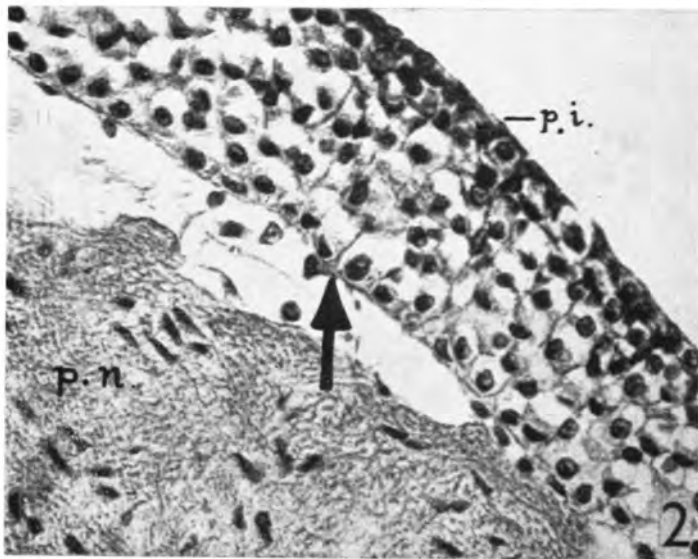
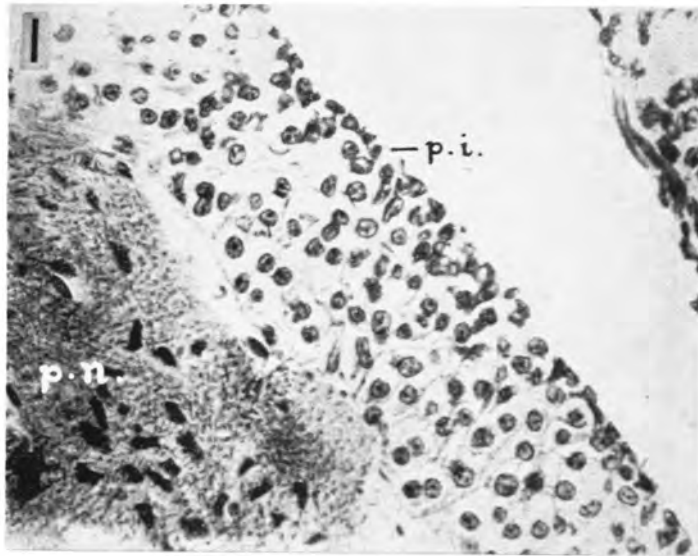
EXPERIMENTAL PROCEDURE

Eight female kittens, weighing 896 ± 8.5 gms., were used in this study of which 4 were injected with ACTH ('Corticotrophin', Wilson Lab., Chicago, U.S.A.) at the rate of 2 mg. (in 0.5 c.c. of sterile distilled water) twice daily. The injections were given at intervals of 12 hours. This dosage was continued for 5 days and from the 6th day it was increased to 3 injections of 2 mg. daily spaced at intervals of 8 hours. This rate was maintained for 7 days after which the animals were sacrificed. In all, the period of treatment lasted 12 days during which a total of 62 mg. of the hormone was administered to each of the experimental animals. The control animals were injected with 0.5 c.c. of sterile distilled water in a similar manner.

Autopsy followed 24 hours after the final injections. The pituitaries were carefully dissected out and fixed immediately in chilled 80 per cent ethyl alcohol. The serial paraffin sections of the gland were processed according to the technique of Gomori (1941) for the demonstration of alkaline phosphatase. The sites of phosphatase activity in the tissue sections were marked by the deposition of cobalt sulphide in the fine black granules. In order to allow critical observation of these granular deposits no counterstain was used. The sections were dehydrated and mounted in the usual manner.

RESULTS

Controls: The cellular architecture of the pars intermedia of this species has already been described in detail (Karkun *et al.*, 1954). In brief, the basophile cells constitute 95 per cent, chromophobes only 0.5 per cent and the macrophages 4.5 per cent of the total cellular elements of this pituitary component.



The nucleus of the basophile cells shows positive reactions for the enzyme but the cytoplasm stains rather faintly (Fig. 1). A similar pattern is noticeable in the chromophobes but the macrophages exhibit an intense phosphatase activity both in the nucleus as well as in the cytoplasm. The endothelium of the vascular sinusoids also react in a strong positive manner (Table I).

TABLE I.

The distribution of alkaline phosphatase in the pars intermedia of normal and ACTH treated cats.

			Normal	ACTH treated
Basophiles—				
Nucleus	+	++
Cytoplasm	+(F)	++
Chromophobes—				
Nucleus	+	++
Cytoplasm	+(F)	+(F)
Macrophages—				
Nucleus	++	++
Cytoplasm	++	++
Vascular sinusoids—				
Endothelium	++	++

Evaluation:—

- + = Positive reaction.
- +(F) = Positive reaction but faint.
- ++ = Strong positive reaction.

ACTH treated: ACTH treatment evokes considerable hypertrophy of the basophile cells. Their cytoplasmic granules become much coarser and aggregate in a peri-nuclear manner leaving the rest of the cytoplasm as a dense hyaline matter (Karkun *et al.*, 1954). The nucleus of these cells stains more intensely for the enzyme than in the controls. The basophilic granules of the cytoplasm also react in a strong positive manner (Fig. 2). It may be recalled that in the control animals only negligible amounts of phosphatase are visible in the cytoplasm of these cells. The macrophages and the chromophobes do not show any change in size or morphology after ACTH treatment. However, their nuclei stain slightly deeper than in the controls. The endothelium of the vascular sinusoids continues to give a strong positive reaction for the enzyme (Table I).

DISCUSSION

The interesting fact which emerges from the present study is the pronounced increase in alkaline phosphatase activity in the cytoplasm of the basophile cells after ACTH treatment. In contrast to this, the cytoplasmic phosphatase of other cellular elements does not show any response to the hormone though a slight increase in enzyme activity is noticeable in the nuclei of all the cell types. Now, according to recent views protein synthesis proceeds at par with the activity of the phosphatases (Roche, 1950). In view of these, it may be reasonable to assume that the enhanced phosphatase activity in the basophile cells signifies an increased formation of some

protein material, probably melanophore hormone. Interestingly, pars intermedia is regarded as the only source of this hormone in the species under study (Turner, 1948) and the basophiles are the cellular elements concerned with its elaboration (Karkun *et al.*, 1954).

SUMMARY

Intramuscular injections of ACTH into kittens cause a pronounced increase in alkaline phosphatase activity in the cytoplasm and the nucleus of the basophile cells of the pars intermedia. In contrast to this, the chromophobes and the macrophages show only a slight augmentation of enzyme activity only in the nucleus after the hormone treatment. The increase in phosphatase activity in the basophile cells, however, is extremely significant and probably signifies an enhanced synthesis of melanophore hormone.

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EXPLANATION OF FIGURES, PLATE XX

(All figures are photomicrographs and are magnified $\times 350$)

- FIG. 1. Section through the hypophysis of a control cat showing the distribution of alkaline phosphatase in the pars intermedia. Only the basophile cells are shown in the field. *p.i.*, pars intermedia; *p.n.*, pars nervosa.
- FIG. 2. Section through the hypophysis of an ACTH treated cat. Note the Crooke's change in the basophile cells and the increase in phosphatase activity (indicated by arrow). Lettering as in Fig. 1.

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