STUDIES ON CYTOCHEMISTRY OF HORMONE ACTION.

PART IX—*Responses of the adreno-cortical alkaline phosphatase in the Guineapig to various hormones.*

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INTRODUCTION.

The presence of alkaline phosphatase has been demonstrated cytochemically in the mammalian adrenal cortex and in that of the pigeon. In the mice embryos, the capsule and the glomerulosa give a positive reaction for the enzyme but the rest of the gland is entirely negative for the phosphatase (Kabat and Furth, 1941). The same authors also observed that the adult human adrenal cortex exhibits maximum enzyme activity in the glomerulosa but the other two zones contain only moderate amounts of the enzyme. Bourne (1943) reported that the glomerulosa of the guineapig's adrenal cortex shows strong positive reactions for the phosphatase but he has not given any account of enzyme activity in the other two zones. Zorzoli and Stowell (1947) demonstrated the presence of alkaline phosphatase in the adrenal cortex of a number of mammals including the guineapig. The distribution of this enzyme in the cortex exhibits a sexual dimorphism in the rat (Dempsey *et al.*, 1949) and in the mouse (Elftman, 1947). Much more of the enzyme is present in the male than in the female. Dempsey *et al.* (1949) observed that after hypophysectomy the phosphatase disappears from the fasciculata and the reticularis of the rat's adrenal cortex but persists in the zona glomerulosa. Replacement therapy with whole pituitary powder causes a reappearance of the enzyme and a return to a condition approximating that of the normal gland. Soulairac *et al.* (1949) reported that castration caused considerable inhibition of cortical phosphatase activity in the rat. Androgen therapy in castrated animals evoked marked augmentation of phosphatase activity but estradiol benzoate and desoxycorticosterone acetate were less effective for this purpose. Elftman (1947) noted that alkaline phosphatase was absent from the adrenal cortex of the castrated male, the ovariectomised female and the immature male mice. Treatment of such animals with testosterone propionate resulted in the reappearance of the cortical phosphatase.

In the pigeon there are considerable amounts of alkaline phosphatase in the adrenal cortical strands located in the central region of the gland but in the peripheral cortical masses there is very little enzyme activity. Treatment with estrogen or androgen caused a reduction in the cortical phosphatase activity (Kar, 1950*a*). Progesterone or desoxycorticosterone acetate administrations were, however, associated with a pronounced augmentation of enzyme activity in the cortex of this species (Kar, 1951).

In the present paper an attempt has been made to study in detail the distribution and concentration of alkaline phosphatase in the adrenal cortex of the normal guineapigs, and to determine whether cortical phosphatase responded to various hormone treatments.

Experimental Procedure.

Adult male guineapigs were used in this study. A total of 28 animals were taken of which a group of 4 was left uninjected to serve as controls. The remaining 24 guineapigs were allotted in groups of 4 animals each for receiving hormone treatments. All of them were kept in cages under uniform husbandry conditions throughout the duration of the experimental period.

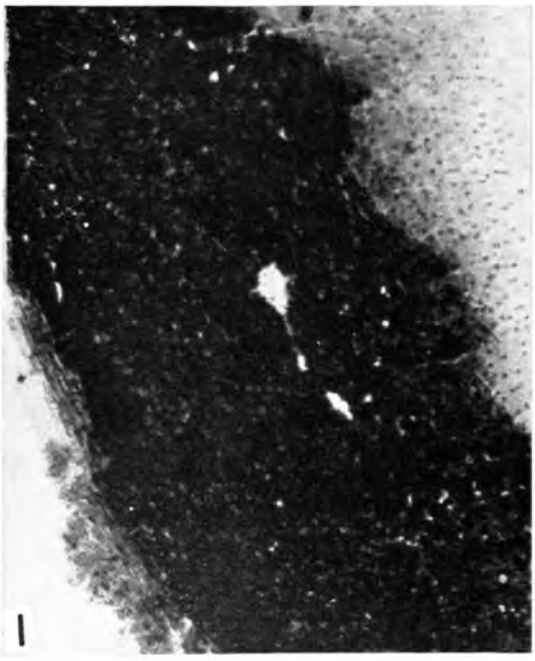
Steroid hormones used in this study were testosterone propionate, progesterone, estradiol dipropionate, diethylstilbestrol and desoxycorticosterone acetate, and the only non-steroid hormone used was serum gonadotrophin. Three injections of each hormone were given to a particular group of animals over a period of 7 days. The steroid hormones were dissolved in sterile sesame oil with the exception of diethylstilbestrol which was administered in an alcoholic solution diluted with 10% physiological saline according to the method of Kar (1947). Testosterone propionate was injected at the rate of 12·5 mgm. per treatment. The dosage for the rest of the steroid hormones, however, was 5 mgm. per injection. Serum gonadotrophin ('Gestyl', Organon Laboratories) dissolved in sterile distilled water, was administered in a dosage of 400 i.u. per injection.

Autopsy followed 24 hours after the final injections. The adrenals were carefully dissected out and fixed immediately in chilled 80% ethyl alcohol and in 10% formol-saline. After dehydration and imbedding in paraffin serial sections were prepared 6 microns in thickness. The tissue fixed in formol-saline was stained with Ehrlich's hematoxylin followed by alcoholic eosin. The sections of the adrenal fixed in ethyl alcohol were incubated in sodium glycerophosphate substrate according to the technique of Gomori (1941) for the demonstration of alkaline phosphatase. The sites of phosphatase activity in the tissue sections are marked by the deposition of cobalt sulphide in fine black granules. In order to allow critical observation of these deposits no counter-stain was used. The sections were dehydrated and mounted in the usual manner.

Results.

Controls. The nucleus of the connective tissue cells in the capsule gives a positive reaction for alkaline phosphatase. Phosphatase activity is also prominent in the endothelium of the capsular blood vessels. In agreement with the findings of Bourne (1943) we observed that in the glomerular zone marked concentration of the enzyme is visible in the component cells. Even the contour of these cells is made invisible by the granular deposits of cobalt sulphide. The entire glomerulosa appears as a dark band and quite well-marked from other cortical zones (Pl. III, fig. 1). In the zona fasciculata the nuclei stain quite intensely but in the cytoplasm the reaction is much less intense and the distribution of the enzyme appears to be rather irregular. The endothelium of the blood capillaries in the fascicular parenchyma also shows pronounced phosphatase activity. In the reticularis the nuclei show only a faint reaction but the cytoplasm and the endothelium of the blood capillaries in this region are virtually negative for the enzyme.

Androgen treatment. There is a pronounced reduction of phosphatase activity in the capsular elements. The connective tissue cells and the endothelium of the capsular blood-vessels are practically negative for the enzyme. In the glomerular zone the intensity of reactions appears to be the same as in the controls and the contour of the cells is totally obscured by cobalt sulphide deposits. In the subglomerular region of the fasciculata the distribution of the enzyme is similar to that in the controls but a definite reduction in phosphatase activity is evident in the rest of this zone (Pl. III, fig. 2). The endothelium of the blood capillaries in the fasciculata, however, continues to give a positive reaction for the phosphatase. Not even a trace of the enzyme is visible in the zona reticularis (Table I).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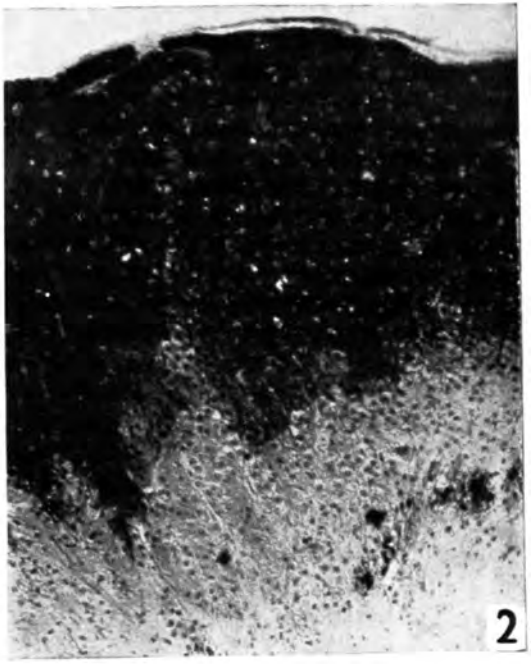
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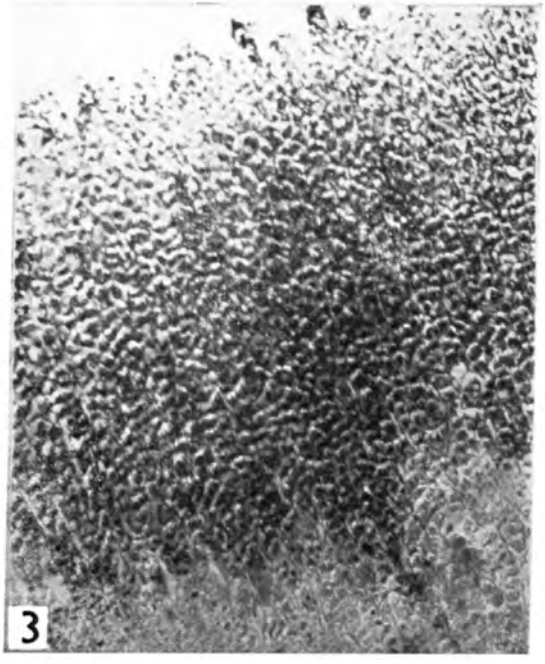
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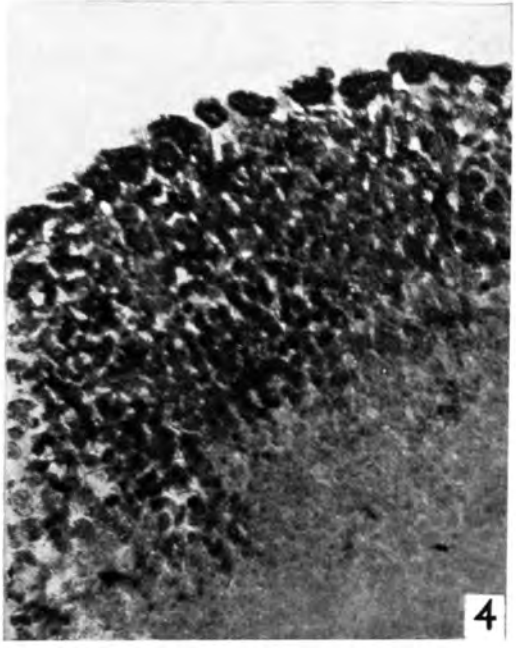
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Progesterone treatment. The capsule gives an entirely negative reaction for the phosphatase. In the glomerulosa only moderate amounts of the enzyme are seen in the nuclei but the cytoplasmic phosphatase activity is practically nil. There is an overall reduction in enzyme activity in the fascicular zone (Pl. III, fig. 3). This enzymatic loss is evident in the fascicular parenchymal cells as well as in the endothelium of the blood capillaries. The reticular zone continues to give a negative reaction for the phosphatase (Table I).

Desoxycorticosterone acetate treatment. The capsule gives a negative test for the phosphatase. Marked cellular atrophy is a notable histological feature of the cortex. In the glomerular zone a pronounced activity of the enzyme is visible but the reactions are less intense than in the control animals. This is evident from the fact that the glomerular masses with their component cells are clearly distinguishable even upon gross examination and do not appear as a dark homogeneous band as in the control animals (Pl. III, fig. 4). The zona fasciculata shows a marked reduction in phosphatase activity. The endothelium of the blood capillaries in this region also stains in a faint manner. The reticular zone is entirely small negative for the phosphatase (Table I).

TABLE I.

The Distribution of Alkaline Phosphatase in the Adrenal Cortex of normal and various hormone-treated Guineapigs

	Controls.	Androgen treated.	Progesterone treated.	DCA-treated.	Estradiol treated.	Stilbestrol-treated.	Gonadotrophic hormone-treated.
Capsule	++	+	−	−	−	−	−
Zona glomerulosa ..	++	++	+^{n}	+	+	+	+^{n}
Zona fasciculata							
Parenchymal cells ..	++^{nn}	± ±^{nn}	+	+	+	+^{n}(F)	+(F)
Blood Capillaries ..	++	+	+	+	+	−	−
Zona Reticularis ..	+(F)^{n}	−	−	−	−	−	−

Legend:— ++ = Strong alkaline phosphatase activity.
+ = Reduced alkaline phosphatase activity.
− = No alkaline phosphatase activity.
± ± = Strong activity in the sub-glomerular zone but no activity in the rest of the zone.
n = Moderate activity only in the nuclei.
nn = Very strong activity only in the nuclei.
(F) = Slight activity.

Estradiol dipropionate treatment. The phosphatase activity in the capsule is practically nil. In the glomerular zone the distribution of the enzyme is more or less similar to that in the DCA-treated animals. The fasciculata shows marked vacuolisation of the parenchymal cells particularly in the region adjacent to the reticularis. There is a definite reduction in phosphatase activity in this zone (Pl. IV, fig. 5). The endothelium of the blood capillaries in the fasciculata is also virtually negative for the enzyme. The reticularis is conspicuous by the absence of phosphatase activity (Table I).

Diethylstilbestrol treatment. No trace of the enzyme is visible in the capsule. Atrophic changes are evident in the cortex and an overall reduction in phosphatase activity is clearly distinguishable. In the glomerulosa moderate amounts of the enzyme are present. Only slight nuclear phosphatase activity is present in the fasciculata (Pl. IV, fig. 6). The endothelium of the blood capillaries in this zone is

also negative for the enzyme. No phosphatase activity is seen in the reticularis (Table I).

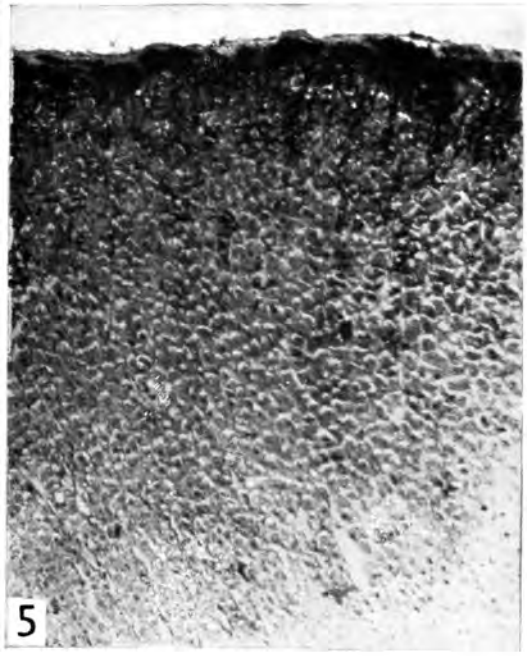
Gonadotrophic hormone treatment. No phosphatase activity is visible in the capsule. There is a marked reduction in enzyme activity in the different zones. In the glomerulosa moderate amounts of phosphatase are present only in the nuclei but the cytoplasmic enzyme activity is practically nil. The fascicular zone is virtually devoid of phosphatase activity (Pl. IV, fig. 7). The enzyme is totally absent in the reticularis (Table I).

Discussion.

The pattern of distribution of alkaline phosphatase in the adrenal cortex of the guineapig differs in several respects from that in the rat and the mouse. In the rat, considerable quantities of the enzyme occur in the glomerular zone, where the endothelium of the sinusoids stains quite intensely but the parenchymal cells show only moderate reactions in the nucleus. The fasciculata and the reticularis, however, exhibit uniform phosphatase activity (Dempsey *et al.*, 1949; Soulairac *et al.*, 1949). In the mouse (Elftman, 1947), the glomerulosa shows negative reactions for the enzyme but the fasciculata and the reticularis give strong positive reactions for the phosphatase. The distribution of the enzyme, however, is somewhat different in the embryonic mouse where the glomerulosa shows intense phosphatase activity but the other zones are entirely negative for the enzyme (Kabat and Furth, 1941). The glomerulosa of the adult human adrenal cortex exhibits pronounced phosphatase activity but the fasciculata and the reticularis contain only moderate amounts of the enzyme (Kabat and Furth, 1941). In contrast to these pictures, the glomerulosa of the guineapig's cortex shows more spectacular phosphatase activity but in the fasciculata the reactions are undoubtedly less intense while in the reticularis the enzyme activity is almost negligible. It is, therefore, evident that the distribution and concentration of alkaline phosphatase in the adrenal cortex show great deal of species variability in the mammals, and in this connection we feel that work on other mammalian species may reveal some hitherto unknown facts regarding the distribution of this enzyme in the cortex.

The enzymatic response of the individual adreno-cortical zones in the guineapig to various hormones is an item of considerable interest. Our results clearly demonstrate that androgen treatment alone retains normal Gomori reactions in the glomerulosa which we may recall here, is the zone of maximum phosphatase activity, but other hormones cause definite reduction in enzymeactivity in this zone (see Table I). A uniform loss of phosphatase activity is, however, encountered in the fasciculata of all the treated groups, whereas the reticular enzyme shows practically no response to various hormonal treatments. Thus, it is evident that the action of various hormones on cortical phosphatase activity in this species is in a general sense, inhibitory. In this connection, we would like to point out once again that the effect of androgen is somewhat different from other hormones since it does not cause any reduction in glomerular enzyme activity but with regard to its action on other cortical zones it falls well in line with the rest of the hormones in our list. Possibly, this effectiveness of androgen in retaining the glomerular phosphatase activity is ascribable to the fact that it is the homologous sexual hormone of our material. However, this explanation is only tentative and we suggest that its validity should be tested experimentally with female guineapigs as the material.

Kar (1950) observed that treatment with estrogen or androgen reduces cortical phosphatase activity in the pigeon, but the degree of this reduction is more pronounced in birds receiving estrogen than in the androgen-treated birds. Progesterone or DCA administrations, on the other hand, cause a marked augmentation of enzyme activity in the cortex of this species (Kar, 1951). The response of the adreno-cortical alkaline phosphatase in the guineapig to estrogen or androgen is in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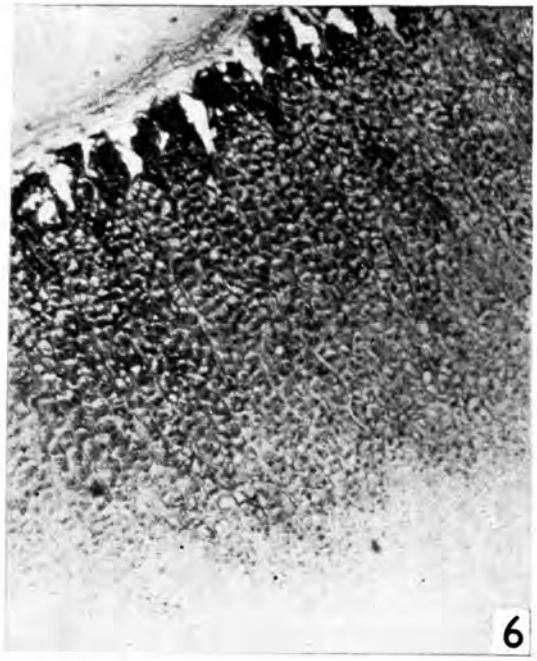
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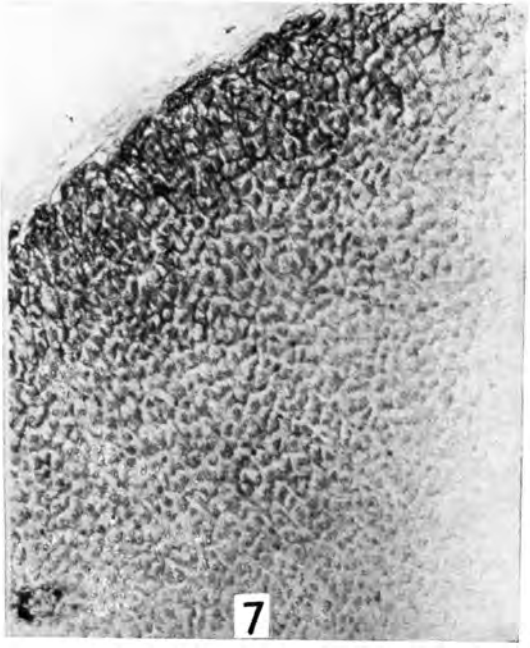
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a sense, similar to that in the pigeon. In this mammalian species also we find that the gonadal hormones cause an overall reduction in cortical enzyme activity but here again, the effect of androgen appears to be less severe than that of the estrogen. The luteoid and the corticoid, however, have altogether different effects. These two hormones definitely inhibit the phosphatase activity of the guineapig's cortex and, therefore, it is evident that their actions are totally unlike in the two vertebrate species studied by us.

Summary.

The distribution of alkaline phosphatase has been studied cytochemically in the adrenal cortex of normal and of various hormone treated guineapigs. In the normal guineapigs spectacular enzyme activity is visible in the glomerular zone but the fasciculata contains only moderate amounts of the phosphatase. The reticularis gives almost negative reactions for the enzyme. Hormonal treatments cause an overall reduction in phosphatase activity in the cortex. The possible significance of this enzymatic reduction is pointed out and discussed.

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Explanation of Plates

(All figures are photomicrographs and are magnified × 70.)

Plate III

Fig. 1. Section through the adrenal cortex of a control guineapig. Note the spectacular phosphatase activity in the glomerular zone.

Fig. 2. Section through the adrenal cortex of an androgen-treated guineapig. Compare with fig. 1.

Fig. 3. Section through the adrenal cortex of a progesterone-treated guineapig. Note the loss of phosphatase activity from the glomerular and fascicular zones.

Fig. 4. Section through the adrenal cortex of a DCA-treated guineapig. Compare with figs. 1 and 3.

Plate IV

FIG. 5. Section through the adrenal cortex of an estradiol dipropionate-treated guineapig. Note the reduction in phosphatase activity from the glomerular and fascicular zones.

FIG. 6. Section through the adrenal cortex of a diethylstilbestrol-treated guineapig. Marked reduction in phosphatase activity is evident.

FIG. 7. Section through the adrenal cortex of a gonadotrophic hormone-treated guineapig. Note the pronounced reduction in phosphatase activity.