STUDIES ON CYTOCHEMISTRY OF HORMONE ACTION.

PART III.

The effect of Progesterone and Desoxycorticosterone Acetate on the Distribution and Concentration of Alkaline Phosphatase in the genital System of female Pigeons.

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(Communicated by Dr. B. Mukerji, F.N.I.)

(Received March 13, 1950.)

INTRODUCTION.

The distribution and concentration of alkaline phosphatase has been studied cytochemically in the ovary of several species of mammals and in the pigeon. In the pig, the dog, and in the human the thecal cells contain abundant phosphatase but those of the granulosum contain none (Corner, 1948). The condition is reversed in the rabbit while in the rhesus monkey the enzyme is present in both the layers (Corner, 1944 and 1948). Alkaline phosphatase is demonstrable in the graafian follicles, corpora lutea, interstitial tissue, and in the blood vessels of the rat's ovary (Dempsey *et al.*, 1949). The enzyme disappears from the ovary of hypophysectomized rats but replacement therapy with pituitary powder causes a reappearance of the enzyme and a return to a condition approximating that of the normal gland. In the ovary of the pigeon alkaline phosphatase is present in the theca, blood vessels, and in the stromal tissue (Kar, 1950 *a*). Androgenic treatment causes a spectacular mobilization of the enzyme in the theca.

Experimental studies in the mouse have shown that estrogen treatment causes an increase in the uterine phosphatase. Progesterone and androgen do not have this effect (Atkinson and Elftman, 1946 and 1947). Karnell and Atkinson (1948) have found only negligible quantities of alkaline phosphatase in the vaginal epithelium of the ovariectomized mice. Estrogen therapy in such animals results in a marked increase in the vaginal phosphatase activity. In ovariectomized rhesus monkeys pre-treated with estrogen, the enzyme is found chiefly in the endometrial glands and in the surface epithelium (Atkinson and Engle, 1947). Progesterone treatment causes considerable reduction of phosphatase in the surface epithelium of these monkeys. The enzyme dissappears completely from the vagina of rats after ovariectomy but is increased in the uterine horns (Thibault and Soulairac, 1948). Estrogen therapy re-establishes a normal phosphatase activity in the vagina but the normal enzyme conditions are restored in the uterine horns only if progesterone is administered following estrogen therapy. Dempsey *et al.* (1949) described the disappearance of phosphatase from the uterus of the rat after hypophysectomy and its restoration on replacement therapy with estrogen or pituitary powder. In the oviduct of juvenile pigeons there is practically no phosphatase, but in the birds of the same age treated with estrogen, the phosphatase concentration is markedly increased in the hypertrophied oviduct (Kar, 1950 *b*). Androgen treatment,

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however, is less effective in augmenting the amount of oviducal phosphatase in the pigeon.

The present report has been concerned with an attempt to study the effect of progesterone and desoxycorticosterone acetate on the distribution and concentration of alkaline phosphatase in the genital system of female pigeons.

Experimental.

Female pigeons, 110 days old were used in this study. A total of 15 birds were involved of which 5 were injected with progesterone, 5 with DCA, and the remaining 5 were left uninjected to serve as the controls. The birds were kept in cages under uniform husbandry conditions throughout the duration of the experimental period.

Progesterone in sterile sesame oil was administered intramuscularly. The daily injections (0·1 mg.) were made into the breast muscles and continued for a period of 24 days. An equal amount of DCA (0·1 mg. in sterile sesame oil) was injected in the similar manner and over the same period of time. The sites of injection alternated on successive days between the right and left sides of the breast.

Autopsy followed 24 hours after the final injections. The ovary and the oviduct were fixed immediately in chilled 80% ethyl alcohol and in Bouin's fluid. After dehydration and imbedding in paraffin, serial sections were cut 6 microns in thickness. The tissues fixed in Bouin's fluid were stained with Mallory's trichrome stain. The sections of the ovary and the oviduct fixed in ethyl alcohol were incubated in sodium glycerophosphate substrate (*p*H 9·5) according to the technique of Gomori (1941) for the domonstration of alkaline phosphatase. The sites of phosphatase activity in the tissue sections are marked by the deposition of cobalt sulfide 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.

A. *Controls.*

Ovary. There is very little phosphatase in the granulosa cells of the ovary. The endothelium of the thecal blood vessels shows strong reactions for the enzyme. Moderate amounts of phosphatase are present in the nucleus of the thecal cells but the cytoplasm of these cells contains only a trace of the enzyme. The phosphatase activity is evident in the endothelium of the stromal blood vessels and in the connective tissue cells but the interstitial cells show negative reactions for the enzyme (Pl. VII, fig. 1).

Oviduct. No phosphatase activity is visible in either the serosa or in the muscularis. The epitheliun bordering the mucosal folds and the connective tissue stroma, however, show the presence of small amounts of phosphatase. The maximum concentration of the enzyme is seen in the young tubular glands of the oviduct (Pl. VII, fig. 2).

B. *Progesterone treatment.*

Ovary. The inhibition of the ovarian growth similar to that reported by Kar (1949) following progesterone treatment is also observed in the present material. The suppression of the follicular development due to the luteoid treatment is evident upon microscopical examination. There is no trace of phosphatase in the granulosum or in the thecal region (Pl. VII, fig. 3). Small amounts of the enzyme are present in the connective tissue cells and in the endothelium of the stromal blood vessels. Interstitial cells, however, continue to give a negative reaction for the phosphatase.

Oviduct. The consequences of the luteoid-induced ovarian inhibition is clearly reflected in the marked hypoplasia of the oviduct. Histological examination discloses an immature condition of the duct with low and non-glandular mucosal

folds. The sub-mucus region presents the appearance of a thin connective tissue lamina and the muscularis appears as an extremely thin peripheral layer. Test for alkaline phosphatase is entirely negative in the component parts of the oviduct (Pl. VII, fig. 4).

C. *Desoxycorticosterone acetate treatment.*

Ovary. The ovary shows signs of hypertrophy. These are evidenced by somewhat accelerated development of the follicles and the stromal growth. The enzyme is practically absent in the granulosum. There is, however, a spectacular mobilization of phosphatase in the theca (Pl. VII, fig. 5). The nucleus and the cytoplasm of the thecal cells as well as the endothelium of the thecal blood vessels show high concentration of alkaline phosphatase. Only small amounts of the enzyme are present in the stromal connective tissue cells and in the endothelium of the blood vessels. The interstitial cells are prominent by the absence of the enzyme.

Oviduct. The oviduct shows induced growth in the serosa, muscularis, and in the mucosal folds. There is, however, no glandular development. The enzyme is absent from the component parts of the oviduct, except the stroma and the mucosal epithelium where only small amounts are present (Pl. VII, fig. 6).

Discussion.

A number of recent studies have revealed a relationship between the endocrine status and the activity of the selected enzymes (*vide* Dempsey *et al.*. 1949). These studies have clearly indicated the possibility that the relationship between the hormones and the enzymes has a causal significance. Moreover, a series of investigations have proved beyond doubt that the activity of the phosphatases vary under different physiological conditions (Dempsey *et al.*, 1949; Karnell and Atkinson, 1948; Atkinson and Engle, 1947; Thibault and Saulairac, 1948; Kar, 1950, *a*, *b* and *c*; and others). In view of this, it is not surprising that the ovarian phosphatase activity in the pigeon should change after hormonal treatments. Thus, progesterone administration in the female pigeon is known to cause inhibition of the ovarian growth and the suppression of the follicular development (Kar, 1949). Concomitantly, the phosphatase activity is considerably diminished in the stroma and totally disappears from the ovarian theca of the luteoid-treated birds. DCA treatment, on the other hand, causes a spectacular mobilization of the enzyme in the theca of a somewhat hypertrophied ovary. It is interesting to note that testosterone propionate also causes a similar mobilization of alkaline phosphatase in the ovarian theca of the pigeon (Kar, 1950 *a*). The action of DCA on the phosphatase activity in the pigeon's ovary, therefore, appears to be androgenic.

The consequence of the loss of phosphatase activity in the ovary of progesterone-treated birds is unmistakably reflected in the change that ensued in the oviduct. This is clearly evident from the total absence of the enzyme from the component parts of the duct. However, a different picture is presented if we reckon the effects of DCA administration. Histologically, there is induced growth in the serosa, muscularis, and in the mucosal folds, but there is no functional development, which can be gauged from the fact that the tubular glands are absent from the mucosa. Cytochemically, only small amounts of phosphatase are present in the oviduct. Here again, the situation is comparable to a similar one that is encountered in the pigeon's oviduct after testosterone propionate treatment. Induced growth of the duct except however, the development of the tubular glands are the noteworthy histological alterations, while cytochemically, there is little augmentation of enzymatic concentration (Kar, 1950 *b*), although the ovary shows a marked increase in phosphatase activity (Kar, 1950 *a*). The findings on the oviduct of the DCA-treated pigeons, therefore, appear to extend the observations made on the ovary and indicate that the corticoid is undoubtedly androgenic in influencing the

distribution and concentration of alkaline phosphatase in the genital system of female pigeons.

Summary.

Intramuscular injections of progesterone ensues a loss of alkaline phosphatase activity in the genital system of female pigeons. Desoxycorticosterone acetate treatment, however, causes a spectacular mobilization of the enzyme in the ovarian theca but there is little augmentation of phosphatase activity in the oviduct. The similarity between the corticoid and testosterone propionate in influencing the enzymatic activity in the genital system of the pigeon is pointed out and discussed.

Acknowledgments.

The author wishes to express his gratitude to Dr. B. Mukerji, Director, Central Drugs and Pharmacognosy Laboratories, for constant help and encouragement. Grateful acknowledgment is made to Dr. K. H. Gruschwitz of Ciba Pharma, Ltd., Calcutta, for the generous contribution of progesterone (Lutocyclin) and desoxycorticosterone acetate (Percorten) used in this study. Thanks are due to Sri P. C. Pathak for taking the photomicrographs which illustrate this paper.

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Explanation of Plate VII.

(All figures are photomicrographs and are magnified $\times 200$.)

Fig. 1.—Section through the ovary of an untreated pigeon. Note the distribution of alkaline phosphatase.

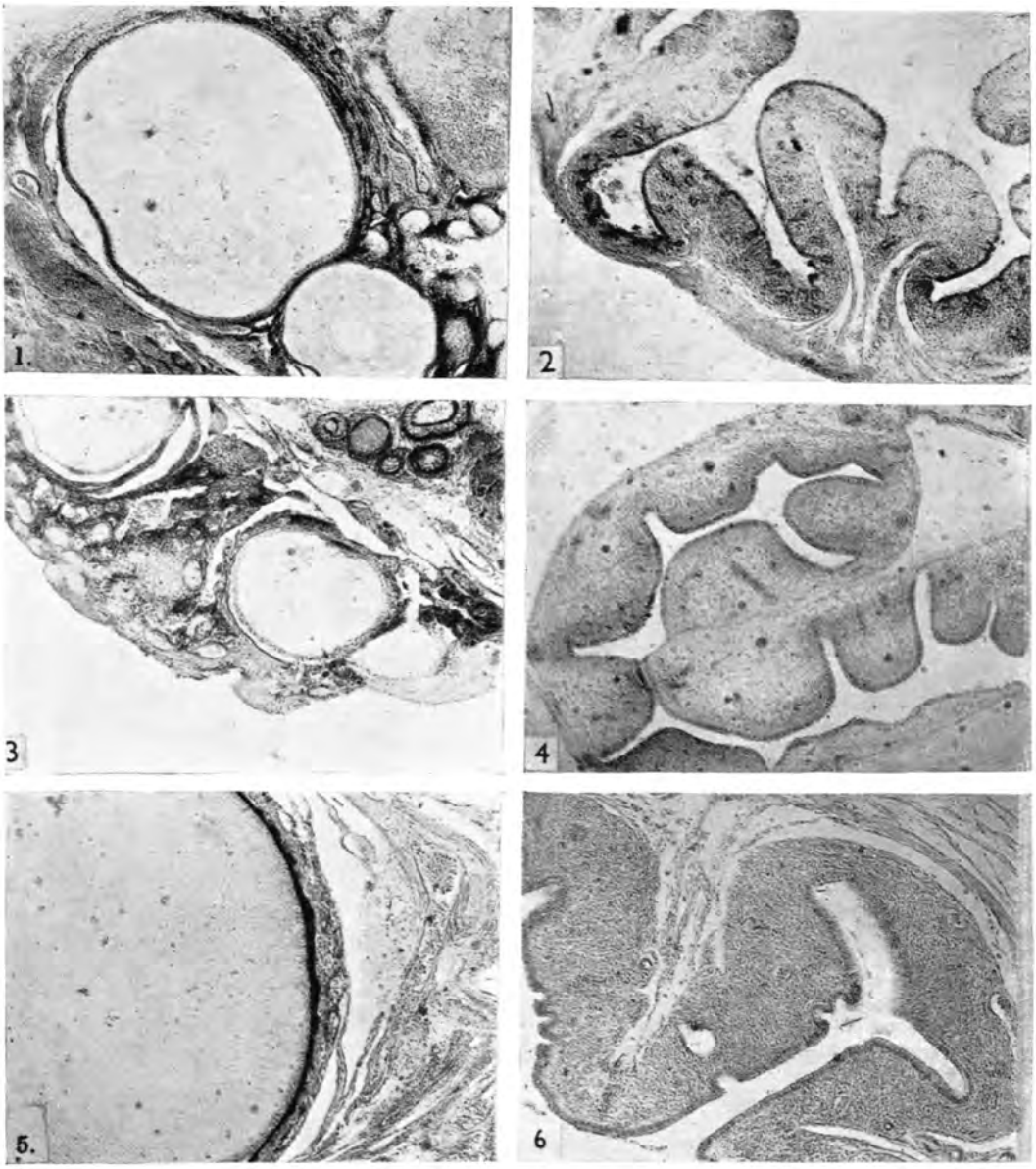
Fig. 2.—Section through the oviduct of an untreated pigeon. Phosphatase is present in the tubular glands.

Fig. 3.—Section through the ovary of a progesterone-treated pigeon. Compare with fig. 1.

Fig. 4.—Section through the oviduct of a progesterone-treated pigeon. Phosphatase is absent.

Fig. 5.—Section through the ovary of a DCA-treated pigeon. Note the spectacular mobilization of alkaline phosphatase in the theca.

Fig. 6.—Section through the oviduct of a DCA-treated pigeon. Compare with figs. 2 and 4.



(For explanation of plate, *see* p. 180.)