STUDIES ON CYTOCHEMISTRY OF HORMONE ACTION— PARTS I AND II.

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

(*Received October 28 ; after revision December 13; read November 25, 1949.*)

PART I. ALKALINE PHOSPHATASE IN THE UROPYGIAL GLAND OF NORMAL AND OF ANDROGEN TREATED PIGEONS.

INTRODUCTION.

Selye (1943) observed that the uropygial gland in the fowl depressed under the influence of testoid compounds. Kar (1947) made an extensive study of the relation of sex hormones to this sebaceous type of gland in the fowl. He showed that castration produced atrophic changes in the gland which, however, were prevented by replacement therapy with testosterone propionate. He further noted that testosterone depressed the uropygial gland of normal cockerels, but estrogen treatment failed to influence this gland. This ineffectiveness of estrogen was explained by the assumption that the female sex hormone failed to influence the uropygial gland of fowls with functioning testes. Recently, the validity of this assumption was tested by Kar (1949) with female Indian spotted munia, *Uroloncha punctulata* (L.) as the experimental material. It was noted that estrogen treatment stimulated the uropygial gland of this passerine species and thereby indicated the sex-specific nature of action of this hormone on the gland.

Montagna and Hamilton (1947) demonstrated that diffuse quantities of alkaline phosphatase were present in the sebaceous cells and the sebum of the golden hamster. In the glands of the ovariectomized female the enzyme was absent, but it was restored on andorgen therapy. Since the uropygial gland in birds resembled the mammalian sebaceous glands both microscopically and physiologically, it seemed desirable first to study the location and distribution of alkaline phosphatase in this gland, and secondly to determine the effects of androgenic treatments on phosphatase activity in this sebaceous type of gland.

EXPERIMENTAL.

Female pigeons, 90 days old, were involved in this study. A total of 8 birds were used of which 4 were injected intramuscularly with testosterone propionate (2·5 mgm. daily for 10 days) and the remaining 4 were left uninjected as controls. All the birds were kept in cages under uniform husbandry conditions throughout the duration of the experimental period.

The birds were autopsied on the day following the last injections. The uropygial glands were carefully dissected out, weighed to the nearest mgm. and fixed in cold 80% (5° to 10° C.) alcohol and in 10% formalin. After dehydration and imbedding in paraffin, serial sections were cut 6 microns in thickness. The

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tissue fixed in formalin was stained in Mallory's. The sections of the gland fixed in alcohol were incubated in sodium glycerophosphate substrate (pH 9·5) according to the technique of Gomori (1941) for the demonstration of alkaline phosphatase. In order to increase the enzyme activity 0·01M. magnesium sulfate was added to the substrate (Atkinson and Engle, 1947). The site of phosphatase activity in the tissue sections is marked by the deposition of cobalt sulfide in fine black or brown granules. No counterstains were used in order to avoid obscuring these deposits. The sections were dehydrated and mounted in the usual manner.

Results.

Controls.—The microscopical structure of the uropygial gland in the fowl has been described by Kar (1947). In general, the histological features of this gland in the pigeon agreed with those of the fowl. The phosphatase was present in diffuse quantities in the gland. The connective tissue forming the basement membrane of the alveoli showed negative reactions for the enzyme. In the blood vessels of the basement membrane the phosphatase was present only in the endothelium. The cuboidal cells underlying the alveolar wall showed maximal reactions for phosphatase activity (Pl. II, Fig. 1). In the rest of the alveolar epithelium the enzyme activity was variable. In one or two layers of cells next to the cuboidal epithelium the enzyme reactions were stronger in the nucleus while the cytoplasmic phosphatase activity was very low (Pl. II, Fig. 1). Beyond these layers, the progressively degenerating cells showed very weak enzymatic reactions in the cytoplasm The phosphatase activity in the nucleus of these cells was practically absent.

Androgen treatment.—Atrophic changes similar to those reported in the uropygial gland of the fowl after androgen treatment (Kar, 1947), were noted in the gland of the pigeons treated with testosterone propionate. The disintegrated cellular mass, characteristic of the alveoli of the atrophic glands was present in the central region of the alveoli (Pl. II, Fig. 2). The basement membrane showed negative reactions for phosphatase activity. In the blood vessels the enzyme was restricted to the endothelium as in the control pigeons. In the cuboidal cells beneath the basement membrane there was slight loss of phosphatase activity in the cytoplasm, while the nuclear enzyme activity was unaffected. In the rest of the alveolar epithelium the enzyme was practically absent from the cytoplasm of the component cells (Pl. II, Fig. 2). The nuclear phosphatase, however, was present in the cells. It is interesting to note that the disintegrated cellular mass in the central region of the alveoli showed slight reactions for the enzyme.

Discussion.

The present studies have clearly demonstrated the presence of alkaline phosphatase in the uropygial gland of the pigeon. The enzyme is present in diffuse quantities in the gland of the normal birds. Androgenic treatments, however, depress the activity of the phosphatases, but do not abolish it, since appreciable amounts of the enzyme are present in the component tissues of the gland. The phosphatase concentration in the uropygial gland of untreated pigeons appears to be in level with that of the sebaceous glands in the golden hamster. In this mammalian species the enzyme is present in diffuse quantities in the sebaceous cells and the sebum (Montagna and Hamilton, 1947). The phosphatase disappears from the glands of castrated female hamsters, but is restored on replacement therapy with testosterone propionate.

A question may now be raised concerning the possible physiological rôle of alkaline phosphatase in the uropygial gland. It is well known today that diverse type of endocrine factors exist and that they selectively regulate the normal

functioning of various specific organs. A typical example of this diversity of endocrine function is provided by the hormonal control of the normal holocrine cycle in the uropygial gland (Kar, 1947, 1949). Unfortunately we have next to no knowledge about the mechanisms by which the hormones exert their effects.

Following the recent recognition that biochemical conversions are controlled by enzymes, the attention has been focussed upon the relationship of the hormones to these regulatory mechanisms. A number of recent studies, for instance, have revealed a relationship between the endocrine status and the activity of the phosphatases. Atkinson and Elftman (1947) studied the mobilization by estrogen of alkaline phosphatase in the uterus of mouse; Talmage (1949) demonstrated the great increase in phosphatase activity in the entire genital tract of the rat after estrogen treatment; the enzyme dissapears rapidly from the uterus of rat after hypophysectomy and is restored on replacement therapy with estrogen or pituitary powder (Dempsey *et al.*, 1949); the immature oviduct of juvenile pigeons show practically negative reactions for the phosphatase, but in birds of the same age treated with sex hormones the phosphatase activity is markedly increased in the hypertrophied oviduct (Kar, unpublished data); and many other examples in the same line may be cited. In the light of these findings, therefore, it now seems probable that the relationship between the phosphatases and the hormones has a causal significance.

The present study adds a new instance in which the alkaline phosphatase activity is shown to be hormonally controlled. Since the phosphatases play a vital rôle in the intermediate metabolism of lipids (Dempsey and Wislocki, 1946), it is not unlikely that the holocrine lipiodal metabolism in this typical avian gland is mediated by the enzyme through the influence of the gonadal hormone. The evidence in support of this concept is provided by the loss of phosphatase activity in the uropygial gland of androgen treated pigeons. This decreased enzyme activity due to testosterone propionate treatment is undoubtedly associated with the disturbance of the normal holocrine order and the consequent atrophy of the gland.

PART II. THE DISTRIBUTION AND CONCENTRATION OF ALKALINE PHOSPHATASE IN THE OVARY OF NORMAL AND OF ANDROGEN TREATED PIGEONS.

INTRODUCTION.

The presence of alkaline phosphatase has been demonstrated cytochemically in the ovary of several species of mammals. In the pig, the dog, and in the human the thecal cells contain abundant phosphatase and 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 occurs ubiquitously in the ovary of the rat. It is demonstrable in the graffian follicles, corpora lutea, interstitial tissue and the blood vessels (Dempsey *et al.*, 1949). The enzyme disappears from the ovary of hypophysectomized rats but is restored on replacement therapy with pituitary powder.

Since the presence of alkaline phosphatase has not hitherto been reported in the avian ovary, it seemed desirable first to study the distribution and concentration of the enzyme in the ovary of normal pigeons, and secondly to note the effects of androgenic treatments on phosphatase activity in the gland.

EXPERIMENTAL.

The ovaries of the same normal and androgen treated pigeons which were involved in the experiment reported in the Part I of this series, were carefully dissected out and fixed immediately in chilled 80% alcohol. Paraffin sections, 6 microns in the thickness, were prepared and processed according to the technique

of Gomori (1941) for the demonstration of alkaline phosphatase. Sodium glycerophosphate was used as the substrate which was buffered to *p*H 9·5. The preparations were incubated in the substrate for 3 hours at 37°C. No counterstains were used in order to allow critical observation of the granular deposits of cobalt sulfide. The sections were dehydrated and mounted in the usual manner.

Results.

Controls.—There was very little alkaline phosphatase in the granulosa cells of the ovary. The endothelium of the vascular wreath situated in the theca showed strong reactions for the enzyme. Moderate phosphatase activity was evident in the nucleus of the thecal cells, while the enzymatic activity in the cytoplasm of these cells was negligible (Pl. II, Fig. 3). In the stroma, the endothelium of the blood vessels and the connective tissue cells showed phosphatase reactions, but the amount of enzyme in the interstitial cells was very slight.

Androgen treatment.—The ovary was greatly hypertrophied after androgen treatment. The induced maturation of the oocytes and the marked stromal growth were evident upon microscopical examination. The phosphatase activity is the granulosa cells of the ovary was practically absent. There was, however, a spectacular mobilization of the enzyme in the theca (Pl. II, Fig. 4). The nucleus and the cytoplasm of the thecal cells as well as the endothelium of the blood vessels showed strong positive reactions for the phosphatase. The nucleus of the interstitial cells in the stroma showed slight phosphatase activity, but the enzyme was totally absent from the cytoplasm of these cells. Moderate reactions for the phosphatase were given by the stromal connective tissue cells.

Discussion.

It is becoming increasingly evident that the hormones exert their regulatory effects on tissues and organs by influencing the rate of enzymatic processes. A number of recent studies have revealed a relationship between the endocrine functioning and the activity of the phosphatases (*vide* Dempsey *et al.*, 1949). These studies have clearly indicated the possibility that the relationship between the hormones and the phosphatases has a causal significance. Moreover, a series of investigations have proved beyond doubt that the phosphatases vary under different physiological conditions.

In view of the above, it is not surprising that the distribution and concentration of alkaline phosphatase in the ovary of the pigeon should change after androgenic treatments. It is well known that the injection of androgen at a suitable level causes stimulation of the avian ovary (for references see Kar, 1948). The histological consequences of this induced ovarian stimulation are the accelerated development of the follicular system and the marked stromal growth. To these, we add here an important cytochemical data, that is, the spectacular mobilization of alkaline phosphatase in the ovarian theca.

The story of the attempts to locate the possible sites for the production of the estrogenic hormones is now one of the classics of endocrinology. Efforts to collect and assay the various follicular components for estrogens showed that high concentrations occurred in the theca interna rather than in the granulosa (*vide* Dempsey, 1948). Moreover, it has been shown that lipid is a constant component of the theca interna (Dempsey, 1948). In view of these facts, it is not unlikely that the phosphatase plays some unknown rôle in the synthesis of estrogenic hormones in the ovary. The marked increase in the enzymatic activity in the ovarian theca of the testoid recipients provides an evidence in support of this concept. However, pending further studies little is to be gained by speculation concerning the possible rôle of the phosphatases in the production of estrogenic hormones in the ovary.

SUMMARY.

Part I.—Alkaline phosphatase is present in diffuse quantities in the uropygial gland of the pigeon. Androgenic treatments depress the activity of the enzyme but do not abolish it. It is suggested that the holocrine lipoidal cycle in this gland is mediated by the phosphatase through the influence of sex hormone.

Part II.—In the ovary of the normal pigeons alkaline phosphatase is demonstrable in the theca, blood vessels, and the stromal tissue. Androgenic treatments cause a heavy mobilization of the enzyme in the theca. The possible rôle of alkaline phosphatase in the ovary is discussed.

ACKNOWLEDGMENTS.

The author wishes to express his indebtedness to Dr. B. Mukerji, Director, Central Drugs and Pharmacognosy Laboratories, for providing facilities for research and for constant encouragement. Grateful acknowledgment is made to Dr. K. H. Gruschwitz of Ciba Pharma, Ltd., Calcutta, for the generous contribution of testosterone propionate (Perandren) used in this study. Thanks are due to Sri P. C. Pathak for the photomicrographs which illustrate this paper.

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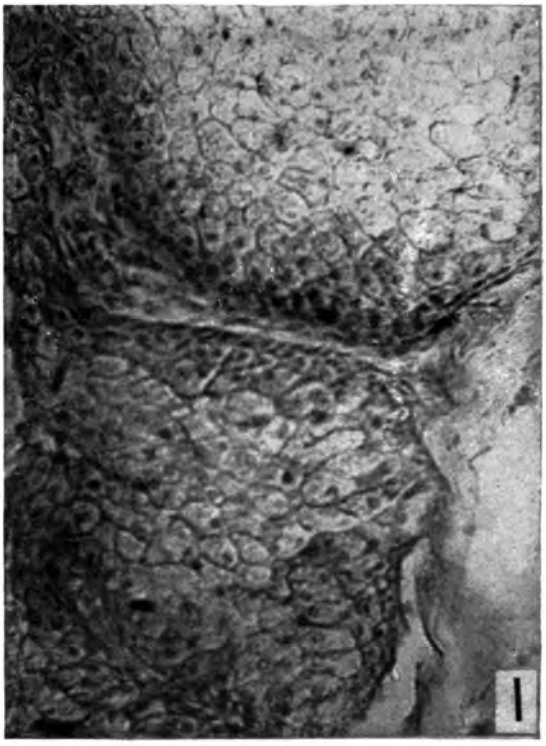
EXPLANATION OF PLATE II

Fig. 1. Photomicrograph of the section through the uropygial gland of a control pigeon (X 420). Note the presence of alkaline phosphatase in the alveoli.

„ 2. Photomicrograph of the section through the uropygial gland of a pigeon treated with androgen (X 420). The enzyme has practically disappeared from the stratified epithelium of the alveoli. Note presence of disintegrated cellular mass in the central region of the alveoli.

„ 3. Photomicrograph of the section through the ovary of a control pigeon showing the presence of alkaline phosphatase (X 140).

„ 4. Photomicrograph of the section through the ovary of an androgen treated pigeon (X 140). Note the heavy mobilization of alkaline phosphatase in the theca of the hypertrophied ovary.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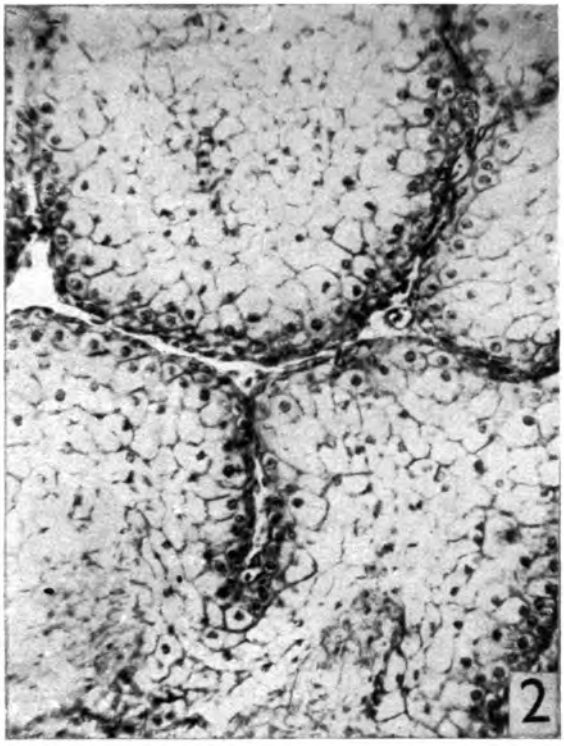
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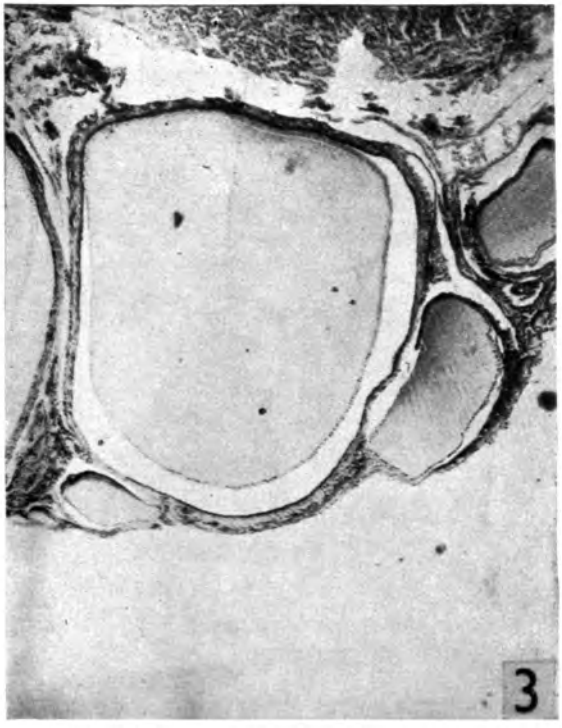
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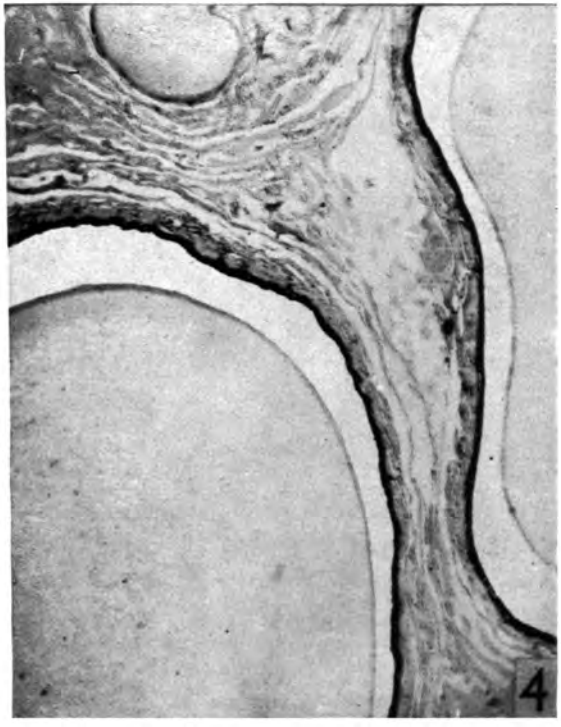
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