STUDIES ON CYTOCHEMISTRY OF HORMONE ACTION.

PART XI. THE DISTRIBUTION OF ASCORBIC ACID IN THE ADRENAL CORTEX OF NORMAL AND ESTROGEN-TREATED PIGEONS.

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INTRODUCTION.

Brisk chemical investigations are making it increasingly evident that ascorbic acid is intimately associated with the secretion of steroid hormones by the adrenal cortex (Giroud and Ratismamanga, 1942; Sayers *et al.*, 1945; Ludewig and Chanutin, 1947; and others). Moreover, many substances including the estrogens (Masonyi, 1936; Mlinko, 1938) and the ACTH (Sayers *et al.*, 1946) are now known to reduce the adreno-cortical ascorbic acid. It is believed that this reduction in ascorbic acid level reflects the inability of the cortical cells to synthesise steroid hormones (Deane and Morse, 1948). Sayers and Sayers (1948) also regard the ascorbic acid level as an useful indicator of the secretory activities of the adrenal cortex. The important rôle of this vitamin in the adreno-cortical physiology therefore, appears to be well recognised and in view of this we decided to investigate cytochemically the distribution of ascorbic acid in the adrenal cortex, using not only normal animals but also animals that had been treated with estrogenic hormone. The pigeon served as our animal of choice since the cytochemical demonstration of ascorbic acid in the adrenal cortex of this species has not hitherto been reported.

In the mammalian adrenal cortex, cytochemical studies have revealed that large quantities of ascorbic acid uniformly occur in the cells of the fasciculata and the reticularis but is either absent or present in small quantities in the glomerular zone (Gough and Zilva, 1933; Bessey *et al.*, 1934; Bourne, 1934 and 1935; Westergaard, 1934; Giroud and Leblond, 1935*a* and *b*, 1936; Leblond and Gardner, 1938; Barnett and Bourne, 1940; Tuba *et al.*, 1946; Deane and Morse, 1948).

EXPERIMENTAL PROCEDURE.

Adult pigeons were used in this study. A total of 12 birds were used of which 6 were injected with estrogen and the remaining 6 were left uninjected to serve as the controls. All of the birds were kept in cages under uniform husbandry conditions throughout the duration of the experimental period. Estradiol dipropionate in sterile sesame oil was intramuscularly injected (2·5 mgm. or 25,000 dipropionate units daily) for a period of 10 days. 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 animals were killed by a blow on the head in order to allow least possible ante-mortem trauma to the adrenal. The glands were fixed and processed according to the technique of Deane and Morse (1948) for the demonstration of ascorbic acid. The details of the technique as laid down by these authors were followed meticulously and only 50 seconds were allowed to elapse between killing an animal and placing the adrenal in the fixative. In order to make a critical observation of the silvered deposits

signifying ascorbic acid or reducing substance of similar activity, no counterstain was used. The sections were dehydrated and mounted in the usual manner.

RESULTS.

Controls.—Before presenting our observations on the distribution of ascorbic acid in the pigeon's adrenal cortex, we propose to make a brief comment on the microscopic organisation of the avian adrenal cortex since it differs in many respects from that of the mammalian cortex. In these animals the cortical tissue is found throughout the gland in islands and amastomosing strands interspersed with groups of medullary cells. The cortical masses are usually larger at the periphery than in the centre of the gland. Between the strands of cortical and medullary tissue is an intricate network of capillaries. The detailed cytological and cytochemical features of the avian adrenal cortex have been described in papers by Miller and Riddle (1942) and by Kar (1947 *a* and *b*, 1950, 1951).

Following fixation with the acid silver nitrate-alcohol solution granules of precipitated silver indicating ascorbic acid or reducing activity as great as that of ascorbic acid are consistently encountered in the cytoplasm of the cortical cells. The granules are absent in the nuclei and their cytoplasmic distribution appears to be irregular (Pl. XI, fig. 1). In addition to their intracellular location, the silver precipitates also occur in the vascular sinusoids. The granules are mostly irregular in outline and appear fairly crowded in the cortical masses. The distribution of the silvered particles in the peripheral and central cortical masses are more or less uniform.

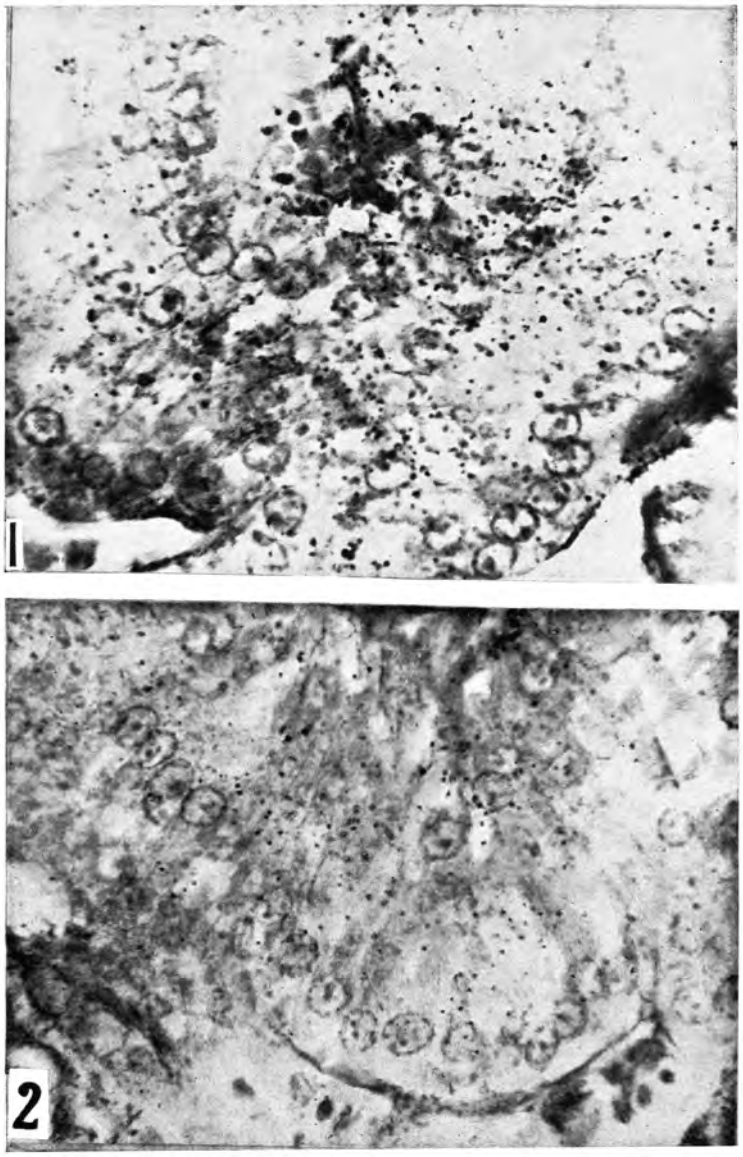
Estrogen treated.—There is a definite reduction in the amount of reduced silver in the cortex of the estrogen recipients. However, the gross pattern of distribution of the particles remains the same as in the controls. The silvered granules appear much finer and considerably sparser than in the controls (Pl. XI, fig. 2). In some cortical cells only a few particles are encountered. There is also a marked loss of the precipitated silver from the vascular sinusoids.

COMMENTARY.

The present studies have indicated clearly that ascorbic acid (or reducing substance of similar activity) is normally present in the parenchymal cells of the pigeon's adrenal cortex. Treatment with estrogenic hormone considerably depletes cortical ascorbic acid level. Our cytochemical findings, therefore, appear to be in agreement with the chemical observations of Mosonyi (1936) and Mlinko (1938) who reported a similar loss of ascorbic acid from the adrenal cortex after estrogen treatment. Moreover, in the light of the current concept that the decline in ascorbic acid level is associated with the inability of the cortical cells to synthesise steroid hormones (Deane and Morse, 1948), it would appear that estrogen treatment considerably affected steroid hormone production in the pigeon's adrenal cortex. However, we venture to make this speculation with considerable reservation, since according to Sayers and Sayers (1948), it has not been possible yet to decipher the precise rôle this vitamin plays in the series of reactions leading to the transformation of cholesterol into cortical hormone(s). In this connection, Lowenstein and Zwemer's unconfirmed report (1946) that the cortical steroid may exist as a conjugate of ascorbic acid appears to be suggestive, but until confirmation of this finding is available it would be unwise to speculate on its significance.

SUMMARY.

The distribution of ascorbic acid has been studied cytochemically in the adrenal cortex of the pigeon. Estrogen treatment considerably depletes adreno-cortical ascorbic acid. The significance of this vitaminic depletion is discussed.



(*All figures are photomicrographs and are magnified* $\times 700$).

FIG. 1. Section through the adrenal gland of a control pigeon. Note the distribution of ascorbic acid in the form of dark granules.

„ 2. Section through the adrenal gland of an estrogen treated pigeon. Ascorbic acid is markedly reduced in amount. The granules appear finer and sparser than in the control animals.

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