CHANGES INDUCED IN THE ADRENAL CORTICAL ZONES BY OVARIAN HORMONES

DONALD J. KIMELDORF AND A. L. SODERWALL
From the Department of Biology, University of Oregon
EUGENE, OREGON

THAT THE FEMALE gonadic hormones influence the adrenal glands in rodents has been demonstrated for the female rat (Ellison and Birch, 1936; Anderson, 1934), mouse (Danner, 1938) and guinea pig (Allen and Bern, 1942). Changes in the adrenal cortex have also been noted during the reproductive cycle indicating a correlation between ovarian physiology and adrenal cortical functions (Anderson and Kennedy; 1932; Zalesky, 1934; Zuckerman, Bourne and Lewes, 1938). In all of these studies the initial effect of estrogen injection was a marked hypertrophy of the adrenal cortex. Similar treatment using progesterone yielded conflicting pictures with Clausen (1940) reporting an actual decrease in cortex while Selye, Brown and Collip (1936) and Selye (1940) noted no effect.

Accepting the hypothesis presented by Bennett (1939) and Deane and Greep (1946) indicating specialized zones for active production of adrenocortical hormones, it is the purpose of this report to present data concerning the influence exerted by natural ovarian hormones on the individual cortical layers of the adrenal.

The adrenal gland in the guinea pig presents suitable material for both macroscopic and histological examinations. The three cortical layers are particularly well-defined and have proven very sensitive to hormonal influences. The relatively large size of the gland lends an added advantage.

MATERIALS AND METHODS

Thirty-five virgin female guinea pigs of age $4\frac{1}{2}$ to $5\frac{1}{2}$ months and weight 450–650 grams were observed for normal cyclic estrous behavior. Twenty-three of this group were spayed and allowed a recuperative period of 21 days. Each was then tested for normal estrous response to administration of ovarian hormones[1] utilizing the standardized technique of Boling, Young and Dempsey (1938). All animals exhibited capacity to respond normally to female sex hormones.

The spayed animals were placed in the following groups: Group I included nine animals which received estrogen injections. Three spayed ani-

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[1] Estrogen, in the form of Progynon-B, and progesterone, in the form of Proluton, were supplied by the Schering Corporation, Inc., Bloomfield, N. J., through the courtesy of Dr. Erwin Schwenk.

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mals received three subcutaneous injections of five R. U. estrogen at 48 hour intervals. Forty-eight hours after the last injection they were sacrificed, the adrenals removed, sectioned and stained for histological examination. Three spayed animals of the same age and weight class as above received ten R. U. estrogen at 48 hour intervals. This dosage has previously been found adequate to sensitize the guinea pig prior to injection of progesterone for inducing the behavioral heat reaction (Collins, Boling, Dempsey and Young, 1938). The glands were removed and prepared for study. Three spayed animals of the same class received twenty R. U. estrogen at 48 hour intervals.

Group II included nine progesterone-injected spayed females: Three animals received three subcutaneous injections of 0.05 mg. at 48 hour intervals. Three animals received 0.1 mg. at similar intervals. This dosage is sufficient to induce heat in an estrogen-conditioned animal (Collins, Boling, Dempsey and Young, 1938). Three animals received 0.2 mg. progesterone.

Group III consisted of 12 normal animals in the 450–650 gram weight class. This constituted the normal control group. All animals in this group were known to be in the diestrous condition when sacrificed.

Group IV was composed of five animals standardized similarly to group III but were spayed and sacrificed in the same manner as the experimental groups. Groups III and IV received injections of sesame oil instead of the hormones administered to the experimental groups.

All glands were fixed in Bensley's bichromate-sublimate-formalin mixture for 24 hours and hardened in 3% bichromate for three weeks. The left adrenal was used in each case as a further standardization. Sections were made at four to eight micra depending upon the subsequent histological or cytological studies. Two routine stains were employed: Regaud's iron-hematoxylin or Krichesky-Mallory triple stain. For some of the glands, frozen sections were stained with Scharlach-R for examination of lipoid inclusions within the cortex.

Each gland was weighed to the nearest tenth-milligram. These weights were compared with the body weights and a table prepared showing the relative milligram weight of adrenal to gram weight of body for a description of effects. Histological examination included the observation and measurements of the three cortical layers. Sections with largest cross-sectional area were projected on a screen and outline tracings made of the cortical zones. These tracings were measured by a planimeter set at a constant arm length and are summarized in Tables 1 and 2. These measurements were reproducible within an error of one per cent.

In the evaluation of results, Fisher's test for significant differences between small groups was applied. When the t-value of the two groups being compared was less than 2.2, the difference was not considered statistically significant.

RESULTS

Table 1 shows the average relative weight of adrenals as influenced by the treatment indicated. (Relative adrenal weight refers to milligram of adrenal per gram body weight. For simplification of presentation this figure was multiplied by a constant, 10^7.) Each figure represents the average value for the particular group. The control group was significantly the lowest with an average relative weight of

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2345. A comparison of the estrogen-injected group with the controls shows clearly the marked effect exercised by the female hormone on the relative adrenal weight. This increase in relative weight is correlated with a substantial increase in cortical width as shown by the column "Entire Cortex".

The relative adrenal weight of the progesterone-treated animals,

TABLE 1. SUMMARY OF GROUP AVERAGES FOR RELATIVE ADRENAL WEIGHTS, CORTEX SIZE AND MEDULLA SIZE. THE "t" VALUES* REFER TO COMPARISONS WITH THE NORMAL CONTROLS. CORTEX AND MEDULLA MEASUREMENTS ARE IN PLANIMETER UNITS

Condition of animals	No.	Adr. wt. / Body wt. $\times 10^7$	"t" value	Cortex size	"t" value	Medulla size
Controls	12	2345	—	16,949	—	558
Castrates	5	2558	1.6	19,364	0.5	528
Estrogen treated	9	3554	59.6	25,710	23.6	483
Progesterone treated	9	2874	46.4	20,427	10.3	519

$$* t = \frac{\bar{x} - \bar{y}}{\sqrt{\frac{n_1 Sx^2 + n_2 Sy^2}{n_1 + n_2 - 2}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}.$$

TABLE 2. SUMMARY OF GROUP AVERAGES FOR THE SIZE OF THE INDIVIDUAL CORTICAL ZONES. THE "t" VALUE HAS THE SAME REFERENT AS IN TABLE 1

Condition of animals	No.	Glom. zone	"t" value	Fasc. zone	"t" value	Ret. zone	"t" value
Controls	12	622	—	6331	—	2924	—
Castrates	5	746	1.8	6001	1.1	3265	1.3
Estrogen treated	9	718	8.1	4236	92.8	5542	57.8
Progesterone treated	9	824	74.8	6283	1.7	3319	16.5

like those receiving estrogen, was significantly greater than the normal controls and castrates. As indicated in Table 1 this effect in the cortical width was considerably less than the estrogen-treated group.

In confirmation of other workers (Allen and Bern, 1942) it is to be noted that the planimeter determinations indicate no significant effect on the medulla following castration or sex hormone administration. The castrate group showed no significant difference in relative adrenal weight and cortex size when compared with the normal controls.

Table 2 summarizes planimeter measurements relative to the individual cortical layers in each experimental and control group. The results of the measurements give a picture of a significantly increased width of the zona glomerulosa following injection of the hormones. Of the hormones administered, progesterone exerted a much greater influence on the glomerulosa area than did estrogen. It is of interest to note that the respective dosages usually employed to condition and induce heat in the guinea pig were found to produce

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the greatest effect on the glomerulosa. The actual significance of this observation awaits further confirmation.

The effects produced by the individual hormones on the fascicular zone revealed a very marked decrease in area of the estrogen-treated females when compared to the controls. No such effect was observed in the progesterone-treated animals. With reference to the zona reticularis the estrogen effect is very pronounced as an increased zonal area in comparison to the controls. The same zone was significantly enlarged in the progesterone-treated animals. No apparent change in size of the individual zones was noted after castration in the guinea pig.

The cellular detail of each zone was compared among the three groups. In the estrogen series the fascicular cells became hypertrophied. The microliposomes became more consistently of a large size. Even more pronounced is the frequency of large macroliposomes. In those animals which received 20 R. U. of estrogen, the fascicular layer appeared to be fenestrated with macroliposomes. The progesterone series resembled more closely the controls in the number of macroliposomes but approached the estrogen series in cell size. Frozen sections stained with Scharlach-R revealed an increased amount of lipoid inclusions in the hypertrophied cells.

The reticularis zone of the controls consisted largely of small irregular, deeply staining cells with an occasional light staining vacuolated cell. These dark shrunken cells had all the characteristics of degeneration and pycnosis. It was these dark staining cells which increased tremendously in number to produce the enlarged reticular zone of the estrogen-injected animals. The progesterone-injected animals showed little increase in the frequency of these cells.

The glomerulosa zone consisted of spherical clusters of cells. Each cell contained very finely dispersed lipoid vacuoles. These cells were hypertrophied particularly in the progesterone series.

Within each experimental group there was a shift in the widths of cortical zones which is worthy of note. In response to the estrogen treatment, a dramatic alteration in zonal areas was noted. The fasciculatis zone became greatly reduced and was accompanied by a comparably strong shift towards a widened reticularis zone. In response to progesterone, there was a pronounced enlargement in the glomerulosa with a smaller increment in the reticularis zone. No significant change was noted in the fascicular zone size.

DISCUSSION

Studies of the adrenal during pregnancy, during estrous periods, after administration of toxic substances, and in the present type of investigation indicate that the mechanism of hypertrophy and increased weight might be effected by a similar mode of action upon the adrenal cortex. Evidence that the pituitary is responsible for cor-

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tical hypertrophy has accumulated (Swann, 1940; Tepperman, Engel and Long, 1943). That such hypertrophy means increased cortical secretion has not yet been demonstrated. In the present study the increment of cell-size rather than hyperplasia appears to be responsible for the increased size of the cortex. The cellular hypertrophy was correlated with enlarged microliposomes and an increase in the number of macroliposomes.

Hoerr (1932) studying regeneration in the adrenal cortex concluded that cells were continually dying off in the reticular zone and being replaced by cells produced in the outer levels of the cortex. He concluded that the light and dark cells of the reticularis represented degenerating cortical cells in the last stage of senescence. This concept of centripetal migration of adrenal cortical cells has been supported by the study of Wotton and Zwemer (1943). In line with this concept Bennett (1939) utilizing histochemical reactions for detection of cortical hormones, designated the sub-capsular cortex as a pre-secretory zone, the outer portion of the fasciculata as secretory zone, and the portion of the cortex between the secretory zone and medulla as post-secretory zone which is characterized by the presence of numerous degenerating senescent cells. The increase noted in the frequency of necrotic cells in the enlarged reticularis zone following estrogen administration, is similar to the action of a toxic substance. In Hoerr's work, wherein guinea pigs were subjected to noxious gases and other toxic chemicals, similar increase in number of pycnotic cells was noted in the reticularis zone.

The increase in the dark-celled reticularis of estrogen-injected animals suggests the possibility of toxic effects of estrogen compounds. It has been found that estrogens are toxic to adrenalectomized rats and ferrets (Gaunt and Hays, 1938; Gaunt, Potts and Loomis, 1938; Cavanaugh and Gaunt, 1937; Schacher, Browne and Selye, 1937). The toxic effect of estrogen upon the cortical cells may stimulate compensatory secretion of the adrenocorticotropic hormone resulting in hypertrophy of the cortex.

Progesterone is known to be life-maintaining in adrenalectomized animals (Gaunt and Hays, 1938; Anderson and Kennedy, 1933; Greene, Wells and Ivy, 1939; D'Amour and D'Amour, 1937; Collings, 1939). This is suggestive in part for the differences observed in the effects between estrogen and progesterone on the adrenal cortical zones of the spayed female guinea pig. Histologically the administration of progesterone did not decrease the size of the fascicular zone of the adrenal cortex.

SUMMARY

The injection of moderate amounts of estrogen into spayed female guinea pigs for a short period of time instigated hypertrophy of the adrenal cortex with an increase of relative adrenal weight over that of the normal and castrated controls. Histological study revealed

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that cortical hypertrophy was due to an increase in cell size in the fascicular and glomerulosar zones. Planimeter measurements were made of cross-sections through all glands. In the estrogen series the reticular zone of degenerate cells was greatly increased in size, the fascicular zone was reduced, while the glomerulosar zone was increased as compared to the controls.

A review of the recent literature indicates that the hypertrophy may be due to mediation of the anterior pituitary. In view of studies indicating differential activities of the cortical zones, the suggestion is made that estrogen causes accelerated degeneration of the cortical cells. This, in turn, stimulates compensatory secretion of an adrenocorticotropic factor to produce hypertrophy of the secretory cortical cells.

The injection of moderate amounts of progesterone for short periods of time caused an increase in relative adrenal weight and an enlargement of the glomerulosar and reticularis zones. Histologically the cortical cells showed an increment of size but otherwise resembled the controls. The life-maintaining properties of progesterone and the toxicity of estrogens are reflected in this study by the differential adrenal responses to injection of these two sex hormones.

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