THE EFFECT OF ADRENALECTOMY ON THE THYROID
GLANDS AND BLOOD OF MALE CHICKENS

by

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INTRODUCTION

That the thyroids of birds might be affected by the removal of the adrenal glands is suggested from the fact that one endocrine gland frequently affects another. In some cases, the abnormal functioning of one endocrine gland affects all or nearly all the other endocrine glands and there may even be reciprocal effects. This illustrates the closeness with which the functions of the endocrine system are related.

The experimental work described in this thesis was done to determine if the thyroids of fowls are altered after removal of the adrenals. This work follows that of Herrick and Torstveit (1958) in which they determined that adrenalectomy in fowls resulted in degeneration of the gonads and alteration of the secondary sexual characteristics. The thyroids from the experimental birds of these workers were made available for the study reported in this thesis.

REVIEW OF LITERATURE

The first adrenalectomy in birds was performed by Gourfein (1936) who found the operation was fatal to pigeons, his experimental animals, within four to 24 hours. Parkes and Selye (1956) reported that adrenalectomy in adult ducks and fowls resulted in a short survival period if bilateral adrenalectomy was accomplished in one operation. Herrick and Torstveit (1958) used a 0.9 per cent sodium chloride solution for drinking water to keep alive for long periods of time, male chickens deprived of their adrenals.
Other workers in this field have found that extirpation of the adrenals caused death of the animals soon afterward. Jaffe (1926) removed the adrenals from 80 rats, the majority of which died before the thirteenth day. Bogoff and Stewart (1926) using 74 dogs determined the average survival period of their animals to be about seven days. Elliott (1914) operated on cats allowing an interval of three to nine months to elapse between the removal of the left and right adrenal. With this technique he found the survival period of cats to be six to 25 days.

That the adrenal cortex is necessary for life has been shown by a number of investigators. Crowe and Wislocki (1914) removed the left adrenal gland from cats, dogs, and rabbits, removed one-half of the right gland, and cauterized the medullary substance in the remaining half of the gland. The animals remained in good health for long periods. Subsequent histological examination revealed no medullary tissue present in the remaining gland. Houssay and Lewis (1925) found dogs to remain normal after splitting the left adrenal, scooping out the medulla, and ten days later excising the right gland.

Life is maintained in the experimental animal if only a small portion of the adrenal cortex is left. Hoskins (1915) found that dogs survive after removal of as much as seven-tenths of the total mass of the glands. Wislocki and Crowe (1922) concluded that about one-fifth is necessary, while Whipple and Christman (1914) gave examples of dogs in which one-sixth or perhaps even a smaller fraction of the adrenal tissue sufficed.
That it is the cortical tissue and not the medullary that is responsible for the fatal outcome, which follows adrenalectomy, is now generally accepted as an incontrovertible fact according to Grollman (1936).

Banting and Cairns (1926) observed that dogs, deprived of their adrenals and surviving less than 50 hours, showed the following symptoms:

- rapid pulse
- elevated temperature
- thirst
- restlessness
- salivation
- weakness
- loss of appetite
- drop in blood sugar
- anuria
- vomiting
- diarrhea
- convulsions

Hoskins (1915) found that partial adrenal deficiency results in a depression in the irritability of the sympathetic nervous system. He concluded that this depression is probably only one phase of a generalized interference with fundamental metabolism.

Rogers (1922) determined experimentally that feeding to dogs derivatives of the entire adrenal gland, especially the adrenal nucleoproteins and a slightly aqueous extract known as the adrenal residue, causes the animal's thyroid to gain from 50 to 70 per cent or more in its iodine content within a few weeks. He attributed the gain in percentage of iodine to the cortex, for adrenalin crystals fed in corresponding amounts had no appreciable effect upon the thyroid. Black, Hupper, and Rogers (1922) report the same findings upon administering adrenal extracts to dogs.

It is believed by Swingle, Pfiffner, et al., (1935) that the chief function of the adrenal cortical hormone is the regulation and maintenance of a normal circulating volume of fluid within the vascular system.
In the absence of the hormone, fluid is continuously lost from the circulation, presumably from transudation through the capillary walls, with the results that the adrenalectomised animal is unable to maintain his normal blood volume, and eventually dies from circulatory collapse due to insufficiency of circulating fluid.

Sun (1927) found the epinephrin content in thyroidless rats to be higher than in those of control rats.

In studying the effect of diet upon young and old adrenalectomized rats Swann (1935) found a fresh milk and bread diet resulted in greater length of life among their operated animals.

Zwemer and Truszkowski (1937) produced the various manifestations of the syndrome of adrenal insufficiency by injecting the solutions of potassium salts in amounts which raise and maintain the plasma potassium levels found in adrenalectomized animals during similar phases of the syndrome.

Kutz, McKeown, and Selye (1935) treated 21 adrenalectomised female rats with Rubin salt mixture. From 13 of these animals which were maintained in good health by salt, 14 remained normally oestric. The Rubin (1935) salt mixture in this case consisted of 0.0329% CaCl₂, 0.015% MgCl₂, 0.7% NaCl, and 0.085% KCl.

Davis and Hastings (1935) regarded the results of their study to suggest a relationship between the adrenal and the thyroid gland which Marine and Sowaann (1921) at first explained as follows: "They supposed that the adrenal exerted some inhibitory influence on the thyroids and
that, when this inhibition was removed, the activity of the thyroids was increased." More recently Marine (1950) suggested that the increased production of thyroid hormone in Graves' disease was a physiological attempt toward compensation of deficiency of some function of the adrenal cortex and sex glands.

Mahorner (1932) attempted to produce hyperplasia in the thyroid glands of dogs by destroying a part of the adrenals and by infection. He removed a small part of the thyroid by which to compare the results. Using 10 dogs for his experiment he reported that seven showed no change, one showed retrogression, and two showed slight hyperplasia. One of the dogs showing hyperplasia died of pneumonia; thus he concluded that infection may have played a part in the change. His criteria for judging changes in the thyroid were an apparent decrease in the colloid and a heaping up of the epithelial cells which he called hyperplasia.

Zweener (1925) removed the thyroids and left adrenal at one operation, 10 days later the right adrenal was removed. The parathyroids were replaced by transplantation in the neck muscles to prevent tetany. He found the survival period to be three times longer in his animals than when the thyroids are present in adrenalectomized animals. Cameron and Sedskiak (1921) fed thyroid to adult white rats and found an increase in the weight of the adrenal glands, and decrease in the weight of the thyroid.

Hoskins, E. P. (1916) and Hoskins, R. C. (1910) obtained adrenal hypertrophy after feeding with thyroid. That there is nothing specific
about the increase in size of the adrenals is evident, since similar hypertrophy is found in other organs, including the pancreas.

Small doses of adrenalin were injected into cats by Cannon and Cattell (1916) by which they produced an action current in the thyroid and secretory activity in the gland. Stimulation of the splanchnics leading to adrenal discharge, produces the same results. Levy (1916) observed that the injection of adrenalin sensitised the vasomotor system to the action of succeeding injections of adrenalin. He produced evidence to show that this sensitisation is due to thyroid discharge produced by the first injection of adrenalin.

Marine and Bauman (1922) reported partial destruction of the suprarenal cortex in rabbits with intact thyroids usually led to increased heat production. Removal of the thyroids prevented or lessened the increased heat production.

Best, Supernaw, Lieberman, and Munro (1928) studied the effect on the thyroid and adrenal glands due to lack of sleep in rabbits. They state that:

In normal rabbits the thyroid nuclei lie in the central or slightly basal part of the cells. They are round or slightly oval depending upon the shape of the cells, and have an average diameter of about six micra. The nuclear membrane is well defined while the content usually takes a diffuse basic stain. The chromatin is quite uniformly distributed. The chromatin net knots and nucleoli are visible but not especially prominent. The nucleoli are from one to four in number, .... In the thyroid glands of exhausted rabbits the nuclei are swollen and measure seven to eight micra in diameter. The chromatin is not diffusely distributed as in the normal nucleus but is present in small masses lying close to the nuclear membrane while the nucleoli retain their normal position.
In five cases of Addison's disease, in which the adrenals were atrophied, Susman (1950) reported lymphocytic infiltration of the thyroid with varying anatomical changes occurring.

Chourke (1950) studying the thyroid glands of ovariectomized female guinea pigs and found that castration causes neither an appreciable change in the proliferative activity nor in the structural characteristics of the thyroid gland. The structure of the thyroid gland seemed to show a tendency toward an increase in the amount of colloid and toward a flattening of the acinar epithelium with advancing age. Kippen and Loeb (1956) removed the gonads of guinea pigs. At an early stage of maturity there was stimulation of the thyroid gland as evidenced by an increase in the average number of mitoses and also phagocytic cells in the colloid. The phagocyte cells are well preserved in the softer colloid of the stimulated gland. The increase in the number and preservation of the phagocytes they considered a sign of stimulation of the gland. Localized accumulation of lymphocytes between the acini in the thyroid gland were found. The greatest number of mitoses were found in the first week after gonadectomy. These workers noted that the stimulation of the thyroid accomplished by this means was moderate and comparable to the effects obtained by the administration of iodine.

Crile (1928) presented a view concerning the relationship of the adrenals and thyroid to the organism:

After a latent period of twelve or more hours iodine and thyroid extract increase the electric conductivity and the capacity of the organs and tissues of the body. In physiological terms, a change in the conductivity and capacity of a tissue signifies a change in the function and the activity of that tissue. These
physical changes, like the clinical changes due to thyroid activity, are lasting and not fleeting. Increased conductivity and capacity means increased functional activity. These changes alone would increase oxidation, hence would increase basal metabolism.

Our experiments have shown that the effect of adrenalin on the capacity and conductivity of tissues and organs is wholly different from effect of thyroid extract and of iodine; that is while the effects of thyroid extract and of iodine are not manifested until after a considerable latent period, adrenalin causes an immediate and a striking change in capacity and conductivity. Moreover, the effects of adrenalin last for only a few minutes while the effects of thyroid extract and iodine last for hours and days. The most striking characteristic of the adrenalin effect, however, is its unexpected selective action on the organs and tissues of the body; that is, adrenalin causes a sharp rise in the conductivity, temperature, and capacity of the nervous tissue, but shows the opposite effect on the conductivity, temperature and capacity of all other tissues, with one notable exception, namely, the medulla of the adrenal gland itself. At first this highly selective action of adrenalin on the tissues and organs seemed incomprehensible until it occurred to us that differences in the potential of the organs of the body might well depend on this very effect.

It would appear that the organism as a whole has been evolved on the principle of electric control, and that it is the function of the adrenal glands and of the vegetative nervous system to establish and maintain and adaptively to change the differences in potential among the different organs and tissues, in order to meet the conditions of struggle and survival. If this conception be true, then not only is there a collaboration between the adrenals and thyroid—concerned not only with the struggle of life but with the maintenance of life itself.

In counting the total number of thrombocytes in the blood of 15 normal rats, Shocket, Friedman and Rice (1935) found the average count to be 477,550 per c.mm. Just before death in the adrenalectomized animals the average count was 840,270 per c.mm., an average increase of 76 per cent.

Fox and Whitehead (1935) by injecting cortico-adrenal extract sought to determine its effect on the leucocyte count in the blood of normal adult rabbits. They reported that the white blood cells and a differential count were well within normal limits during the time of their experiment.
Corey and Britton (1932a) found normal pre-operative counts of the red blood cells in the blood of cats to average 9,800,000. With the development of adrenal insufficiency the average count rose to 15,500,000, an increase to nearly 60 per cent. The total white blood cells averaged 12,500 per c. mm. in the normals. After operation the count dropped to an average of 7,000 cells. The lymphocytes increased in percentage to the decrease in neutrophils. These same workers (1932b) found a 100 to 200 per cent increase of the neutrophil count in the blood of rats and cats treated with cortico-adrenal extract. With this increase of neutrophils a pronounced decrease in lymphocytes was observed.

Shaw (1930) sought to eliminate the use of an indirect method in counting cellular bodies in the blood of birds. In order to make differential counts of blood cells, stains were employed, made up as follows:

<table>
<thead>
<tr>
<th>Solution A</th>
<th>Neutral Red (Grubler) 25.0 mgm.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NaCl 0.9 grm.</td>
</tr>
<tr>
<td></td>
<td>distilled water 100.0 cc.</td>
</tr>
<tr>
<td>Solution B</td>
<td>Crystal Violet (Grubler) 12.0 mgm.</td>
</tr>
<tr>
<td></td>
<td>Sodium citrate 5.6 grm.</td>
</tr>
<tr>
<td></td>
<td>Formaldehyde 0.4 cc.</td>
</tr>
<tr>
<td></td>
<td>distilled water 100.0 cc.</td>
</tr>
</tbody>
</table>

MATERIALS AND METHODS

Adrenalectomy, Care, and Histological Preparation

Adrenalectomy as performed on the fowls used in this study is well described by Herrick and Torstveit (1939) who developed the technique. In brief the operation consists of entering the body between the last two
ribs much as in caaponizing, removing the adrenals by means of cautery, pulling the ribs together with threads, and suturing the skin. Two operations were performed in total adrenal removal. One week usually intervened between the removal of the first and the second adrenal gland.

Cortical extract, Eschatin, was given to each bird at the close of the second operation and was continued for two or three days afterward. One cc. was the usual daily amount given. After the discontinuance of the eschatin injections the animals were injected intramuscularly, every day, with 12 to 15 cc. of normal saline solution. Salt was kept in their drinking water at all times after the operation.

Each animal had a separate pen in the Experiment Station animal house. A weekly recording of their weight and comb size was made.

Their food during this time was the same diet given to the normal animals. This diet consisted of: corn meal—39 per cent, ground wheat—29 per cent, dried milk—12 per cent, alfalfa meal—5 per cent, and salt—1 per cent. Cod-liver oil was added to this diet.

The length of time that each animal lived after the operation varied for two reasons. Some adrenalectomized birds died within a short time; while others were allowed to live for several weeks before they were sacrificed. Whenever an animal died or was sacrificed the thyroid glands were immediately removed, placed in Bouin's fixative for 24 hours, removed and run through several changes of 70 per cent alcohol. Final dehydration was affected by passing the tissues through a graded series of alcohol including absolute. These were imbedded in paraffin and sectioned at six micra. All tissues used in this study were stained in
Ehrlich's hematoxylin and eosin.

Blood Counts

For making the blood counts seven White Leghorn male chickens were used; four of these chickens had undergone complete adrenalectomy, the other three were used as controls. Two separate counts of the controls were made a week apart and at approximately the same hour of the day in order to avoid error due to diurnal fluctuations. This provided the standard with which the chickens with their adrenals removed compared. Only one count of the adrenalectomized chickens was possible for, if during examination all the adrenal tissue was found to be removed, accompanied by a reduction in the size of the testes, they were sacrificed for other experiments. The animals deprived of adrenals were given 0.6 cc. of Nembutal prior to the taking of the blood whereas, the controls were not anesthetized.

Avian blood clots quickly. To overcome this a scalpel was employed to open a wing vein. A large flow of blood was readily obtained from which a sample was taken for blood counts. The skin was washed with alcohol before each incision.

The blood cell counts were made using a Levy hemocytometer with Neubauer ruling. After obtaining blood, the stem of the hemoglobinometer was filled to the 0.5 mark immediately followed with Solution A (Shaw 1930) drawing it to fill one-half of the bulb. This was shaken for about 15 seconds then Solution B was added to bring the prepared blood
maple to the mark (101). It was found best to shake the mixture for about 5 minutes. Two drops were allowed to escape; the third and fourth drops were placed in the Levy chamber. The cells were given several minutes to settle before the blood count was made. This consisted of counting the cells in 40 small squares on each side of the hemocytometer. The number of red and white blood cells in these squares were computed and an average made in order to obtain what is called a blood count in this study.

Because fowl blood does clot rapidly an extra measure of precaution was taken to overcome this. After thoroughly cleaning, the hemoglobinometer was placed close to an electric lamp which warmed it considerably. This warming also aided in driving off any water or remaining alcohol. The pipette was then removed and held in the hand until it had cooled to about human body temperature. It was then ready for use in taking a blood sample. The body temperature of fowls is higher than that of the human body so by this rough determination of the temperature of the pipette the operator had an instrument near the temperature of fowl blood as it flowed from the vein.

**OBSERVATIONS AND RESULTS**

**Normal Thyroid**

After the thyroid tissues were sectioned and stained they were studied in detail microscopically using magnifications of near 500 diameters. Since the nature of the epithelium in the acini of the thyroid
gives an index to the activity of the gland, the nature of the epithelium was ascertained as accurately as possible.

The follicular epithelium of the thyroids of normal fowls was cuboidal in most cases (Figure 1) but some low and columnar types were also present. Round nuclei occupied the central portions of the cells. Occasional phagocytes were seen in the colloidal material and a few lymphocytes were found between the acini of the gland. The phagocytes found in the normal thyroid were of the characteristic monocyte type, a small nucleus surrounded by a relatively large amount of cytoplasm. Few follicles showed any vacuolization.

Thyroids of Adrenalectomized Birds

The thyroids from thirteen adrenalectomized birds and one capon were used in this study (Table 2). Eight thyroids showed a heavy infiltration of lymphocytes in the colloid and two showed a lesser number of lymphocytes. The remaining tissues had a few lymphocytes in the colloid. In all tissues these white blood cells could be found between the acini in the gland in fairly large numbers. The lymphocytes were clearly distinguishable by the large nucleus and the rim of clear cytoplasm surrounding the nucleus.

All birds with the exception of A-157 and A-252 had soft colloidal material in their thyroids. It was observed that few phagocytes were found in these glands. Of six thyroids having cuboidal epithelium, five showed much high columnar epithelium as well as the piling up of tissue
Table 1. Results of blood counts in normal and adrenalectomized fowls. (The factor to be used with this table is 20,000 to give numbers in cubic millimeters of blood.)

<table>
<thead>
<tr>
<th>Chicken No.</th>
<th>Rhythrocytes</th>
<th>Mast cells</th>
<th>Monocytes</th>
<th>Thrombocytes</th>
<th>Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-152</td>
<td>15</td>
<td>5.0</td>
<td>4.0</td>
<td>5.0</td>
<td>11.0</td>
</tr>
<tr>
<td>B-177</td>
<td>16</td>
<td>3.0</td>
<td>5.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>B-159</td>
<td>15</td>
<td>1.0</td>
<td>4.0</td>
<td>5.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Average</td>
<td>15</td>
<td>1.7</td>
<td>5.7</td>
<td>4.5</td>
<td>6.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chicken No.</th>
<th>Rhythrocytes</th>
<th>Mast cells</th>
<th>Monocytes</th>
<th>Thrombocytes</th>
<th>Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-145</td>
<td>17</td>
<td>4.0</td>
<td>4.0</td>
<td>2.0</td>
<td>7.0</td>
</tr>
<tr>
<td>B-146</td>
<td>15</td>
<td>2.0</td>
<td>7.0</td>
<td>2.0</td>
<td>5.0</td>
</tr>
<tr>
<td>B-147</td>
<td>11</td>
<td>1.0</td>
<td>12.0</td>
<td>6.0</td>
<td>12.0</td>
</tr>
<tr>
<td>Average</td>
<td>11</td>
<td>1.0</td>
<td>8.7</td>
<td>6.0</td>
<td>7.0**</td>
</tr>
</tbody>
</table>

* First count made July 12.
** Second count made July 15.

Table 2. Study of thyroid gland.

<table>
<thead>
<tr>
<th>Chicken No.</th>
<th>Days</th>
<th>Type of Epithelium</th>
<th>Colloid Consistency</th>
<th>Lymphocyte numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-137</td>
<td>21</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

Adrenalectomized Birds

Gonadectomized Bird
<table>
<thead>
<tr>
<th>Chicken No.</th>
<th>Days</th>
<th>Type of Epithelium</th>
<th>Colloid Consistency</th>
<th>Lympohocyte Numbers</th>
<th>Infiltartion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Bird</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
partially to fill several follicles in each gland. One bird A-60 (Figure 5) had a heavy infiltration of lymphocytes between the acini as well as clusters of lymphocytes surrounded by a growth of epithelial tissue. Whether this growth was formed as a protective measure or was the result of stimulation by these white blood cells is not known.

Hyperplasia was found in the thyroid of the capon, and epithelial growth was noted to have filled some follicles (Figure 2). This gland apparently had been stimulated more than glands from four adrenalectomized animals showing a similar picture but in lesser degree.

The characteristic condition found in the thyroids of birds deprived of their adrenals, was low or flat epithelium (Figure 4). The nuclei were flat and the cytoplasm appeared next to adjacent cells rather than apically or basally. The follicles were distended and filled with colloid. Phagocytes were present in great numbers. The areas of phagocytic invasion were localized and vacuolization was more prominent near the blood vessels of the gland. Table 2 shows in a composite list the entire results of the study of the thyroids regarding the (1) type of epithelium, (2) consistency of colloid, and (3) relative numbers of lymphocytes.

Blood Picture

An erythrocyte count of three normal male chickens was made (Table 1). The average count was found to be 149 in 40 of the small squares of the Levy hemocytometer. The white cell number which included mast cells,
lymphocytes, and monocytes averaged 11; thrombocytes averaged 4. There were no leucocytes in the foal blood which could be recognized as either neutrophils or basophils. For this reason a differential count could not be included. White blood cells in these counts were characteristic of the monocyte group.

The adrenalectomized birds used were few in number. Because of varying factors in each count it was impossible to obtain satisfactory picture of blood cellular changes following extirpation of the adrenals. Two birds had been opened a short time previous to a blood count. In one of these birds a considerable reduction in erythrocytes was found (Table 5). Two showed a larger number of erythrocytes than the normals. All gave a small increase in white blood cells compared to normals.
Fig. 1. Photomicrograph of thyroid tissue from normal adult male chicken.

Fig. 2. Photomicrograph of thyroid tissue from castrate male chicken.
Fig. 3. Photomicrograph of thyroid tissue from adrenalectomized male chicken showing high epithelium.

Fig. 4. Photomicrograph of thyroid tissue from adrenalectomized male chicken showing low epithelium.
SUMMARY

1. Changes occurred in the thyroid glands of both gonadectomized and adrenalectomized birds.

2. The thyroid gland of the gonadectomized bird exhibited a more hyperplastic condition than the thyroid of the adrenalectomized birds.

3. All adrenalectomized animals showed an invasion of the colloidal material in the gland by lymphocytes. Normal thyroids exhibited only an occasional lymphocyte in the colloid of the gland.

4. The greatest infiltration of lymphocytes into the thyroid colloid of adrenalectomized birds was correlated with low epithelium and distended follicles.

5. Lymphocytes were found between the acini of the glands in all adrenalectomized animals.

6. Of thirteen thyroids from adrenalectomized fowls, eight showed involution while five contained high columnar epithelium indicative of hyperplasia.

7. The change from the normal thyroid to the conditions found is regarded as evidence of an adrenal-thyroid relationship the nature of which has not been made clear by this study.

8. There was a small increase in the number of white cells in the blood of adrenalectomized birds.
ACKNOWLEDGMENT

The writer wishes to express his appreciation to Dr. E. H. Merrick for his suggestions and criticisms during the progress of this work, and to Mr. Olaf Torstveit and Mr. John Finerty for their assistance in many ways.
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