CYTOLOGICAL CHANGES IN THE ANTERIOR PITUITARIES OF ADRENALECTOMIZED AND CASTRATE MALE FOWLS

by

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INTRODUCTION AND LITERATURE

When the adrenal glands are surgically removed in male fowls, characteristic changes occur, the most striking of which is a reduction in the size of the testes. Accompanying this is a degeneration of the tubules which results in reduced testicular activity. These changes were first described by Herrick and Torstveit (1938) who observed the changes in the gonads, decrease in size of comb and wattles, and associated changes in body shape and feathering, similar to those in capons. The physiological mechanism through which the testes are affected by removal of the adrenal glands has been a matter of speculation. There has been no indication as to whether the influence is directly from the adrenals on the gonads, or whether there is an indirect influence through the effect of adrenalectomy on some other organ.

Because of the function of the pituitary gland as a regulator of the endocrine system, and its definite relationship with testicular activity, it was suspected that the pituitary might be an intermediary in the adrenal-testis complex.

Much work has been done in animals on cytological studies of the pituitary after physiological alterations:

Either normal ones, such as oestrus or pregnancy; or experimental removal of other endocrine organs, especially the gonads or thyroid. During the past few years there have been reports by many different workers of changes in the cytology of the pituitary in various forms of animals. However, most of the work has been done on mammals, with only a few scattered papers on birds. Fichera (1905) observed mitosis and an increase in size and number of the eosinophiles in a castrated hen. Marrassini and Luciani (1911) described increase in size and relative number of eosinophiles. At this time reports were made of increases in eosinophiles in other animals, too, but since then, it has been shown that the enlarged cells were really basophiles. The only recent outstanding work on birds has been that of Schooley and Riddle (1938) on pigeons. This work and that done on other animals confirm the belief that some kinds of changes undoubtedly do occur in the pituitary.

Before considering the specific changes in cytological structure of the pituitary, it might be well to discuss briefly the types of cells present and their suspected functions. There are three normal types of cells. The acidophiles (eosinophiles, or alpha cells) are cells about 8-12 micra in diameter which contain acid staining granules, and have a centrally located nucleus. Because of their in-

crease in size, number, and activity in cases of acromegaly, they are accepted as the producers of the growth hormone, (Cushing and Davidoff, 1927; Benda, 1900). The basophiles (beta cells) are larger cells, about 10-15 micra in diameter, which contain basic staining granules, and whose nuclei are usually excentric. They are considered the source of the gonadotropic hormones because of the increased gonadotropic potency of castrate pituitaries which contain larger, more active, and more numerous basophiles. These enlarged cells, although they had been reported previously and called "signet ring cells", or "castration cells", were first described as modified basophiles by Addison (1917). The third type of cell is the chromophobe, a small, undifferentiated cell, which with most techniques takes a light gray stain, or none at all. It is believed by most workers that the chromophobe is a reserve cell which may be converted into either of the chromophiles.

Probably the most accepted and best understood change which has been noted is that which follows castration. It is also the most important in a study of this kind because it proves the pituitary-gonad relationship. If a change such as this does occur, there must be some explanation of the mechanism which causes the basophiles to increase and become enlarged. The scarcity of mitotic divisions in the pituitary rules out the possibility of a simple increase in

the actual numbers of cells, so there must be some way in which the cells are changed from one type to another. In his paper on the cytology of the anterior pituitary of the rat, Severinghaus (1933) reviewed the theories which have been put forth concerning interrelationship of the cell types. There are two general theories, both of which are still supported in the current literature. The one assumes that chromophobes, acidophiles, and basophiles are three distinct types of cells, while the other looks upon these cells as representing different physiological stages of a single cell type. In this paper, Severinghaus also described his own theory of cell interrelationships in the anterior pituitary, using the Golgi apparatus as a criterion.

This theory, which is now the most widely accepted one, states that in the embryo the cells are of one type, the undifferentiated chromophobes; as the individual develops these cells divide and form two other kinds of chromophobes, which can be distinguished by their Golgi bodies. These are the acidophilic and basophilic chromophobes. The former may become granulated and develop into functional secretory acidophiles, while the latter may develop into secretory basophiles. When secretion occurs, the granules are released and the cells revert to the chromophobic state. Each chromophobe may remain a reserve cell for an indefinite

time, or may immediately begin a new accumulation of granules, but only granules of the same nature. A small proportion of these degranulated cells regularly degenerates and dies.

Some of the changes in the relative numbers of cells described after physiological alterations in animals are as follows. Hohlweg and Junkmann (1933) and Severinghaus, Smelser, and Clark (1934) reported loss of acidophiles and castration-like changes in the basophiles after experimental thyroidectomy. Marine, Rosen, and Spark (1935) confirmed the loss of acidophiles in the thyroidectomized rabbit. Zeckwer, et al. (1935) described the loss of acidophiles and the appearance of large colloid-filled "thyroidectomy cells" after thyroidectomy in the rat. Siler (1936) reported reduction of acidophiles and castration-like changes in the basophiles, after thyroidectomy of the garter snake. Schooley and Riddle (1938) compared the pituitaries of castrate and thyroidectomized pigeons. The castrates showed a decrease in acidophiles and increased activity of the basophiles; while the thyroidectomized individuals showed a degenerate type of basophiles similar to those of sexually inactive birds.

Kraus (1923 and 1927) found a marked decrease in numbers of basophiles in Addison's disease. Those basophiles which were present exhibited various aspects of degenera-

tion -- pycnotic nuclei, degranulation, shrunken and irregular form, indistinct cell borders, and a tendency to flow together into honeycombed masses.

Crooke and Russell (1935) consider the reduction of the basophile cells in the anterior lobe of the pituitary to be a constant change and the most significant change in the ductless glands following destruction of the suprarenal cortex in Addison's disease. They suggest that this reduction is the cause of the low blood pressure and possibly of the hypoglycemia in this disease. In the same paper they state that in nine of the thirteen published examinations of the pituitary in Addison's disease, a diminution of basophiles has been described. They refer to the work of Martin (1932) and Moehlig and Bates (1933) as the only animal experimentation on adrenal deficiency, but that in both cases the results do not agree with the autopsy reports in human beings.

Moehlig and Bates (1933) report hyperplasia of the pituitary, especially the basophiles after bilateral adrenalectomy of eleven dogs.

Martin (1932) in a study of the effect of suprarenalectomy on the oestral cycle of the white rat found that complete suprarenalectomy caused a decrease in size and staining reaction of the acidophile cells, with a variable

effect on the basophiles. He found, however, that injection of pituitary extract restored the normal oestral cycle of these rats, but did not prevent death from suprarenal insufficiency.

Nicholson (1936) concurred with the results of the other workers on the diminution of basophiles in human Addison's disease, but found no cytological changes in the anterior pituitary in twenty-five bilaterally adrenalectomized dogs.

Shumacker and Firor (1934) using one dog with incomplete adrenal deficiency obtained basophilic decrease, and this work was followed by that of Grollman and Firor (1935) who found similar changes in dogs, cats, and rats with chronic adrenal insufficiency. The symptoms of their animals could not be altered by adrenal cortical hormone therapy, but the observed symptoms could be relieved by injection of anterior pituitary extracts.

The results of these and other experiments which show pituitary-gonad, pituitary-thyroid, and pituitary-adrenal relationships led to the belief that possibly the changes in the testes of adrenalectomized male fowls were caused by some such change in the cell proportions of the anterior pituitary. Especially since Kraus (1923), Crooke and Russell (1935), and others found degenerative changes in the basophiles in Addison's disease, and as one of the

symptoms of this disease is atrophy of the gonads, (Wolf, 1936, p. 332; Grollman, 1936, p. 227), it seemed reasonable to suspect some pituitary influence on the testes resulting in their degeneration after adrenalectomy (experimentally induced Addison's disease).

In order to determine if there were associated changes in the cytology of the pituitary accompanying the observed testicular degeneration in the adrenalectomized male fowls, these experiments were conducted. It was hoped that if some such changes did occur, there might also be some clue as to whether the gonad changes were caused directly by removal of the adrenals, or secondarily as a result of altered pituitary activity.

MATERIAL AND METHODS

The adrenal glands were destroyed in ten adult male chickens during two operations, by the method of Herrick and Torstveit (1938). Immediately after the second adrenal was removed, the comb and left testis were measured, the bird was weighed and 1 cc. of Eschatin (Parke, Davis and Company adrenal cortex extract) was injected to prevent death from the sudden lack of adrenal secretion. The chickens were provided with an adequate diet; and an unlimited supply of drinking water containing sixteen grams sodium

chloride per liter. When the comb showed signs of becoming limp, pale and reduced in size, the bird was reopened and the left testis was measured. If the testis had regressed to about one-half its previous size, the bird was sacrificed, its pituitary removed and prepared for cytological examination. The weight of each chicken was recorded again to show that the degenerative changes were not due merely to malnutrition.

Ten normal adult male chickens of approximately the same ages as the experimentals were sacrificed, and their pituitaries removed for cytological study, to serve as controls.

In order to compare the effects of adrenalectomy and castration on the pituitary, five male chickens were caponized, and their pituitaries also prepared for examination.

The method of procuring the pituitary gland was to remove the top of the skull with a hack-saw; lift the anterior part of the brain exposing the optic chiasma and the surrounding area; cut away parts of the spongy bone composing the sella turcica, just posterior to the chiasma; and separate the gland from its meningeal coverings.

To study a complex gland, such as the anterior pituitary, a very specialized and specific technique must be employed in order to preserve the tissue and differentiate

the types of cells. Many different techniques have been devised in the study of this gland, but there are two which are most commonly used. One method is that of Severinghaus (1932) who used Champy fixation, followed by osmication according to Nassonov's modification of Kolatchew's procedure. This is followed by staining similar to Altmann's method. The second common method of staining is that advocated by Rasmussen (1930) which, although it does not show the Golgi apparatus, gives extremely good differentiation of cell types, and is much quicker and less difficult. It consists of a modification of Mallory's trichrome stain after fixation in formaldehyde or saturated corrosive sublimate solution. Besides giving excellent differentiation, the advantages of this technique are its simplicity and the short length of time between fixing of the tissue and completion of the slide.

After repeated trials, a modification of the latter method was perfected which gave excellent differentiation, and could be completed in three days. The entire method in detail is as follows:

1. Fix tissue immediately after removal in Heidenhain's "susa" — 6-10 hrs.
   - Mercuric chloride (Saturated solution in physiological salt solution) — 50 cc.
   - Trichloroacetic acid — 2 gm.

	Formalin	20 cc.
	Glacial acetic acid	4 cc.
	Distilled water	30 cc.
2.	Place in 50% dioxan - 50% absolute alcohol.	4 hrs.
3.	Change to pure dioxan (Add few drops of iodine from time to time until no more decolorization occurs).	4 hrs. (or longer)
4.	Infiltrate in: Dioxan (1 part) - warm paraffin (2 parts).	2-4 hrs.
5.	Melted paraffin (5-6 changes of twenty minutes each).	
6.	Imbed in paraffin.	
7.	Section serially at five micra.	
8.	Mount sections on slides and allow to dry.	
9.	Remove paraffin with xylol.	About 1 minute each
10.	50% xylol - 50% absolute alcohol.	
11.	Absolute alcohol.	
12.	95% alcohol.	
13.	85% alcohol.	
14.	70% alcohol.	
15.	Transfer to distilled water.	5 min.
16.	Alum haematoxylin.	20 sec.
17.	Rinse in tap water.	
18.	Rinse in distilled water.	

19. Solution number 1. 4 min.
(Acid fuchsin 0.25 gm.)
(Distilled water 100 cc.)

20. Rinse in distilled water.

21. Solution number 2. 20 min.
A. Aniline blue 2.0 gm.
Distilled water 100.0 cc.
B. Orange G 1.0 gm.
Distilled water 100.0 cc.
C. Phosphotungstic acid (1%).
(Keep A, B, and C separate until just before use. Then mix equal parts of each).

22. Rinse rapidly in 95% alcohol, then in 100% alcohol.

23. Clear in absolute alcohol-xylol, then in xylol.

24. Mount in balsam.

In pituitaries fixed and stained according to this technique, the acidophilic granules are red; the basophilic granules are blue; the chromophobes are gray; the nuclear membranes of all cells are sharply defined by the haematoxylin; chromatin material is red; colloid, or hyaline material, is bright blue; erythrocytes are orange; and connective tissue is blue.

After the slides had been prepared, the number of sections of each gland was counted. The total number of sections of a gland was divided by five in order to get four sections at equidistant levels. The glands had been sagittally sectioned in the lateral plane so that the four

levels were at equidistant intervals from the dorsal to the ventral surface.

The number of degenerating basophiles was counted in each of these chosen sections. Only those cells which were distinctly degenerate basophiles were counted. Any cellular material which was doubtful was not included.

After the cell counts had been completed, each section was measured to obtain its area. This was done by projecting the section with a micro-projector, and measuring the projected area with a polar planimeter. The planimeter which was used measured the area in square inches which were easily converted into square millimeters by multiplying the result by 645.2. To compute the actual area, a stage micrometer was used. A measured square (4 square millimeters) was projected with the same magnification, and its projected area was measured. It was found to be 9.71 square inches, or 6265 square millimeters.

6265 : 4 = area of projected section : area of the section

Area of the section = area of projected section x .0006385

Using the preceding formula, the area of each of the eighty sections was determined.

The total number of degenerating basophiles in the four sections of each gland was then divided by the total area of the four sections to determine the average number of

those cells per square millimeter. These figures (Table 1) were then combined to obtain the average number per square millimeter of all the adrenalectomized chickens, and of all the normal chickens.

DISCUSSION OF RESULTS

The results of adrenalectomy were substantially the same as those which were described by Torstveit (1937) in that the combs in all but one case were smaller than at the time of adrenalectomy, and testes in all cases were much smaller, usually about one-half their former size (Table 1). The weights of all birds showed that the reduction in size was not due to malnutrition.

An average of 23.60 degenerating basophiles per square millimeter was found in the pituitaries of the controls, while in the adrenalectomized chickens there was an average of 66.54 degenerating basophiles per square millimeter. This suggests that the number of actively secreting basophiles was being depleted three times faster in the experimentals than in the controls.

In order to fully understand the significance of this it may be necessary to discuss some of the history of a typical basophile as well as something of its interaction with other cells.

Although with the technique employed it was impossible to distinguish between the two types of chromophobes, there were many chromophobes present; approximately half of all the cells were of this type. Basophiles in all stages of granulation and degranulation were easily observed. Severinghaus (1936) gives his own theory of cell interrelationship as well as those of Trautmann (1909) and Collin (1928). According to their theories a certain amount of these basophiles, instead of becoming granulated again, become infiltrated with colloid, or hyaline material. It was difficult, at first, to recognize any of these cells because of their relative scarcity in normal chickens, but when the adrenalectomized chickens were studied, the deep blue colloidal material was easily recognized. A perfectly integrating series was observed of the degenerating basophiles (Plate 1), from a solid, dark blue, hyaline cell with regular cell boundaries through progressive vacuolization until only a few strands of blue connective material were left in the cell space. After becoming more familiar with the material, the same phenomena were observed in the anterior pituitaries of the control birds as well, although to a lesser extent.

With alterations in cellular proportions go changes in the capacity of the gland to produce given responses. If the colloid infiltration and vacuolization of these

secreting basophiles is their way of degenerating and dying, then these cells would be losing their activity three times as fast in the birds in which the adrenal glands had been destroyed. If this continued, the proportion of basophiles would rapidly decrease. The basophilic granules are considered to be precursors of the gonadotropic hormones, so following their diminution, less gonadotropic secretion would be expected, which would not prevent regression of the gonads. This provides an explanation for the degeneration of testis tubules, and reduction of testis size in the experimental male fowls.

However, it does not overcome the possibility that these changes in the pituitary are a result of the testis degeneration after adrenalectomy rather than the cause of it. To find out if such changes would follow gonad ablation, five male fowls were castrated and, after the characteristic capon comb and feathering had developed, were sacrificed and their pituitaries studied. These glands exhibited a very different appearance from the other two series. No cell counts were made because the changes were so evident that detailed study seemed unnecessary. As described by many workers on other animals there was a very definite increase in the size and number of the basophiles, with an apparently slight decrease in the number of granular

acidophiles (Fig. 13). It is an accepted conclusion that this is caused by the sudden cessation of male hormone which inhibits excess gonadotropic hormone secretion in normal individuals. When the inhibition is removed, the pituitary becomes overactive in secreting gonadotropic hormones as a result of the increase in size and number of the basophiles.

The fact that after castration the basophiles enlarge and increase while after adrenalectomy they degenerate and decrease seems to indicate that there is a fundamental difference between the two conditions and that adrenalectomy changes in the anterior pituitary are causes rather than results of gonad decline.

Even though the pituitary changes are caused differently in capons and adrenalectomized birds, the appearance of their secondary sexual characteristics should be expected to be, and are, the same in both conditions. In either case, there is a cessation of male hormone secretion, which results in the characteristic capon comb and feathering.

The possibilities of application to the clinical aspects of extreme adrenal deficiency (Addison's disease) should not be overlooked. Most of the investigators, who have studied the anterior pituitaries of individuals after

death from this disease, have described degenerative changes in the basophiles, and a decrease in their number. In animal experimentation of this condition (in rats and dogs), the results have not been uniform.

Up to this time the experiments of Grollman and Firor (1935) have been the only work in which the results have agreed with those found during autopsies of human beings after death from Addison's disease. There have been at least eight different published accounts of experimental work in which the results disagreed. This work with chickens shows that there is decrease and degeneration of the basophiles, as found in dogs by Grollman and Firor and in human beings by many workers.

The atrophy of reproductive organs which is associated with Addison's disease may thus be considered an effect of cytological changes in the pituitary rather than from other sources. It may be possible from further study to make more clear the causes of the other symptoms and obtain clues to their possible alleviation.

SUMMARY

1. It was found that in normal adult male chickens there was an average of 23.60 degenerating basophiles per square millimeter, while in adrenalectomized individuals of

the same age there was an average of 66.54 degenerating basophiles per square millimeter of the pituitary section.

2. The degenerative changes in the gonads of adrenalectomized male fowls are apparently caused by cytological alterations in the basophile cells of the anterior pituitary gland.

3. The changes in secondary sex characteristics of adrenalectomized chickens are believed to be a result of gonad degeneration.

4. Increase in size and number of basophiles has been previously described in other castrate animals, but this is the only work in which these changes have been observed after castration of male chickens.

5. The results of cytological examination of the pituitary gland in induced Addison's disease in experimental chickens agrees with the autopsy findings in Addison's disease of human beings.

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Table 1. A comparison of the combs, gonads, and number of degenerating basophiles in adrenalectomized and normal chickens.

Chicken	Age (in days) When adrenalectomized	Age (in days) When killed	Days on experiment	Comb (in mm.) When adrenalectomized	Comb (in mm.) When killed	Left testis (in mm.) When adrenalectomized	Left testis (in mm.) When killed	Weight (in grams) When adrenalectomized	Weight (in grams) When killed	Number of degenerate basophiles (per sq. mm.)	Number of degenerate basophiles (per sq. mm.) (average)
Controls:											
A440	---	138	---	---	116 x 61	---	32 x 15	---	---	25.56	
A430	---	166	---	---	132 x 77	---	38 x 19	---	---	29.66	
A409	---	166	---	---	124 x 70	---	43 x 23	---	---	39.16	
A416	---	166	---	---	122 x 68	---	40 x 19	---	---	29.00	
A441	---	124	---	---	108 x 54	---	43 x 22	---	---	13.61	
A396	---	124	---	---	120 x 74	---	44 x 23	---	---	33.61	
B171	---	215	---	---	146 x 81	---	49 x 23	---	---	17.01	
B155	---	237	---	---	133 x 86	---	46 x 23	---	---	5.77	
A407	---	124	---	---	118 x 77	---	41 x 20	---	---	24.21	
A412	---	152	---	---	99 x 62	---	33 x 17	---		22.51	23.60
Adrenalectomized:											
T2000	105	158	53	74 x 39	64 x 29	23 x 11	12 x 5	964	1213	66.23	
T1985	105	135	30	94 x 50	92 x 60	34 x 13	15 x 8	1005	good cond.	74.25	
T1976	110	124	14	95 x 55	91 x 53	31 x 15	15 x 7	1069	good cond.	41.29	
B195	95	144	49	99 x 53	76 x 37	26 x 14	9 x 5	797	930	71.96	
B193	95	144	49	129 x 65	111 x 59	34 x 14	16 x 6	1051	1543	60.49	
B178	185	213	28	135 x 74	121 x 79	55 x 23	35 x 21	1519	1477	86.29	
B162	190	213	23	123 x 82	115 x 86	36 x 15	26 x 14	1725	good cond.	92.54	
A127	406	477	71	130 x 94	123 x 89	35 x 18	30 x 16	---	---	96.46	
B80	133	223	90	113 x 66	120 x 71	24 x --	17 x 9	1147	1402	57.03	
B194	95	124	29	92 x 51	88 x 49	32 x 16	20 x 10	941	good cond.	32.51	66.54

EXPLANATION OF PLATE I

All drawings made with aid of camera lucida.

Fig. 1. Normal basophile cell from the anterior pituitary of an adult male chicken.

Figs. 2-7. Progressive changes in the vacuolization of a colloid infiltrated basophile cell.

PLATE I

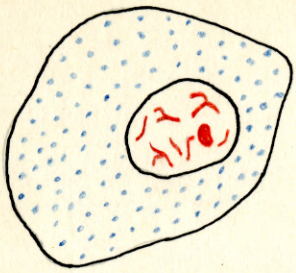


Fig. 1

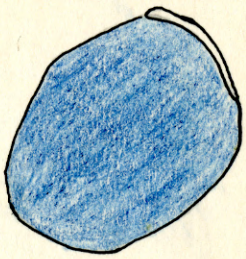


Fig. 2

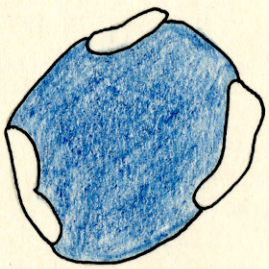
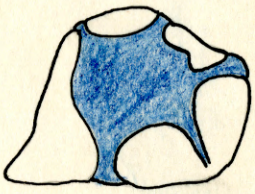


Fig. 3



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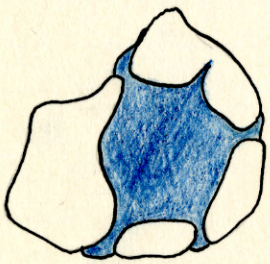

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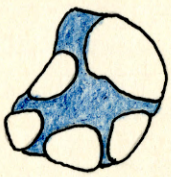

Fig. 6

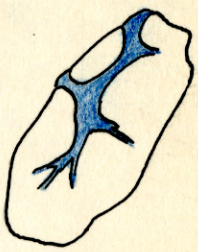

Fig. 7

EXPLANATION OF PLATE II

Fig. 8. Comparison of the comb and wattles of an adult male chicken with those of the same chicken 82 days after adrenalectomy.
——— Before adrenalectomy.
------- After adrenalectomy.

Fig. 9. Comparison of the testes of normal adult male chickens with those of chickens which had been adrenalectomized 17 days before.
A-B Normal.
C-D 17 days after adrenalectomy.

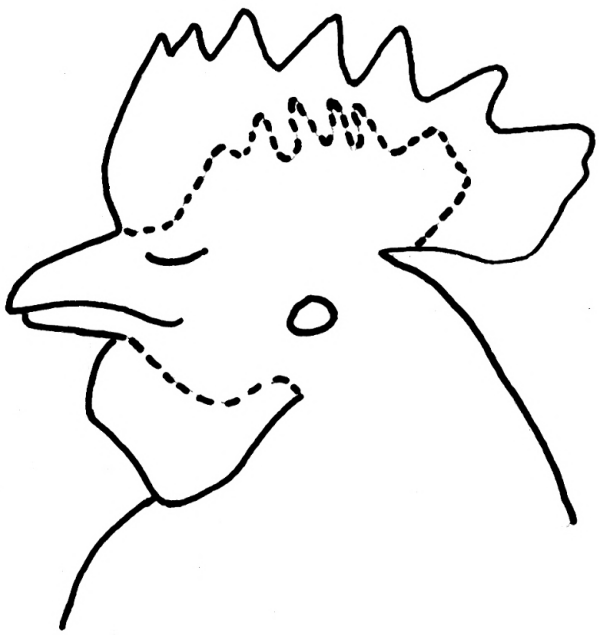

Fig. 8

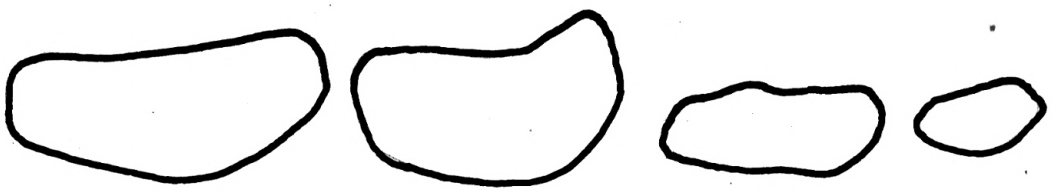

Fig. 9

EXPLANATION OF PLATE III

Fig. 10. Normal adult male chicken.

Fig. 11. Adrenalectomized adult male chicken after deficiency changes had occurred.

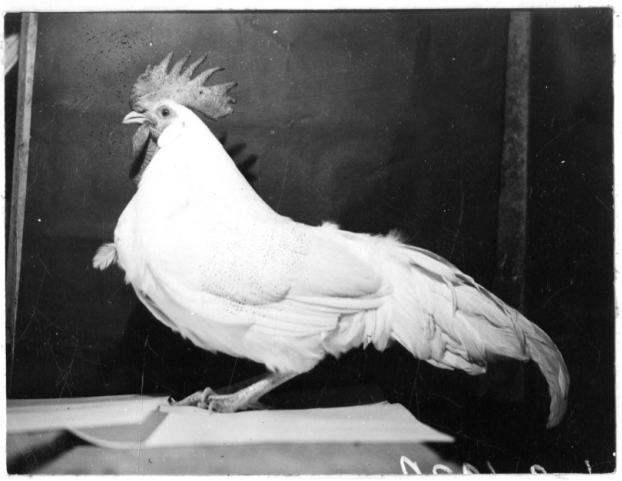

Fig. 10

Fig. 11

EXPLANATION OF PLATE IV

Fig. 12. Histological appearance of the anterior pituitary of a normal adult male chicken. 500X. Photomicrograph.

Fig. 13. Histological appearance of the anterior pituitary of a castrate adult male chicken. 500X. Photomicrograph.

Fig. 12

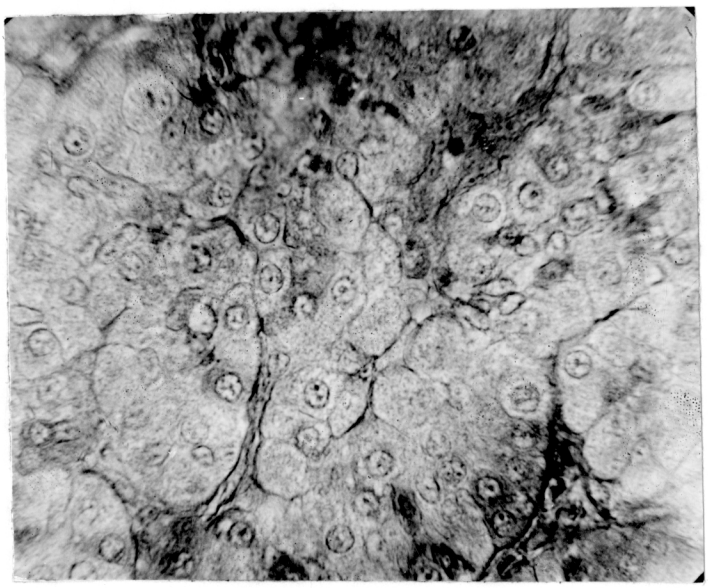

Fig. 13

EXPLANATION OF PLATE V

Fig. 14. Histological appearance of the anterior pituitary of an adrenalectomized adult male chicken. 500X. Photomicrograph.

PLATE V

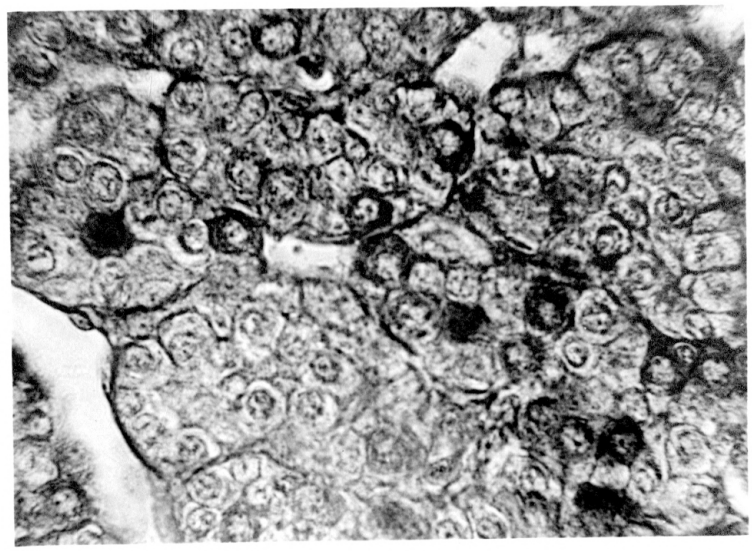

Fig. 14