PHYSIOLOGICAL CHANGES IN THE ADULT WHITE LEGHORN HEN INFLUENCED BY DIENCEPHALIC LESIONS

by

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INTRODUCTION

It was originally supposed that the pituitary gland was essential to life. Experiments designed to study the functional role of the pituitary gland in the body have mainly involved hypophysectomy with and without replacement therapy and recording the subsequent physiological changes. Mitchell (1929) found that complete hypophysectomy in immature chicks caused their death within two weeks after operation. Near complete hypophysectomy resulted in stunted growth and delay in the appearance of adult plumage. No specific inhibition of the gonads was noted. The thyroids showed lack of pituitary support but the adrenals and parathyroids were not affected.

It has been known for many years that the removal of the anterior lobe of the pituitary in mammals would lead to atrophy of the gonads, but this generalization was not extended to birds until the excellent work of Hill, Corkill and Parkes (1934) and Hill and Parkes (1934). These investigators showed that hypophysectomy of the laying hen resulted in the cessation of ovulation and the subsequent atrophy of the ovary, oviduct and comb. They concluded that these results were due to a temporary pituitary deficiency. Schooley, Riddle, and Bates (1941) reported that the pituitary removal from 54-day-old Carneau pigeons reduced body weight by 20%; thyroids by 50% and testes by 66%. Miller and Riddle (1942) found that atrophy of the pigeon's adrenal cortex following hypophysectomy is characterized by small shrunken lipoid-filled cells with a decreased number of mitochondria and atrophic Golgi apparatus. Nalbandov

and Card (1943) report that the thyroids respond macroscopically to lack of pituitary secretions after as short a time as ten to twenty days. After more prolonged apituitarism, when adiposity of the neck region becomes pronounced, it becomes impossible to separate these glands from the surrounding tissue. These authors also report that the adrenals respond rapidly to hypophysectomy, i.e. they undergo initial and almost complete degeneration followed by a possible partial recovery. Shirley and Nalbandov (1956) removed the posterior lobe of the pituitary gland of chickens and observed no apparent measurable effect on either growth rate or reproduction. Their operation did not delay the age of sexual maturity when it was performed on immature females, neither did it diminish the rate of ovulation or oviposition after sexual maturity was reached. Results by Newcommer (1958) indicate that hypophysectomy of young chicks markedly inhibit but do not wholly prevent an acidophilia following stress. The exact mechanism by which stressors cause an acidophilia in chickens is not known. Newcommer, in a more recent paper (1959), disagrees with earlier investigators (Schooley, Riddle and Bates. 1941, Miller and Riddle. 1942) with regard to significant gross weight change of the bird's adrenal glands following hypophysectomy.

All of these investigations plus the hundreds reported on mammals (Ganong and Forsham, 1960) clearly describe the important role the pituitary gland plays with regard to adrenal, thyroid, and reproductive physiology. These studies have in turn stimulated a phenomenal amount of research dealing with hypothalamic nuclei as probable control centers over the pituitary gland. Basic morphological studies support a physiological relationship between the hypothalamus and the anterior lobe of

the pituitary. The neurohypophysis is embryonically and functionally a part of the brain. The adenohypophysis, on the other hand, is derived from Rathke's pouch and has only a vascular link with the hypothalamus provided by the portal hypophyseal vessels.

Although the major portion of the literature on this subject has involved mammals, there appears to be sufficient evidence from many sources to suspect that the central nervous system in birds exerts a similar regulation over the function of the anterior pituitary. Wingstrand (1951) gives a detailed description of the neural and vascular relationships between the hypophysis and hypothalamus of birds. From his excellent monograph it is learned that the neurohypophysis receives four different tracts from the hypothalamus (1) tractus hypophyseus anterior which includes all hypophysial fibers behind the chiasma that do not belong to the second two; (2) tractus supraoptico-hypophyseus, or neurosecretory tract to which also belong the axones from the nucleus paraventricularis; (3) tractus tubero-hypophyseus whose fibers arise from the tuber nuclei; and (4) tractus hypophyseus posterior whose origin is assumed to be in the posterior hypothalamus. According to Wingstrand (1951) there are no nervous connections between the hypothalamus and the adenohypophysis. Furthermore there is no direct vascular connection between the neurohypophysis and the adenohypophysis. Wingstrand (1951) also drew attention, because of the large accumulation of neurosecretory material, to a special section of the ventral wall of the infundibulum, the median eminence. From there the vessels of the hypothalamo-hypophysial portal system pass to the pars distalis of the adenohypophysis. In the absence of other anatomical connections he suggested that the portal system must be the mechanism through which the hypothalamus exerts its

control over the adenohypophysis.

Legait (1955a, b) found that the histological appearance of the neurosecretory system of the Rhode Island Red hen can be influenced by the use of hormones. She found that an injection of gonadotrophin led to a decrease in neurosecretory activity in the hypothalamic nuclei, in the hypophysial stalk, and in the neurohypophysis. Estrogen in large doses reduced the activity of the hypothalamic neurosecretory cells in which the neurosecretory material is stored. Deoxycorticosterone and cortisone were also effective in bringing the neurosecretory system into a complete condition of rest. Legait (1957) found further that thyroid hormone or thyroid extracts reduce the activity of the neurosecretory system although not to the extent of the reduction caused by adrenal cortex hormones. Oksche et al. (1959) made a histological study of the hypothalamo-hypophysial system of the sparrow, Zonotrichia leucophrys gambelii. They found that the median eminence can contain so much neurosecretory material that it may be regarded as a depot of such material second only to the neurohypophysis. Saffran (1958) has presented a related and most complete review paper pertaining to hormonal functions of the hypothalamus in mammals.

Recently, a great many investigators (D'Angelo and Traum, 1958; Ganong, 1959; McCann and Haberland, 1960; Dear and Guillemin, 1960; and others) have employed electrolytic lesioning techniques to locate specific centers in the hypothalamus of mammals that might regulate hormone release from the pituitary. In comparison there are only a few papers in the literature that concern diencephalic lesioning in birds. The first publication to appear (Feldman, et. al., 1957) dealt with aphagia

in chickens. The photographs accompanying this publication show lesions that appear to be more in the thalamic region of the brain than in the hypothalamus. A series of papers by Ralph (1959) and by Ralph and Fraps (1959a, b) involve gonadotrophin release in the domestic hen. Miller (1961) investigated the response to stresses on hypophysectomized pigeons with lesions in the median eminence. His lesioning technique involved aspiration rather than electric coagulation. The work of these four investigators constitute all the research published in the field of avian neuro-physiology using lesioning techniques.

The research reported in this dissertation is a study of some of the physiological changes that take place in the adult White Leghorn hen following specifically placed electrolytic lesions in the diencephalon. From these observations an attempt is made to map area in the hypothalamus that regulate specific trophic hormone releases from the anterior lobe of the pituitary.

MATERIALS AND METHODS

Adult White Leghorn hens between seven and fourteen months of age were used for all experiments. These birds were raised on regular egg laying mash at the University of Arizona Poultry Farm. For each experiment ten birds were housed individually in a special battery of cages equipped with an electric Esterline-Angus recorder to establish accurate egg-laying records. Each cage was large enough for limited movement of the hen. Water and egg-laying mash were available ad libitum. The bottom of each cage was slanted at approximately 30° to permit the eggs to roll immediately after lay to a tray in the front of each cage. Before entering the tray each egg would trip a paddle which in turn would stimulate the appropriate ink stylet in the Esterline-Angus recording machine. The exact time and interval of egg lays were then automatically recorded twenty-four hours a day.

Every bird has a characteristic clutch or egg-laying sequence (Fraps, 1955). Deviations from this characteristic pattern are good response indicators when analyzing physiological data of birds. No less than ten days were used to establish these records for each hen. Any bird that failed to lay was eliminated from the experiment prior to operation time. A post-operative egg-laying record was compared with the pre-operative record for each bird. These results were then correlated with lesioned areas in the diencephalon.

All lesions were made with the assistance of a Johnson stereotaxic instrument (See Fig. 1) especially adapted for chickens. The

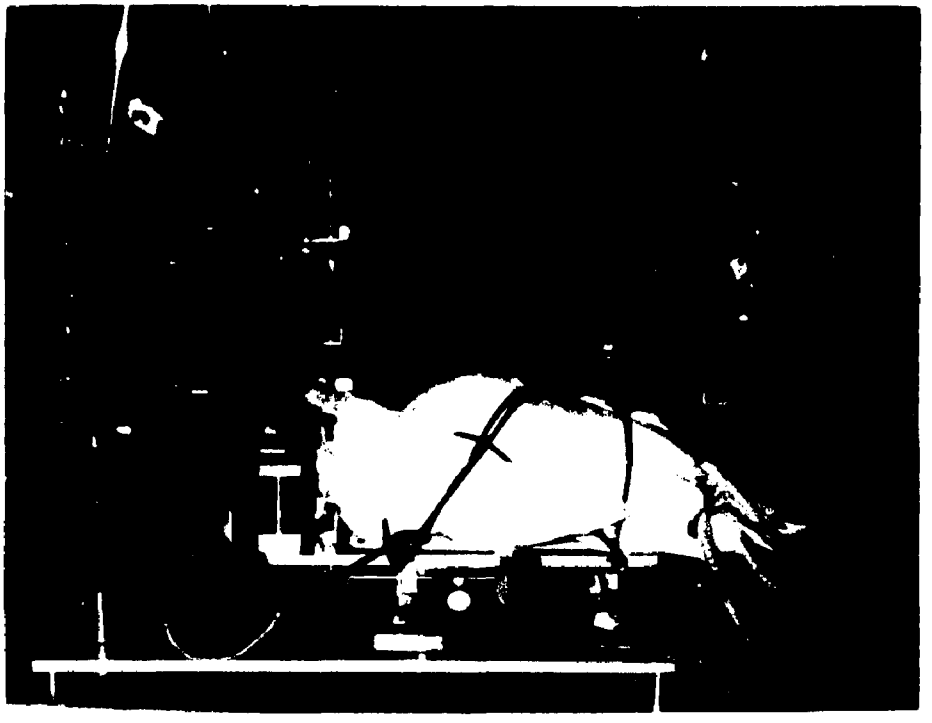

Figure 1. White Leghorn hen secured in a Johnson Stereotaxic Instrument with dental drill in position.

head of the bird was fixed in the holder by plastic ear plugs. The electrodes used for making the lesions were size 0 Bohemian insect pins insulated with three coats of fingernail lacquer. An x-ray machine was used to insure precise placement of the electrodes in the brain.

In order to interpret the x-ray of the chicken's brain with confidence, a preliminary study of its anatomy was made. Part of this study involved making a mid-saggital cut through the head of a freshly killed chicken with the aid of a band-saw. Small specks of lead were then placed in the median eminence and supraoptic areas of the brain. With the two halves of the skull tied together an x-ray was made. The lead specks showed up on the x-ray and permitted orientation of structural landmarks on the skull. Fig. 2 shows an electrode placed in the region of the infundibulum of the chicken brain. Another part of this anatomical study of the skull involved standard histological methods.

A direct-current coagulator was attached to the electrode with one lead and the other lead intramuscularly to the chicken. A current of 3 milliamperes for 15 seconds produced the best lesions. Before the electrodes could be placed in the brain two small bilateral holes were drilled through the skull with the use of a dental drill. All birds were anesthetized with Nembutal before operations.

The pre-operative data that was collected for each bird besides egg-laying records included body weight, comb and wattle measurements, and blood count. Each of these were compared with their post-operative data. If a bird lost considerable weight or appeared not to have recovered from the operation, its data was discarded as invalid.

To determine the comb and wattle size the greatest anteropos-

Figure 2. X-ray showing electrode placed in region of infundibulum. 4x. Elec.= Electrode; E.P.= Ear Plugs; Inf.= Infundibulum; O.C.= Orbital Cavity.

terior length of the comb and height as the perpendicular distance from the base of the comb to the highest point directly above were measured with calipers. These values were then expressed as a comb factor (L x H/2). Similar measurements were made for the wattles and a wattle factor recorded.

Because of the normal presence of nucleated erythrocytes and thrombocytes in avian blood, it has always been difficult to accurately count all the white blood cells. A direct acidophil counting method described by Wiseman (1931) was used for this determination. This method employs a diluting solution of Phloxine which causes maximum staining within one hour. This solution stains acidophilic granules a brilliant red. All other cells take the stain much less brilliantly and are not to be confused with acidophilic leucocytes. Acidophils are comprised of both eosinophils and heterophils. This solution is prepared by mixing 50 mg of Phloxine stain with 5 ml of 40% solution of Formalin and 95 ml Chicken Ringer's solution.

A tip of a comb spike was snipped off after it had been thoroughly cleansed with 95% ethanol. Blood was collected in a red blood cell pipette and diluted 1-200 with the Phloxine solution. This mixture was allowed to stand for at least one hour, and was then shaken for two to three minutes. A subsample was then placed on a hemacytometer and the average of four counts using a 10 mm objective with a 10x occular under strong white light was recorded.

All birds from each experiment were lesioned on the same day. At the end of each operation 100,000 units of Penicillen were injected intramuscularly as a precautionary measure against infection. The birds

were returned to their cages for the remainder of the experiment. All experiments were terminated two to five weeks after lesioning.

At the end of the experiment body weight, blood count, and comb and wattle measurements were again recorded. Each bird was then given a sublethal dose of Nembutal. The adrenal glands were quickly removed, trimmed of connective tissue, and weighed on a Roller-Smith balance. One gland was frozen for future corticoid analysis and the other was preserved in Bouin's fixative for future histological study. The brain was quickly removed and preserved for histological study. Both thyroid glands were dissected free from all connective tissue, weighed and preserved in Bouin's fixative. The oviduct was then removed, the length and width measured and a portion of the albumen gland preserved in Bouin's with the other organs. Eosin and Harris' Hematoxylin were used for all histological preparation. Paraffin embedding was used exclusively.

The ovary of the White Leghorn hen completely surrounds the adrenal glands and their venous connections. Because of this, sampling of the adrenal effluent blood is impractical in the hen. Corticoid analysis was therefore made on mascerated adrenal glands. A Farrand fluorometer was used for routine quantitative analysis of adrenal corticoids (Sweat, 1954., Ganong, personal communication). The reagents used in this analysis included:

1. Chloroform, A. R., Eastman Kodak, redistilled from K_2SO_4.
2. 2,3,4-trimethylpentane (isooctane), practical.
3. Sulfuric acid, A. R., 30 normal solution with glass distilled water.
4. Ethyl alcohol, redistilled from 2,4-dinitrophenylhydrazine.
5. Sodium hydroxide, 0.1 normal solution.

6. Corticosterone[1] free alcohol, for standard solutions.

The equipment included:

1. Fluorometer; Farrand, Model A. Fitted with a 436 mu, Corning Nos. 3389-5113 primary filter and a 530-545 mu, Kodak wratten gelatin Nos. 16-17 secondary filter.

2. Extraction tubes; 50 ml polyethylene centrifuge tubes with stoppers.

3. One and 10 ml pipettes.

The technique consisted of macerating an adrenal gland with a glass rod in a centrifuge tube containing 4.0 ml distilled water. The corticoids were extracted with 1 ml of absolute ethanol. Four ml isooctane were added to the extraction mixture and agitated for one minute before centrifuging for three minutes. The isooctane layer was discarded. Fifteen ml of redistilled chloroform were then added to the extraction mixture and agitated for one minute before centrifuging for three minutes at 2000 rpm. The aqueous layer was removed by aspiration. One ml of NaOH 0.1 N was then added to the tubes containing the chloroform fraction. The tubes were shaken for 15 seconds and centrifuged for 3 min at 2000 rpm. Ten ml of the chloroform extract were then transferred into another 50 ml centrifuge tube containing 2.0 ml of H_2SO_4 30 N. The tubes were shaken vigorously for 30 seconds and centrifuged at 2000 rpm for 3 min. One ml of the acid extract was then transferred into a fluorometer tube and read 30-40 min later (Sweat, 1954) with the fluorometer being set at 80 for the 2γ/ml corticosterone standard. Six standards ranging from 0.1γ/ml to 2.0γ/ml were prepared from a stock solution of Corticosterone

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1. Five gm Corticosterone was donated by Research Laboratories of the Upjohn Company, Kalamazoo, Michigan

in absolute ethanol. The standards were carefully evaporated to dryness by a stream of air. Two ml of distilled water were then added to these standards and then carried through the same procedure as the unknowns. Standard curves were plotted for each determination. These curves fit the regression equation $y=a+bx$. The symbol a, which is the x intercept, represents the minimal background of fluorescence found with the reagents when a water blank is run through the fluorometric procedure. It is of the order ± 10 in these determinations. The amount of fluorescent steroids in a sample is read from this graph. It is expressed as γ corticosterone/100 mg adrenal tissue

RESULTS

Lesions were placed at various points in the diencephalon of actively laying adult White Leghorn hens (See Figs. 4-10 on pages 31-37). These lesions were approximately 0.5 to 2 mm in diameter. If a bird appeared not to have recovered from the operation, its data was discarded as invalid. Some of the birds died during the operation, probably due to an overdose or too rapid an injection of Nembutal. It is understood that many of the lesions are large enough to destroy more than one group of nuclei or fiber tracts. Because of this the lesions were systematically placed to overlap the different areas in the diencephalon. Table 1 summarizes the location of these lesioned areas. The first fifty birds used for this investigation are not included in this table because of insufficient histological data of their brains. In these first fifty birds the pituitary was removed before histological preparation was started. Along with the pituitaries some of the infundibulum containing lesions was accidently removed. Although x-rays for these birds are available, showing the electrodes in position, it is felt that the precise location of the lesions should still be substantiated with histological studies. The data or the egg-laying cycles, adrenal and thyroid weights for these birds provided a good comparison of subsequent experiments.

Fig. 3 shows a schematic representation of the structures seen in saggital section of a chicken brain. For identification of these hypothalamic nuclei reference was made to the works of Oksche *et al.* (1959) and or McDonald (1962)

TABLE 1. SITES OF DESTRUCTIVE LESIONS IN THE DIENCEPHALON

Hen No.	Nucleus Supraopticus A. Preoptic division B. Lateral division C. Dorsal division D. Ventral division	Nucleus Paraventricularis A. Ventral division B. Dorsal division C. Lateral division	Fiber Tracts A. Corticoseptomesencephalicus B. Lateral forebrain bundle C. Periventricularis D. Lateral optic tract	Infundibulum A. Median-Eminence B. Pars-Tuberalis C. Posterior Hypothalamus	Nucleus Mammillaris A. Lateral division B. Ventral division C. Medial division
1			D	B	
2				B	A
3			D	B	
4	C,D		D		
5		A	D		C
6				B	A
7					C
8			C		
9			D	C	
10				C	
11	B				
12			A,D		
13	Lesion was high in the cerebral hemisphere				
14		B	A,B		
15		B and septal area			
16		B	A		
17			A		A
18	D	A,B	C		
19			C	A	C
20		B,C	A,C		
21	A				
22	Lesion was high in the cerebral hemisphere				
23	A				
24	A				
25	A				
26				A,B,C	
27		C			
28				A	
29		B			
30	A	B,C	B,C		
31	A				
32			B		
33	A				

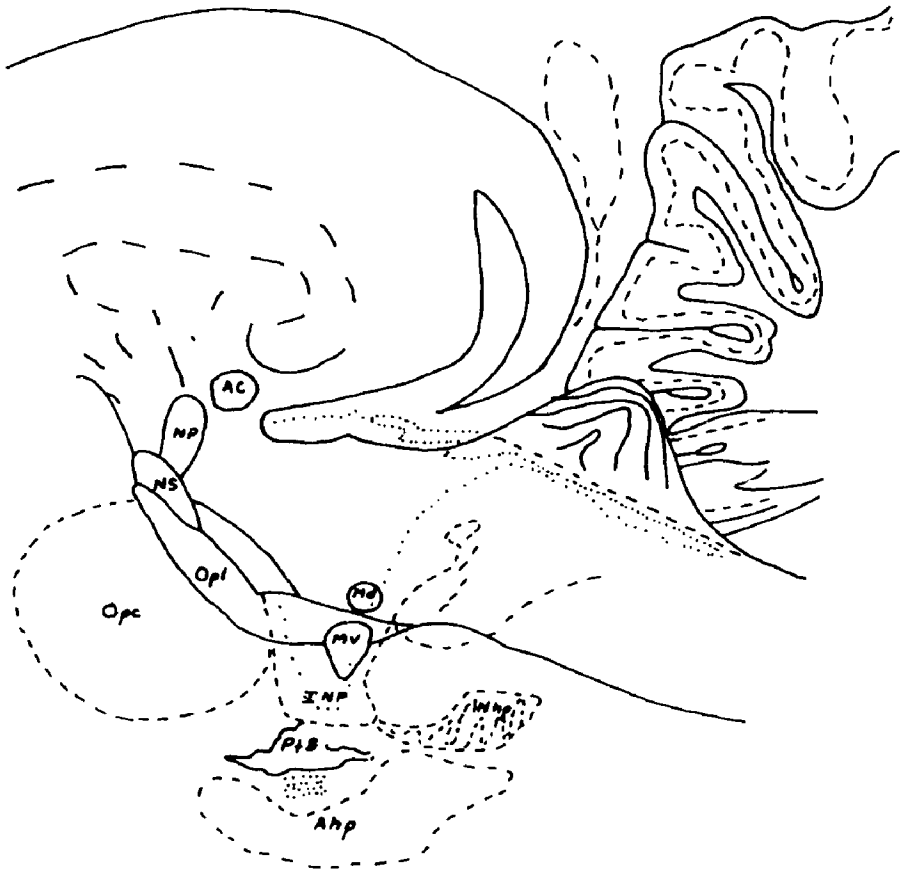


Figure 3. Schematic representation of structures seen in saggital section of brain. Midline structures in dotted lines, lateral structures in solid lines (Modified from McDonald, 1962). Opc. = Optic chiasma; Opl = Lateral optic tract; NS = Nucleus Supraopticus; NP = Nucleus Paraventricularis; AC = Anterior Commissure; Md = Nucleus Mammillaris dorsalis; Mv = Nucleus Mammillaris ventralis; Inf = Infundibulum; Ptb = Pars Tuberalis; Nhp = Neuro hypophysis; and Ahp = Adenohypophysis.

Oviposition and Secondary Sex Characteristics. All birds with lesions in the nucleus paraventricularis, median eminence or pars tuberalis brought about a cessation of the egg-laying cycle accompanied by a percent decrease of the comb factor measurements ranging from 18% eleven days post-operative, to as much as 52.8% just fourteen days post-operative (See Figs. 4,7 and 8). Some of these birds laid eggs on the first day post-operative, but in no cases were lay cycles renewed after day one. Hen No. 8, with a lesion in the posterior hypothalamic nucleus (See Fig. 6), had a nineteen day interruption in her egg-laying cycle. At the end of this period she recycled and appeared to be as normal as before the lesioning. Hen No. 10 laid two eggs twelve and twenty-six days post-operative. She too had lesions in the posterior hypothalamic nucleus. In most birds with lesions in other parts of the diencephalon (See Fig. 9), there was no interruption of the pre-operative egg-laying cycle. Some birds showed a short interruption of their normal clutch followed by an altered, but recycled egg-laying series. Birds with short interruptions in their egg-laying cycles showed slight decreases in their post-operative comb factor values; i.e. 2 to 8% decrease. Decreases in the wattle size paralleled those of the comb. Table II on page 18 summarizes comb and wattle factor measurements of twenty-six birds.

Those birds with over 18% comb factor change had ovaries with atrophic or reabsorbed follicles; some were no larger than small beads. The oviducts from these birds also appeared to be atrophied. The length of the laying hen's oviduct ranges from 50 to 70 cm. Oviducts measuring 15 to 30 cm are considered here to be atrophic. A comparison of sections of the oviducts (in the region of the albumen gland) from normal and non-laying birds is shown in Figs. 13 and 14 on page 39 and 40 respectively.

TABLE II

PERCENTAGE CHANGE IN COMB AND WATTLE FACTOR MEASUREMENTS[1]

Hen No.	Percentage Change in Comb Factor	Percentage Change in Wattle Factor	Post-operative Duration	Length of Oviduct (cm)
11	11.6	-2.1	14 days	35
12	-32.5	-30.7	" "	17
13	- 5.5	7.1	" "	35
14	-38.6	- 1.9	" "	23
15	-49.3	-47.0	" "	17
16	-52.8	-59.6	" "	22
18	-34.6	-26.1	11 days	24
19	-39.6	-57.9	" "	36
20	-29.3	-34.2	" "	23
21	- 2.3	0.0	" "	58
22	- 8.8	-23.7	" "	46
23	- 6.4	10.1	" "	58
24	- 8.7	-14.7	" "	57
25	0.0	13.3	" "	56
26	-20.0	-39.2	" "	38
27	-26.0	- 2.6	" "	32
28	-18.0	- 7.5	" "	21
29	-18.8	-60.9	" "	20
30	-33.6	-24.8	" "	25
31	- 6.5	14.8	" "	54
32	- 3.7	4.3	" "	53
33	1.8	7.6	" "	52
C-1	0.1	2.2	No lesion made	52
C-2	- 1.2	11.9	" " "	48
C-3	1.8	10.9	" " "	51
C-4	4.2	1.7	" " "	47

1. Comb and wattle factor measurements were calculated from one-half the length times the height of the comb or wattle respectively. The percentage change refers to the post-operative factor change divided by the pre-operative factor measurement times 100. Negative values represent a percentile reduction at the end of the experiment.

The condition of the albumen gland from birds in this study is quite similar to that observed by Hill et al. (1934) following hypophysectomy. The large folds of the glandular wall of the normal laying hen almost obliterates the lumen of the oviduct, while the connective tissue cores of the folds are inconspicuous. In those birds with cessation of egg-laying and obvious decrease in their comb size, the lumen of the oviduct is large due to the shrinkage of its glandular walls. The connective tissue cores of the folds are also most conspicuous.

Acidophil Counts. Although the birds used in this determination are the same species and age group, there appears to be considerable individual difference among them with regard to acidophil counts. When the pre-operative and post-operative acidophil counts of each bird are compared, (See Table III), the birds' response to lesioning becomes apparent. Non-lesioned birds showed a slight decrease in acidophil counts or they remained relatively constant. In most determinations the increment of change in acidophils is small. Since the number of acidophils in each hen is also a small value, any slight change in this value would result in an obvious percentile change. A percentage change less than 30% is not regarded as a significant response to treatment.

Six birds out of twenty showed an increase in acidophils greater than 30%. Of these six, three had lesions in the median eminence. Six out of seven birds with lesions in the nucleus supraopticus failed to show a significant percentage increase. The one exception here also had its lesion extending into the nucleus paraventricularis, lateral forebrain bundle, and periventricular fiber system. Three birds, each with large lesions in the nucleus paraventricularis showed an increase greater

TABLE III. ACIDOPHIL COUNT PER 0.9 ml BLOOD

Hen No.	Pre-operative Count	Post-operative Count	Percentage Change	Lesion Location
18	23	44	91	NS(VD),NP(V & DD),PFT
19	22	29	32	ME,NM(MD),PFT
20	19	20	5	CSM,NP(D & LD),PFT
21	15	18	20	NS(PD)
22	25	32	28	CH
23	21	18	-14	NS(PD)
24	20	18	-10	NS(PD)
25	16	18	12	NS(PD)
26	13	31	138	ME,PT,PH
27	26	19	-27	NP(LD)
28	15	26	73	ME
29	23	30	30	NP(DD)
30	14	49	250	LFB,NS(PD)NP(D & LD),PFT
31	20	11	45	NS(PD)
32	12	11	- 8	LFB
33	17	19	12	NS(PD)
C-1	16	16	0	-
C-2	36	33	- 8	-
C-3	12	10	-17	-
C-4	32	23	-28	-

Key to lesioned areas: CH=cerebral hemisphere; CSM=corticosepto-mesencephalicus; LFB=lateral forebrain bundle; ME=median eminence; NM(MD)= nucleus mammillaris (medial division); NP(V,L & DD)-nucleus paraventricularis (ventral, lateral & dorsal division); NS(P & VD)=nucleus supraopticus (preoptic and ventral division); PFT=periventricularis fiber tracts; PH=posterior hypothalamus; PT=pars tuberalis.

Percentage change in acidophils was calculated from the post-operative change divided by the pre-operative count times 100. Negative values represent a percentile reduction at the end of the experiment.

than 30%, while three other birds with small lesions in the dorsal limits of the nucleus showed an increase less than 30%.

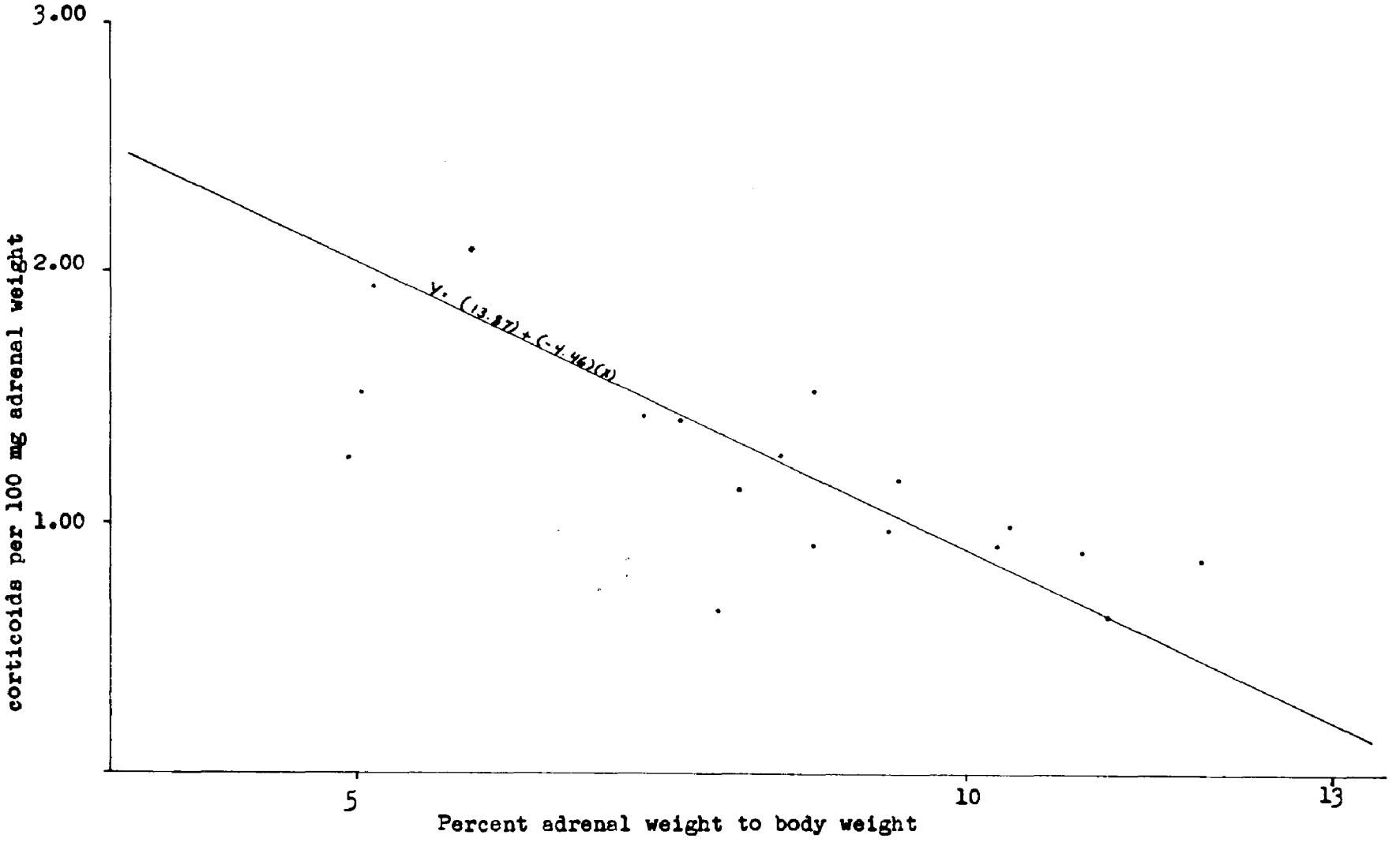
Adrenal Analysis. Data of adrenal corticoid concentration from twenty birds with lesions at different loci in the diencephalon are recorded in Table IV together with their body and adrenal weights. When adrenal corticoid concentration per 100 mg of adrenal tissue is compared with the ratio of adrenal to body weight, a negative correlation of 0.72 is obtained. Graph I on page 23 represents the regression curve of this data. In spite of the apparent inverse relationship these values may be explained differently if the adrenal gland of the White Leghorn hen has a basal level of corticoids that is produced independently of the hypothalamus or pituitary. An increase in the adrenal to body weight ratio apparently is not accompanied by an increase in corticoid concentration. It is suggested that an influence by the pituitary on the adrenal corticoid concentration may exist in the White Leghorn hen, but only at times of stress.

The failure to obtain a better correlation may be explained in either of two ways. First, if there is a basal level of corticoid concentration common to all hens, it seems logical to assume that this level is maintained within a definite range rather than at a precise level. A second explanation might involve the weighing techniques used for this determination. Although a Roller-Smith balance with an accuracy of 1/5 of 1% was used, the weights obtained were wet weights. Since the average adrenal weight was only 70 mg, any small difference in body fluid, or untrimmed connective tissue, might introduce some inaccuracy in the final results.

No difference in the ratio of cortical to medullary tissue of the

TABLE IV. ADRENAL WEIGHTS AND CORTICOID CONCENTRATION

No. Hen	Body Wt (gm)	Absolute Adrenal Wt (mg)	Percent Adrenal Wt / Body Wt	γ Corticoids / 100 mg Adrenal Tissue
18	1903	159.8	8.7	0.90
19	1958	199.0	10.2	0.59
20	1674	82.9	4.9	1.24
21	1919	152.1	7.9	0.67
22	1733	178.0	10.3	0.98
23	2121	106.7	5.0	1.50
24	2114	153.8	7.3	1.42
25	1879	110.3	5.9	2.08
26	1647	182.8	11.1	0.63
27	2000	101.6	5.1	1.93
28	1193	172.5	14.5	0.71
29	1654	139.4	8.4	1.45
30	1341	125.4	9.4	1.19
31	1936	168.6	8.7	1.51
32	1642	143.0	8.7	0.99
33	1604	149.2	9.3	0.99
C-1	1647	196.2	11.9	0.86
C-2	1362	110.2	8.1	1.11
C-3	1661	126.9	7.6	1.40
C-4	1546	169.0	10.9	0.89



Graph 1. Regression curve of corticoid per 100 mg adrenal weight plotted against percent adrenal weight to body weight. ($r = -0.72$).

birds in this study could be detected. The cellular structure was consistently similar in all birds; i. e. none of the cells appeared to be atrophic or hypertrophic.

Thyroids. The average value obtained for percent thyroid weight to body weight is 7.6%. Every bird with a thyroid-body weight ratio between 3.3 to 4.6% had lesions in the nucleus supraopticus preoptic division (See Fig. 10). No bird with a lesion in the preoptic division had a percentage value greater than 4.6%. Two birds with lesions in the lateral, dorsal and ventral divisions of the nucleus supraopticus did not show a decrease in percentage weight from that of the proposed normal weights. Lesions in other parts of the diencephalon appeared to have no affect on thyroid glands.

There does not appear to be any obvious morphological variation in the follicle epithelium between the birds with low and high percentages of thyroid weights. There does appear to be a definite accumulation of intracellular colloidal droplets in the central portion of the glands as well as in the periphery in normal hens. Birds with lesions in the nucleus supraopticus preoptic division are void of these droplets in the central regions of their glands two weeks following operations. Turner (1950) indicates that intracellular colloidal droplets are associated with active thyroid glands. The fact that post mortems were performed just two weeks following lesion placement, may explain why there was no obvious breakdown of the follicle epithelium. Figures 15 and 16 present a comparison of the thyroid from a normal laying hen and one from a hen that has been lesioned in the nucleus supraopticus, preoptic division.

DISCUSSION

The observations recorded above support the view that the physiological role the pituitary plays in the body is dependent upon the hypothalamus. The physiological changes in the hen following electrolytic coagulation of areas in the diencephalon are similar, though more interpretive, to those of hypophysectomy techniques. When one removes the pituitary completely, the post-operative syndrome taken by the animal involves changes in his endocrine glands, body size, behavior and similar responses all at the same time. The lesioning techniques employed in this research have permitted a more isolated study of these responses and their control by the diencephalon. It has been demonstrated with the data above that lesions in different parts of the diencephalon produce different, though specific , responses in the White Leghorn hen. It has also been shown that lesions can be placed in parts of the brain with no apparent affect on certain trophic responses.

Cessation in the egg-laying of hens was brought about by lesions in the nucleus paraventricularis, median eminence and pars tuberalis. Histological studies concerning neurosecretion in birds by Wingstrand (1951) and Oksche et al. (1959) suggest that it is the nucleus paraventricularis that is responsible for the initial control over oviposition in the hen. Wingstrand suggested that the portal system must be the mechanism through which the hypothalamus exerts its control over the adenohypophysis. Oksche et al.(1959) report that the median eminence is a storage site of neurosecretory material second, quantitatively, only

to the neurohypophysis. It can be understood from the work of these investigators how lesions in the median eminence or pars tuberalis could block the control coming down via fiber tracts to the pituitary from the nucleus paraventricularis. These findings also give support to Ralph's (1959) investigations on gonadotrophin release in the hen.

Release of corticotrophin by the anterior pituitary in mammals appears to be under the control of the hypothalamus (Ganong, 1959). In the White Leghorn hen lesions were placed at different loci in the diencephalon with the hope of blocking the corticotrophin release from the anterior pituitary and the subsequent change of corticoid concentration in the adrenal glands. There did not appear to be any correlation between lesioned areas and corticoid titer of any of the birds two weeks following the operations. Although these results do not agree with results from similar studies in mammals, they do support hypophysectomy studies on pigeons by Miller (1961) and a corticosterone study on chickens by Dullin (1955). The data presented by these investigators and that which is reported here suggest that the bird adrenal gland, unlike that in mammals, functions independently from the pituitary or hypothalamus. The histological arrangement of the adrenal glands in birds differs from that of mammals (Hays, 1914) in that the cortical and medullary tissue is intermingled with each other. A histological comparison of the adrenal tissue from lesioned birds with non-lesioned birds did not reveal any difference in their cellular structure.

Some of the lesioned birds in this research showed a significant increase in acidophil counts (See pages 19 and 20). Newcomer (1958) reports that responses to stress are accompanied by an increase in acido-

phils. Since none of the birds in these experiments were subjected to stress,other than the initial operation, the increase in acidophils in those birds with lesions in the above mentioned areas is not clearly understood. One possible explanation might suggest that these lesioned areas control or maintain a "normal level" in each bird, and that when this control center is destroyed, the level of circulating acidophils is increased. The mechanism by which stressors bring about an increase in acidophils might therefore be similar in their blockage of these hypothalamic areas.

Figures 15 and 16 illustrate the apparent influence that the nucleus supraopticus, preoptic division has on the activity of the thyroid gland in the White Leghorn hen. It is understood that the appearance, or lack of, colloidal droplets is a qualitative rather than a quantitative index of activity. The percentage weights of these thyroids to body weights show a similar thyroid picture; and, support these qualitative findings as possible indicies of thyroid response to diencephalic lesioning. With the rapidly growing knowledge of radiation biology, more quantitative experiments should be easy to design in future thyrotrophic research of this kind.

LITERATURE CITED

1. D'Angelo, S. A. and R. E. Traum. 1958. An Experimental Analysis of the Hypothalamic-Hypophysial-Thyroid Relationship in the Rat. Annals of the New York Acad. of Sci. 72(7):239-270.

2. Dear, W. E. and R. Guillemin. 1960. Adrenal Sensitivity to ACTH as a Function of Time after Hypothalamic Lesions and after Hypophysectomy. Proc. Soc. Exp. Biol. and Med. 103:356-358.

3. Dullin, William E. 1955. The effect of Cortisone on the White Leghorn Cockerel and Capon. Poultry Sci. 34(1):73-77.

4. Feldman, S. E., S. Larsson, M. K. Dimick and S. Lepkovsky. 1957. Aphagia in Chickens. Amer. J. of Physiology 191(2):259-261

5. Fraps, R. M. 1955. Egg Production and Fertility in Poultry. In: Progress in the Physiology of Farm Animals. Vol. 2. Butterworths Scientific Pub., London.

6. Ganong, W. F. 1959. Adrenal-Hypophyseal Interrelations. In Comparative Endocrinology, 187. (Gorbman, A. Editor. J. Wiley and Sons, Inc. New York, N. Y.).

7. Ganong, W. F. and P. H. Forsham. 1960. Adenohypophysis and Adrenal Cortex. Annual Review of Physiology 22:579-614.

8. Hays, V. J. 1914. The development of the Adrenal Glands of Birds. Anat. Rec. 8(10):451-474.

9. Hill, R. T., A. B. Corkill, and A. S. Parkes. 1934. Hypophysectomy of birds. II. General Effects of Hypophysectomy of Fowls. Proc. Roy. Soc. London 115(B798):208-220.

10. Hill, R.T. and A. S. Parkes. 1934. Hypophysectomy of Birds. III: Effects of Gonads, Accessory Organs and Head Furnishings. Proc. Roy. Soc. London. 116(B798):221-236.

11. Legait, H. 1955a. Correlations endocriniennes de l'hypothalamus et de la neurohypophyse chez la Poule Rhode-Island. C. R. Society Biol. (Paris) 149(9-10):1016-1018.

12. _____ 1955b. L'hydrencephalocrine neurosecretoire chez la Poule Rhode-Island au cours du cycle annuel et dans diverses conditions experimentales. C. R. Society Biol. (Paris) 149(13-14):1459-1461.

13. _____ 1957. Correlations hypothalamo-hypophyso-thyroidiennes chez la Poule Rhode-Island. C. R. Society Biol. (Paris) 151(4): 760-763.

14. McCann, S. M. and P. Haberland. 1960. Further Studies on the Regulation of Pituitary ACTH in Rats with Hypothalamic Lesions. Endocrinology 66(2):217-221.

15. McDonald, William. 1962. Nuclei and Fiber Tracts of the Diencephalon of the Chicken, Gallus domesticus. M. S. Thesis, University of Arizona.

16. Miller, R. A. and O. Riddle. 1942. The cytology of the adrenal cortex of normal pigeons and in experimentally induced atrophy and hypertrophy. Amer. J. Anat. 71(1):311-341.

17. Miller, R. A. 1961. Hypertrophic Adrenals and their response to stress after lesions in the median eminence of totally hypophysectomized Pigeons. Acta Endocrinologica. 37:565-576.

18. Mitchell, J. B. 1929. Experimental Studies of the Bird Hypophysis. I. Effects of Hypophysectomy in the Brown Leghorn Fowl. Physiol. Zoology. 2(3):411-437.

19. Nalbandov. A. V. and L. E. Card. 1943. Effect of Hypophysectomy of Growing Chicks. J. Exper. Zool. 94(3):387-413.

20. Newcommer, W. S. 1958. Physiologic Factors which influence Acidophilia Induced by Stressors in Chickens. Amer. J. Physiol. 194(2):251-254.

21. _____ 1959. Effects of Hypophysectomy on some Functional Aspects of the Adrenal Gland of the Chicken. Endocrinology 65(1): 135-135.

22. Oksche, A., D. F. Laws, F. I. Kamemoto, and D. S. Farner. 1959. The Hypothalamo-Hypophysial Neurosecretory System of the White Crowned Sparrow, Zonotrichia leucophrys gambelii. Zeitschrift fur Zellforschung 51:1-42.

23. Ralph, C. L. 1959. Some Effects of Hypothalamic Lesions on Gonadotrophin Release in the Hen. Anat. Rec. 134(3):411-431.

24. Ralph, C. L. and R. M. Fraps. 1959a, Effect of Hypothalamic Lesions on Progesterone-induced Ovulation in the Hen. Endocrinology 65(5):819-824.

25. _____ 1959b. Long-term Effects of Diencephalic Lesions on the Ovary of the Hen. American J. of Physiol. 197(6):1279-1283.

26. Saffran, Murray. 1958. XI Hormonal Functions of the Hypothalamus. (Allan Memorial Institute of Psychiatry, Montreal, Canada). Ergebnisse der inneren Medizin und Kinderheilkunde. Neue Folge. 10(11):594-612.

27. Schooley, J. P., O. Riddle, and R. W. Bates. 1941. Replacement

Therapy in Hypophysectomized Juvenile Pigeons. Amer. J. Anat. 69(1):123-157.

28. Shirley, H. V. Jr. and A. V. Nalbandov. 1956. Effects of Neurohypophysectomy in Domestic Chickens. Endocrinology 58(4):477-483.

29. Sweat, Max L. 1954. Sulfuric-Induced Fluorescence of Corticosteroids. Analytical Chem. 26:773-776.

30. Turner, C. W. 1950. Thyrotrophic Hormone. In: Hormone Assay(edited by C. W. Emmens. Academic Press Inc. New York.).

31. Wingstrand, K. G. 1951. The Structure and Development of the Avian Pituitary. Lund-Gleerup.

32. Wiseman, B. K. 1931. An Improved Direct Method for Obtaining the Total White Cell Count in Avian Blood. Proc. Soc. Biol. and Med. 28:1030-1033.

Figure 4a. Brain No. 6. Lesion in Nucleus Mammillaris Lateral Division and Lateral Extension of the Pars Tuberalis. 14x.

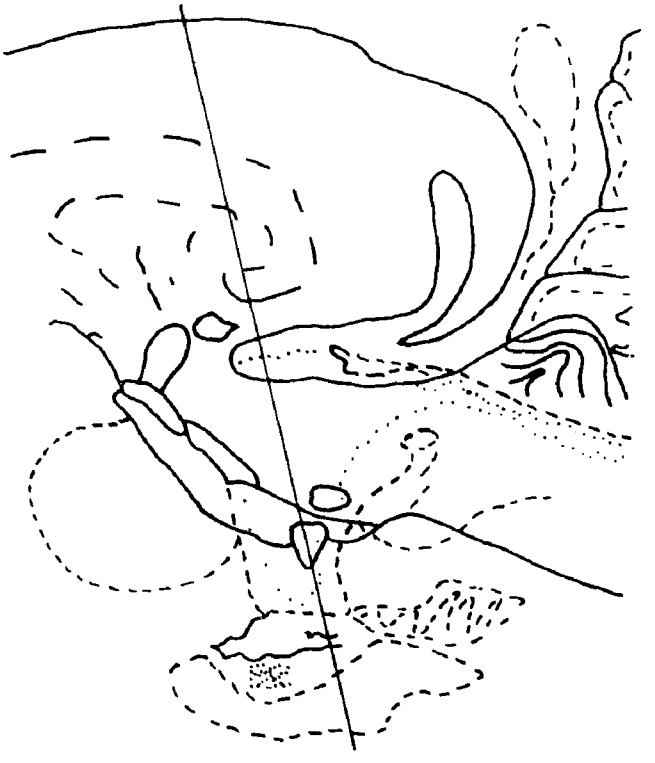


Figure 4b. Schematic of mid-saggital view of the brain showing the plane at which the above section was made.

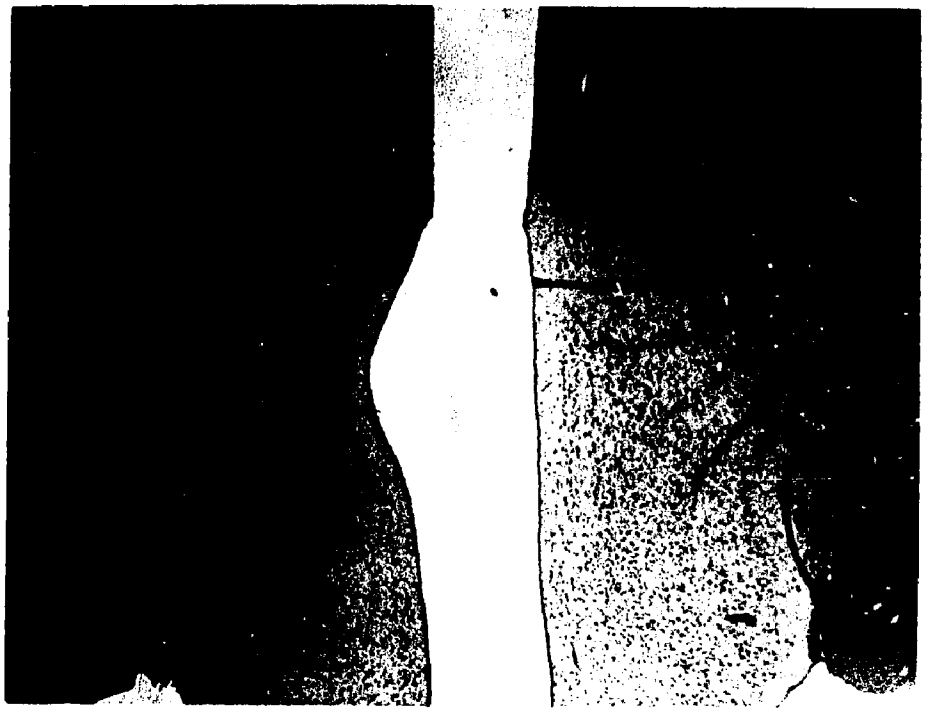


Figure 5a. Brain No. 8. Unilateral lesion in Periventricularis Fiber System. 14x.

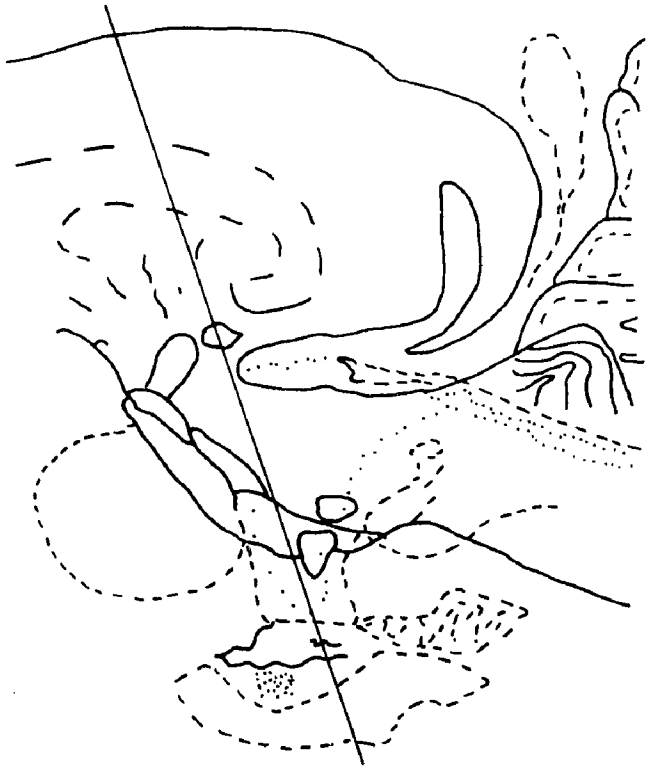


Figure 5b. Schematic of mid-saggital view of the brain showing the plane at which the above section was made.

Figure 6a. Brain No. 10. Lesion in the Posterior Hypothalamus. 14x

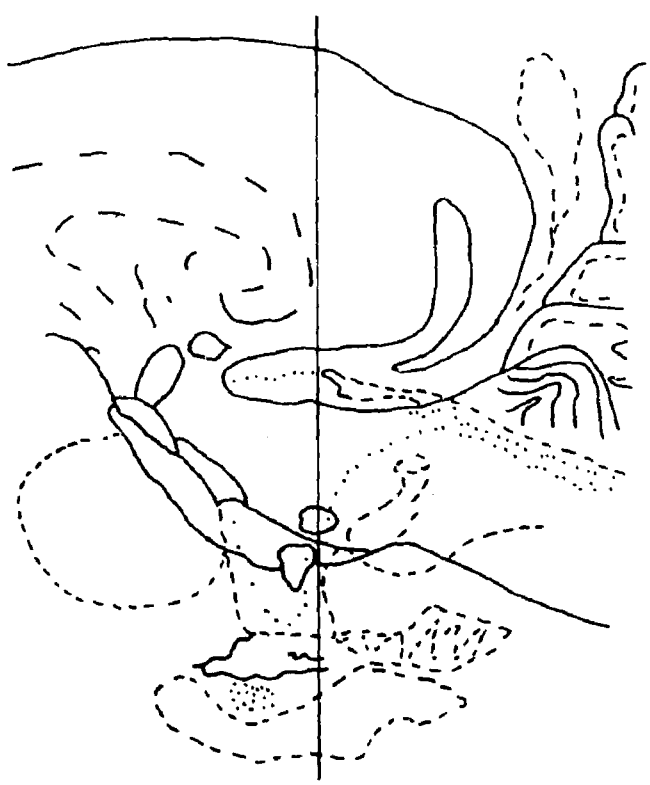


Figure 6b. Schematic of mid-saggital view of the brain showing the plane at which the above section was made.

Figure 7a. Brain No. 18. Large lesion in the Nucleus Supraopticus Ventral Division, Nucleus Paraventricularis Ventral and Dorsal Division and Periventricular Fiber System. 14x.

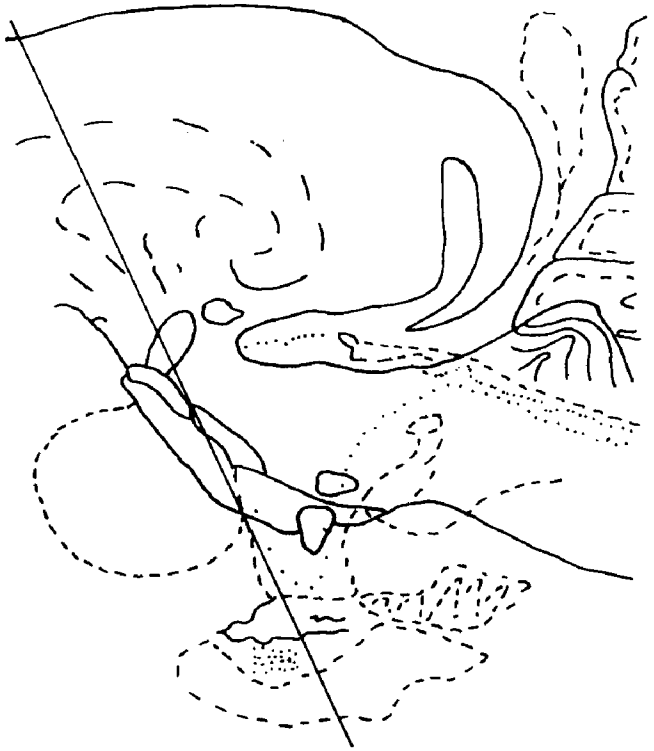


Figure 7b. Schematic of mid-saggital view of the brain showing the plane at which the above section was made.

Figure 8a. Brain No. 20. Lesion in the Nucleus Paraventricularis Lateral and Dorsal Division and in the Corticoseptomesencephalicus and Periventricularis Fiber System. 14x.

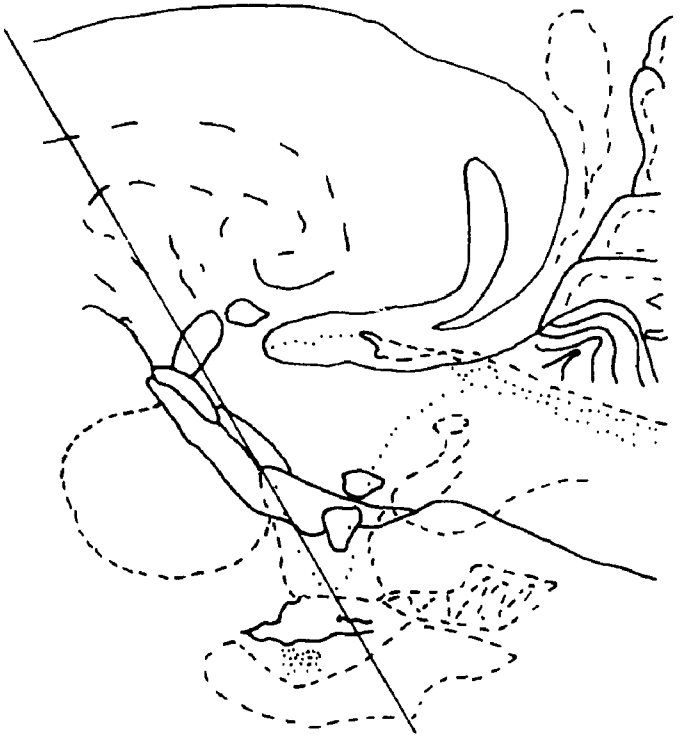


Figure 8b. Schematic of mid-saggital view of the brain showing the plane at which the above section was made.

Figure 9a. Brain No. 22. Bilateral lesion in Cerebral Hemisphere above the Corticoseptomesencephalicus Fiber Tracts. 14x.

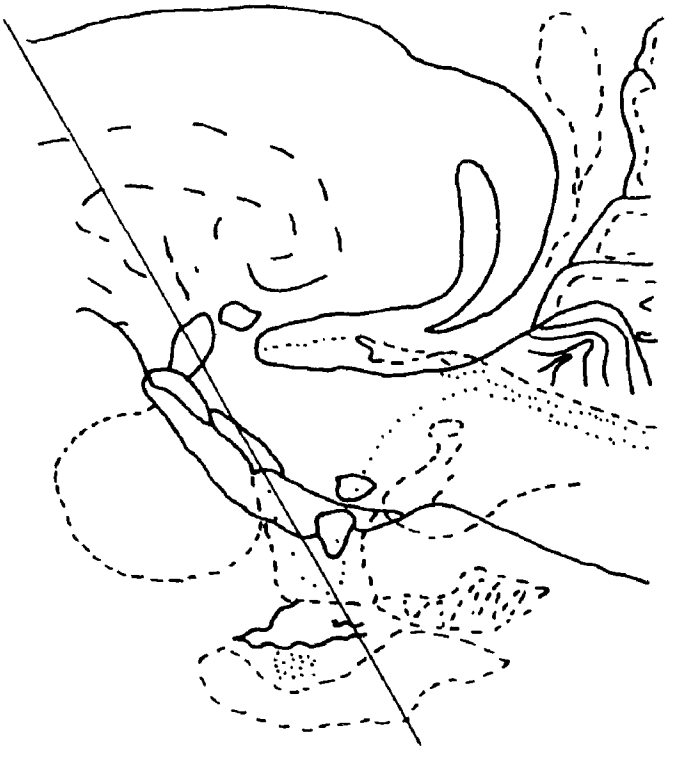


Figure 9b. Schematic of mid-saggital view of the brain showing the plane at which the above section was made.

Figure 10a. Brain No. 23. Lesions in the Nucleus Supraopticus Preoptic Division. 14x.

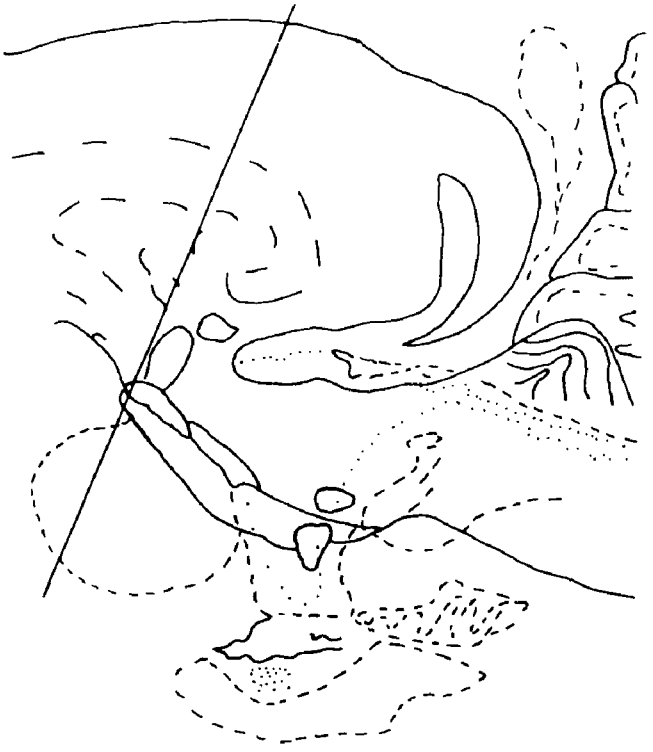


Figure 10b. Schematic of mid-saggital view of the brain showing the plane at which the above was made.

Figure 11. Photograph of normal laying hen (C-4) showing typical comb size.

Figure 12. Photograph of non-laying hen (No. 16) showing atrophic comb.

Figure 13a. Oviduct of a normal laying hen (C-4), region of the Albumen Gland, showing the small lumen. 14x.

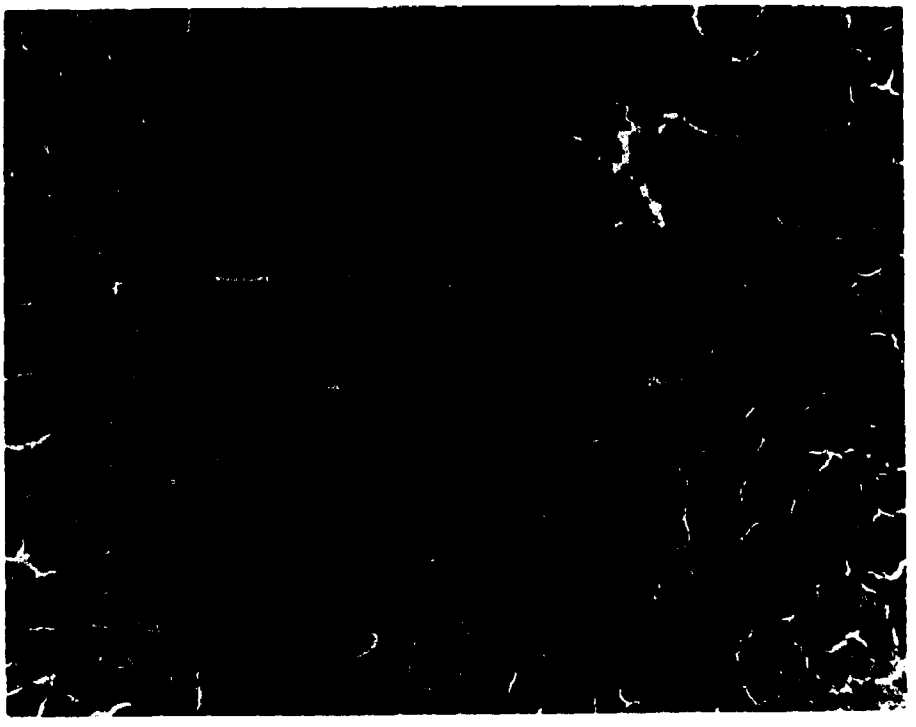


Figure 13b. Enlarged microphotograph of one fold from the oviduct in Fig. 13a showing the rather inconspicuous connective tissue core in the middle. 40x.

Figure 14a. Oviduct of a non-laying hen (No. 16), region of the Albumen Gland, showing the large lumen and its shrunken glandular folds. 14x.

Figure 14b. Enlarged microphotograph of one fold from the oviduct in Fig. 14a showing the relatively large connective tissue core in the middle. 40x.

Figure 15. Cross section through a Thyroid Gland from a normal laying hen. Focused to show colloidal droplets. 15x.

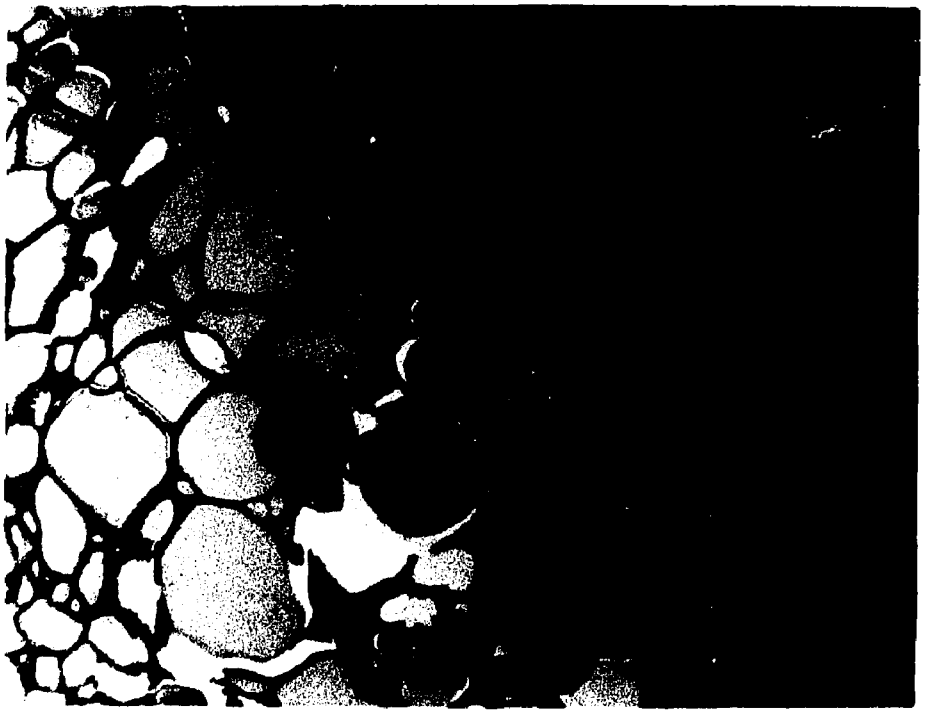


Figure 16. Cross section through a Thyroid Gland from a hen with lesions in the Nucleus Supraopticus Preoptic Division. Note the lack of colloidal droplets in most of the follicles. 15x.