ROLE OF THE HYPOTHALAMUS IN THE CONTROL OF THYROTROPIN PRODUCTION BY THE ANTERIOR PITUITARY OF CHICKENS

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CHAPTER I

INTRODUCTION

The anterior pituitary is essential for normal thyroidal morphology and iodine metabolism in mammals (Taurog et al., 1958) and in birds (Nalbandov and Card, 1943; Tixier-Vidal and Assenmacher, 1962; Hurst, 1963). Without thyrotropin (TSH) from the anterior pituitary, thyroidal iodine metabolism is only a vestige of that seen in intact animals; not only is the synthesis of the thyroidal hormones drastically reduced by hypophysectomy but the distribution of the intrathyroidal iodinated amino acids is also altered in a rather specific manner (Taurog et al., 1958; Tixier-Vidal and Assenmacher, 1962; Hurst, 1963).

It is evident in both mammals and birds that certain apparently neurally mediated stimuli elicit changes in the secretion rate of the trophic hormones from the anterior pituitary. For the past four decades (Smith, 1927) the hypothalamus has been suspected as being the connecting link between the nervous system and the anterior pituitary. The techniques of stalk-sectioning (Uotila, 1939a,b, 1940; Brown-Grant et al., 1957; Donovan and Harris, 1956) and pituitary heterotopic transplantation (Greer et al., 1953; Scow and Greer, 1955; Harris and Jacobsohn, 1952; Nikitovich-Winer and Everett, 1958, 1959), which result in separation of the pituitary from the hypothalamus, have strongly implicated the hypothalamus in the regulation of TSH secretion in the

mammal.

The technique of selectively destroying discrete areas of the mammalian hypothalamus has clearly shown that this structure is essential for normal thyroidal activity (Greer, 1951, 1952, 1955; Ganong et al., 1955; D'Angelo and Traum, 1956; D'Angelo, 1958; Averill et al., 1961). Unresolved, however, is the question of whether the hypothalamus is essential only for "setting" the basic level of TSH secretion or whether it is also essential for bringing about changes in the secretion rate of TSH in response to alterations in the environment, either internal or external (van der Werff ten Bosch and Swanson, 1963; Moll et al., 1961; D'Angelo, 1960; van Beugen and van der Werff ten Bosch, 1961; Yamada et al., 1965; de Jong and Moll, 1965; Andersson et al., 1962a,b; 1963; 1965).

The hypothalamic control of TSH secretion in mammals is apparently humorally mediated via the hypothalamic-hypophysial portal system (Guillemin et al., 1962; Bowers et al., 1965; Guillemin et al., 1965a, b).

The role of the hypothalamus in the bird is less well understood. Although there is a rather vast literature on neurosecretion in the bird (Oksche et al., 1959; Farner and Oksche, 1962; Benoit, 1962) most of the work has been concerned with gonadal control and very little has dealt with the control of the thyroids or adrenals. While the hypothalamic control over the adenohypophysial gonadotropic function in birds appears to be complete, the control over the corticotropic and thyrotropic functions is less striking (Benoit, 1962). Although hypophysectomy in the bird results in a drastic curtailment of thyroid

structure and function, neither stalk-section (Shirley and Nalbandov, 1956; Assenmacher, 1958) nor pituitary heterotopic transplantation (Ma, 1963) appears to appreciably affect the thyroid. Recent evidence has been presented which indicates that certain brain lesions result in reduced thyroidal weights (Egg and Chiasson, 1963).

In view of the facts 1) that the production of TSH by the anterior pituitary in mammals is quite dependent upon the hypothalamus and the vascular connections with it while in the bird the production of TSH appears to be rather autonomous; 2) that most of the assays for secretion rate of TSH, after manipulation of the hypothalamic-pituitary axis, have been based on morphologic changes in the thyroid (an assay which has been determined to be rather insensitive); and 3) that the TSH content of the chicken pituitary has not been reported, it was deemed desirable to investigate further the possible functional relationship between the hypothalamus and the anterior pituitary in the bird.

The purpose of this investigation was to study some of the effects of hypothalamic lesions on thyroidal function and on the content of TSH in the anterior pituitary of the bird. Additional information was also gathered on the effects of hypothalamic lesions on gonadal weights in the bird.

CHAPTER II

REVIEW OF THE LITERATURE

The purpose of this literature review is to present evidence that the hypothalamus exercises some degree of control on the production of thyrotrophic hormone (TSH) by the adenohypophysis of birds. Certain anatomical considerations of the hypothalamic-hypophysial axis will also be presented.

The review is intended to be rather complete in showing the interrelationships between the hypothalamus and the anterior pituitary. Since several recent reviews are available which cover the various aspects of thyroidal physiology, a detailed review of thyroidal function will not be included.

The physiologic regulation of the thyroid and pituitary has been reviewed by Solomon and Dowling (1960), Bodganove (1962), Blanquet (1962), Reichlin (1963), Purves (1964), and Rosenberg and Bastomsky (1965); synthesis of thyroid hormones by Solomon and Dowling (1960), Ingbar and Galton (1963), Rosenberg and Bastomsky (1965), and Rosenberg et al. (1965); peripheral actions and metabolism of thyroid hormone by Solomon and Dowling (1960), Ingbar and Galton (1963), and Rosenberg and Bastomsky (1965); thyroid intermediary metabolism by Ingbar and Galton (1960); the chemistry of thyroglobulin by Edelhoch (1965); and the hypothalamic releasing factor by Guillemin (1964).

Anatomy

The juxtaposition of the adenohypophysis and hypothalamus suggest a functional relationship especially when the embryonic origin of the two structures are considered. The pituitary of the bird is located at the base of the hypothalamus and consists of two lobes, the adenohypophysis and the neurohypophysis. The adenohypophysis, derived from a diverticulum of the oral ectoderm, is composed of two divisions, the pars distalis and the pars tuberalis. The pars distalis has a cephalic and caudal lobe which differ somewhat in cytologic appearance. The pars tuberalis is a very thin segment which, in the postnatal form, forms a collar around the infundibulum. These two structures together are known as the pituitary stalk.

The neurohypophysis is an outgrowth of the neural tube and consist of the infundibulum and its enlarged terminal end, the infundibular process (sometimes called the pars nervosa). The infundibulum may be divided into a more anterior portion, the median eminence, and a more caudal segment, the infundibular stem. It is primarily around the median eminence that the pars tuberalis lies and this is the only anatomic connection between the pars distalis and the infundibulum in birds (Wingstrand, 1951).

Several extensive investigations of the avian adenohypophysis have revealed that this structure is virtually devoid of nerve fibers, (Green, 1951; Drager, 1944, 1945) except for a few strands which are derived from the sympathetic nervous system and which are assumed to be vasomotor fibers (Wingstrand, 1951).

The vascular supply to the avian adenohypophysis has been de-

scribed as being derived from a dense capillary network which covers the median eminence and which is drained by portal vessels, via the pars tuberalis, into a secondary capillary plexus in the pars distalis (Green, 1951). It is not known whether the hypothalamic-hypophysial portal system represents the sole route of blood supply to the anterior pituitary of the bird as it does in the rat (Goldman and Sapirstein, 1962).

A very peculiar innervation of the median eminence has been reported in various avian species in which fine nerve fibers turn perpendicular to the axis of the infundibulum and form loops in the superficial layer of the median eminence in close contact with the primary capillary plexus of the hypothalamic-hypophysial portal system (Wingstrand, 1951; Benoit, 1962 [originally reported in 1951]). A large number of neurosecretory droplets can be seen along these nerve fibers. Benoit (1962) believes that these droplets originate in the neurons of the magnocellular anterior hypothalamic nuclei; their transfer into the portal veins has been reported (Arizone and Okamoto, 1957 [quoted from Benoit, 1962, p. 256]).

It would appear, therefore, that the neural loops in close association with the primary portal plexus form an anatomic arrangement ideally situated for transmission of neurohumoral substances from the hypothalamus to the adenohypophysis. It is also probable that various parts of the primary capillary plexus go to circumscribed parts of the adenohypophysis (Adams et al., 1964). This would suggest that a mechanism is available by which groups of anterior lobe cells of similar function could receive specific neurohumors produced by particular

groups of hypothalamic nerve cells. This would not be absolutely necessary, however, since a neurohumor could possibly be specific for a particular target cell-type in the adenohypophysis. In fact, electron microscopy revealed that the majority of the synapses in the anterior hypothalamus contained two types of vesicles, this suggested that a single synapse might be able to liberate more than one transmitter substance (Pellegrino de Iraldi et al., 1963).

It appears, from the above observations, that even though there is no, or at the best a very limited, nerve supply to the adenohypophysis there is a rich portal blood supply. The blood supply is derived from an area which is richly innervated with fibers which contain neurohumoral droplets. Thus it is readily apparent that information from the nervous system could be transmitted to the anterior pituitary.

The structure of the avian hypothalamus has been studied by several investigators (Huber and Crosby, 1929; Kuhlenbeck, 1937; Oksche et al., 1959) and recently an atlas of the avian brain has been published (van Tienhoven and Juhasz, 1962). Most of the anatomic studies of the diencephalon of the bird have concerned the nuclei and less attention has been given to the fiber tracts, most of which are fine and difficult to trace.

The hypothalamus contains a dozen or more of more-or-less discrete pairs of nuclei. According to Farmer and Oksche (1962), the hypothalamic neurosecretary system of birds consist of: a) two neurosecretary nuclei, the supraoptic nucleus and the paraventricular nucleus; b) two neurosecretory tracts, the supraoptico-hypophysial tract and the paraventriculo-hypophysial tract; c) the neurosecretion-rich infundibular

branches of the neurosecretory tracts in the median eminence; and d) the neural lobe of the hypophysis. Although most of the fibers of the neurosecretory tracts pass to the neural lobe there are many which turn ventrally, at various levels, to penetrate the superfical layer of the median eminence. In addition the neurosecretory tracts are augmented by fibers derived from more caudal divisions of the neurosecretory nuclei. It appears, therefore, that the median eminence receives nerve fibers from a large portion of the hypothalamus, especially the anterior portions.

Interrelationship Between the Anterior Pituitary and the Thyroid

There is now no doubt that the thyroid is almost completely dominated by the anterior pituitary in both mammals and birds as shown by hypophysectomy studies both with and without substitution theraphy. This domination is mediated by the thyrotropic hormone (TSH) which affects not only all phases of thyroidal iodine metabolism but also a vast array of other intrathydroidal metabolic reactions.

The function of the thyroid gland is to produce the thyroidal hormones which are thyroxine (T_4) and triiodothyronine (T_3). The formation of these hormones involves the following generally accepted steps in the sequence given: 1) active transport of iodide into the thyroidal cells, 2) oxidation of the accumulated iodide, 3) synthesis of thyroglobulin (TGB), 4) iodination of the tyrosine groups in TGB to form monoiodotyrosine (MIT) and diiodotyrosine (DIT) groups, 5) coupling of two molecules of the iodotyrosines to form T_4 and T_3, 6) pinocytosis

of TGB from the colloid into the follicular cells, 7) proteolysis of TGB into the iodothyronines and iodotyrosines, 8) the liberation of the iodothyronines from the follicular cell, and 9) the deiodination of the iodotyrosine within the follicular cells.

Not only does TSH affect the thyroid but the thyroidal hormones also act back on the anterior pituitary and inhibit its secretion of TSH. This inhibition is apparently at the level of the anterior pituitary (Bogdanove and Crabill, 1961; Kendall, 1962; von Euler and Holmgren, 1956a; Harrison, 1961; Reichlin and Boshans, 1964). However, some investigators have reported that T_4 when injected into the hypothalamus inhibits thyroid function after a latent period of several hours (Yamada, 1959; Yamada and Greer, 1959).

The question of whether secretion and synthesis of TSH are synonymous or whether the two may be independent of each other is still somewhat controversial but it appears that the two may vary independently and that T_4 inhibits the secretion rate of TSH (Contopoulos and Koneff, 1963; Bakke and Lawrence, 1964; Salaman, 1964; Solomon and McKenzie, 1966).

Evidence is accumulating which indicates that it is not the level of circulating thyroid hormones *per se* which inhibits TSH secretion but rather the rate of peripheral deiodination of the thyroid hormones (Escobar del Rey and Morreale de Escobar, 1961; Morreale de Escobar and Escobar del Rey, 1962; Tanabe *et al.*, 1965; Werner *et al.*, 1961; Grinberb *et al.*, 1963). The hypothalamus has been implicated in the degradation of T_4 by the anterior pituitary (Reichlin *et al.*, 1966).

Interrelationship Between the Hypothalamus and the Anterior Pituitary. Stalk-Section Studies

As pointed out elsewhere probably no place in the body is there a more advantageous anatomic arrangement for humorally mediated control of an organ than the arrangement between the adenohypophysis and the hypothalamus of birds and/or mammals. Furthermore, various responses of the pituitary-thyroid axis apparently are mediated through the central nervous system and since the only connection between the central nervous system and the adenohypophysis is via the hypothalamus and the "pituitary stalk" the last mentioned structures would seem to be the logical place to commence an investigation of the interrelationship between the two systems.

Smith (1927) was one of the first to suggest that the hypothalamus might play a part in controlling the endocrine organs, especially the gonads.

Some of the earliest work performed in an attempt to determine whether the anterior pituitary depended upon the hypothalamus for synthesis of TSH employed sectioning of the hypophysial stalk (pars tuberalis and infundibulum). Uotila (1939 a,b; 1940) found that the thyroid gland of stalk-sectioned rats remained histologically normal, that they responded to an increased circulating level of T_4 by atrophying, and that they responded to a decreased circulating level of thyroid hormones by hypertrophying but that they were unable to respond normally to cold exposure. After seven days of exposure to a temperature of 5° C (46 days after stalk-section) the sham-operated controls showed a 60% increase in thyroidal follicular cell index

while the stalk-sectioned animals showed only a 5.5% increase. There was no difference in the thyroid weights.

He concluded that the TSH function of the anterior pituitary had a basic rhythm which for the most part was controlled humorally by variations in the T_4 level of the organism without the mediation of the hypothalamic-hypophysial pathways. However, under certain circumstances (such as cold exposure) "impulses" through the pituitary stalk can modify the basic thyrotropic secretion rate.

In 1947 Brolin reported that stalk-sectioning inhibited the appearance of thyroidectomy cells in the rat and four years later it was reported that thyroid hypertrophy due to goitrogen feeding was diminished in stalk-sectioned animals. Thus the original conclusions of Uotila were beginning to be questioned.

These early observations were open to question since permanency of the stalk-sections was not ascertained at autopsy. Also, the use of thyroid morphology wasn't a very sensitive assay for TSH secretion. Therefore, Harris and his associates (Brown-Grant et al., 1957; Donovan and Harris, 1956) conducted a series of experiments in which permanency of stalk interruption was assured by insertion of waxed paper plates between the severed pituitary and the hypothalamus in rabbits. They found that the 48-hour iodide uptake by the thyroid was reduced to about two-thirds of normal and that the rate of I^{131} release from the thyroid was decreased to about 1/2 of normal. These values, nevertheless, were above those for hypophysectomized animals.

When rabbits with plates and without plates were compared it was found that the latter group did not have its 48-hour uptake impaired

while the former group did. The release rate was equally impaired in the two groups and it was intermediate between that of the hypophysectomized and normal controls. Thus it appeared that uptake was less sensitive to stalk interruption than was secretion of the thyroid hormones.

Although stalk sectioning resulted in a 25% weight loss by the pars distalis these workers could find no evidence of fibrous or ischemic changes in the pars distalis and noted that the gland showed evidence of good vascularity.

It was also demonstrated in these permanently stalk sectioned rabbits that the inhibition of I^{131} release normally seen after restraint was blocked while the same response following injection of T_4 or laparotomy was not blocked. It was thus concluded that the hypothalamus, through the mediation of the pituitary stalk, maintains the activity of the thyroid gland and modifies this activity in response to stimuli acting through the nervous system while an increased level of circulating thyroid hormone (and physical trauma) appears to affect the release of TSH by a direct action on the pituitary gland.

That complete section of the pituitary stalk resulted in altered iodine metabolism which was intermediate between that of normal and hypophysectomized animals has been shown. The percent of total iodide taken up by the thyroid which was present as T_4 was 6.9 in sectioned animals compared with 10.3 for normal and 1.8 for hypophysectomized controls and the percent of thyroidal I^{131} found in protein was 15-20% for stalk sectioned rats compared with 70-80% for normal and 3-7% for hypophysectomized controls (Blanquet, 1962).

Thus, from the foregoing experiments it appears that even basal levels of TSH are not maintained without the hypothalamic connection and that those stimuli which act through the central nervous system are blocked. However, apparently the thyroid hormones can act directly on the pars distalis and affect its secretion rate of TSH.

It has been shown that the hypofunction of the anterior pituitary after stalk-sectioning is not due to a decreased blood flow to surviving pars distalis parenchyma (David et al., 1965).

Using thyroid morphology as a criterion in the assay for TSH secretion, Assenmacher (1958) was unable to observe any change in thyroid weight or histology following sectioning of the hypophysial portal veins in the duck (a species in which the portal veins are separate from the pars tuberalis connections between the pars distalis and the infundibulum). She did find that the hypertrophy of the thyroid due to PTU feeding was blocked. She also showed that while there was no morphologic change in the thyroid after portal vein sectioning there was a significant decrease in the radioiodide release rate (Assenmacher and Tixier-Vidal, 1959).

Pituitary Transplantation Studies.

In the case of pituitary transplantation studies, as in the case of stalk-sectioning experiments, many of the early reports were conflicting. The investigators in the early experimental work were restricted to morphologic changes in the thyroid for evaluating the results of their experiments, an assay which has since been recognized as the least sensitive of all the thyroid responses to TSH. Further-

more, they did not report on the completeness of hypophysectomy.

Greer and his associates (Greer et al., 1953; Scow and Greer, 1955) using intraocular pituitary transplants in hypophysectomized mice found that the thyroid weights were reduced to hypophysectomized levels but that the iodine metabolism of the thyroid was somewhat higher and that the pituitary could respond to either an increased or decreased circulating level of T_4.

Harris and Jacobsohn (1952) transplanted pituitaries to several different places in the rat and although all the transplants showed a good blood supply only those animals with transplants placed near the median eminence and which had become revascularized from the primary capillary plexus had normal thyroid histology. The others showed signs of thyroid atrophy. These findings argued in favor of the hypothesis that the secretion of TSH was under hypothalamic control which was mediated by the portal vessels.

When pituitaries which had been transplated under the renal capsule were re-transplanted under the median eminence thyroidal I^{131} uptake was considerably increased and thyroid weight was restored to normal (Nikitovich-Winer and Everett, 1958). This occurred despite the fact that the anterior pituitary when transplanted to the kidney underwent marked degeneration and upon re-transplantation to the median eminence the degeneration was more severe. However, the surviving parenchyma reorganized rather rapidly and one week after transplantation reorganization of the graft was essentially complete (Nikitovitch-Winer and Everett, 1959).

On the other hand, Florsheim and Knigge (1961) found that neonatal

rat anterior pituitaries transplanted to the eye chamber of hypophysectomized recipients supported thyroid function and morphology to a considerable degree as judged from T/S ratio, PBI, I^{131} uptake, thyroid weight, and thyroid cell height. The transplanted pituitaries maintained these indices at 60, 57, 53, 71 and 78%, respectively, of control value. Only 6, 45, 35, 46 and 22%, respectively, of control values were maintained by hypophysectomized controls. They believed that their intra-ocular homotransplants were just as capable of maintaining normal thyroid function as were Nikitovitch-Winer and Everett's (1958) auto-re-transplants and, therefore, they questioned the importance of direct hypothalamic-hypophysial vascular connections for maintenance of normal thyroid function.

These observations (Florsheim and Knigge, 1961) are in line with those of von Euler and Holmgren (1956b) in which the latter workers showed that, although the thyroid gland of the hypophysectomized rabbit bearing an intra-ocular pituitary graft took up much less I^{131} than the normal control, it took up a significantly larger amount than that of the hypophysectomized animal. Furthermore, the release rate (slope of curve) of I^{131} was identical in normal control and hypophysectomized, intra-ocular pituitary graft bearing rabbits. The I^{131} release was inhibited by T_4 in both groups (although less T_4 was required to produce an inhibition of I^{131} release in graft-bearing than control animals) and by enucleation of the graft-bearing eye. However, exposure to cold (5^{o} C) failed to increase the I^{131} release rate although the same temperature greatly increased this rate in the intact animal. (It was also reported that graft-bearing hamsters were unable to accel-

erate TSH secretion upon exposure to cold (Knigge and Bierman, 1958).

Furthermore, results obtained by Florsheim and Knigge (1961) cast doubt on the possibility that the hypothalamus influences the pituitary transplant via the peripheral circulation. They showed that lesions in the hypothalamus of graft-bearing animals did not alter thyroid function in these animals when compared with nonlesioned, pituitary-grafted ones.

Not only can the transplanted pituitary be inhibited in its secretion of TSH by T_4 (von Euler and Holmgren, 1956b) but it can also respond to decreased levels of thyroid hormone (produced by partial thyroidectomy) by increasing its secretion rate of TSH (Khazin and Reichlin, 1961).

These observations indicated that the pituitary when removed from its normal connections with the hypothalamus could secrete TSH (although at a reduced rate) and that the secretion rate could be increased in response to a lowered blood level of thyroid hormones, or decreased in response to an elevated thyroid hormone level but that it was not responsive to neurogenic stimuli which normally caused an elevated TSH secretion rate.

Recently Florsheim et al. (1963a) presented evidence that the hypothalamus can influence the pituitary when the latter is far removed from the former but where the hypophysial blood supply is unaltered. Pairs of rats joined in parabiotic union were used. One partner had a hypothalamic lesion in the area which had previously been shown to block the goitrogenic response to PTU and the other partner was hypophysectomized. The fact that the lesions did not interfere with the blood flow to the anterior pituitary was established. It was

found that the intact hypothalamus of the hypophysectomized partner could act across the parabiotic union and remove the goiter block induced by the lesion in the lesioned partner.

That the lesions were effective was subsequently shown (Florsheim et al., 1963b) by also lesioning the hypophysectomized partner, in which case the goiter block persisted.

The work of Florsheim et al. (1963a) has come under attack by Greer et al. (1966). Like Nikitovitch-Winer and Everett (1958), Greer et al. (1966) found that pituitary homotransplants (young donors) under the median eminence of hypophysectomized rats enabled the thyroid to hypertrophy in response to PTU. If the grafts were merely in the near vicinity of the median eminence (but not touching it) the goiter block persisted although normal testicular size and structure were maintained. They concluded that the median eminence was essential to allow a thyrotropic releasing factor of the hypothalamus to reach the adenohypophysis in sufficient quantity to cause an increased TSH secretion in response to PTU feeding and that if the thyrotropic releasing factor could not reach the pituitary in the near vicinity of, but not touching, the median eminence then it was very unlikely that it could reach the pituitary across a parabiotic union.

Another approach used to ascertain whether the hypothalamus was essential for normal pituitary function was implantation of the adenohypophysis directly into the hypothalamus. In abstract form only, Yasumura (1964) reported that 2 weeks after making such transplants in the rat the T/S for implanted rats was 5-10 times higher than for hypophysectomized ones and after 4 weeks on feed containing PTU the

implanted rats showed some tendency to form goiter. Unfortunately, he did not have controls with pituitaries transplanted elsewhere than the hypothalamus.

In a more definitive experiment Halasz et al. (1962) showed that anterior pituitary tissue grafted into the hypothalamus, other brain areas or under the renal capsule in the rat resulted in maintenance of the thyroid only when the graft was in the ventral hypothalamus. Grafts in other areas lead invariably to atrophy. Further, only those transplants which were contiguous with the median eminence were able to produce compensatory, thyroidal hypertrophy due to methylthiouracil treatment. On the other hand the T/S was observed to be maintained even when the transplant was located outside of the hypothalamus (Halasz, 1965a). Flament-Durand (1965) showed that pituitary tissue transplanted into the hypothalamus of hypophysectomized rats restored the thyroid weights to normal.

Although the preceding reports are confusing, the overall consensus appears to be that the median eminence connections between the hypothalamus and the pituitary in mammals are necessary for optimal secretion of TSH. However, the pituitary when separated from the hypothalamus is still able to maintain a minimum of thyroid function and to respond to changes in the circulating level of the thyroid hormones.

This general scheme derived from mammalian experimentation seems to be somewhat different in the chicken. Ma (1963) found that pituitaries which had been autotransplanted under the kidney capsule in White Leghorn cockerels maintained both thyroid weight and uptake of I^{131} by the thyroids at nearly normal levels (95% and 82% of controls,

respectively). These autotransplants were also capable of responding to exogenous T_4. A daily dose of 30 ug T_4/bird depressed thyroid function and morphology to hypophysectomized levels while smaller doses did not. Thus it appears that these autotransplanted pituitaries were secreting T_4 at a rate which was the same as the T_4 secretion rate reported for the intact New Hampshire hen by Stahl et al. (1961).

Thus it appears that either the adenohypophysis is more autonomous in regard to TSH secretion in chickens than it is in mammals or that the thyrotropin-releasing factor (TRF) can act more effectively via the systemic circulation. On the other hand these autotransplanted pituitaries were unable to secrete gonadatropic hormones in sufficient quantity to maintain testicular or comb size.

Brain Lesion Studies.

Certainly more fruitful and instructive than the controversial transplantation studies have been those studies concerning the hypothalamic control of the anterior pituitary utilizing the technique of electrolytic lesions in the hypothalamus. Nonetheless, lesioning studies have not been without their share of conflicting reports. As it turned out, much of the conflict revolved around PTU treatment and the sensitivity of the assay technique which was employed to determine the secretion rate of TSH.

Greer (1951) was one of the first to show definite effects of hypothalamic lesions on the thyroid gland. He found that lesions located between the optic stalk and the infundibulum blocked the goitrogenic response to PTU feeding but the T/S was not impaired.

Since that time numerous investigators have employed lesioning techniques in attempts to localize the hypothalamic site(s) which is (are) responsible for the control of TSH secretion, to determine if the hypothalamus is involved in the negative feedback regulation of TSH secretion, to determine if the hypothalamus is essential for normal thyroid function, and to determine if the hypothalamus is essential for the thyroidal responses to neurogenic stimuli.

In 1952 Greer localized further the thyrotropic area in the hypothalamus of the rat (anterior to the ventromedium nucleus near the ventral surface). He noticed that the lesioned animals lacked the goitrogenic response to PTU feeding but that they retained their magnitude of T/S. He decided that one of two explanations was possible: either 1) two TSH's were produced by the pituitary, one controlling growth and the other iodide metabolism, and that the latter was not under the control of the hypothalamic areas lesioned while the former was; or 2) the T/S was more sensitive to TSH than was the thyroidal weight. To test the latter hypothesis he gave graded doses of PTU to rats and found that as the dose of PTU increased the T/S and thyroid weight increased in parallel throughout the whole dose range. This indicated that the T/S was not more sensitive to TSH. Therefore, he assumed that the former hypothesis was correct (i.e., two TSH's).

Greer also showed that the effects of the lesions on the thyroids were due to their interference with TSH secretion and not due to a direct interference with the thyroid since the thyroids of lesioned rats were still capable of responding to exogenous TSH (Greer, 1955).

By using the drug, amphenone, which blocks both thyroidal and

adrenal hormonogenesis and concomitantly causes hypertrophy of both glands, Greer and Erwin (1956) were able to show that the thyrotropic center and the corticotropic center in the hypothalamus were separate. In this last report the thyrotropic area was further localized in the rat to be in either the paraventricular nucleus or in the midline between this nucleus and the infundibulum.

The findings of Greer (1951, 1952) were confirmed by Bogdanove and Halmi in 1953. Extended observations by these latter investigators showed that histologically the thyroid glands of the lesioned animals appeared normal while their pituitaries showed evidence of a lack of thyrotrophs. These investigators found that the thyrotropic and gonadotropic areas were separate with the former being in the general vicinity of Greer's thyrotropic center while the gonadotropic center was basally located between the anterior limit of the median eminence and the premammillary nucleus.

Although Bogdanove and Halmi (1953) confirmed Greer's experimental findings of lesion-blocked goitrogenesis without T/S impairment, they interpreted their results differently. In view of the observation of Halmi et al. (1953) that PTU strongly potentiated the effects of TSH on the iodide concentrating ability of the thyroid without altering the morphological response to the hormone, they believed that the lesions permitted enough TSH to be liberated so that this hormone could stimulate the PTU-potentiated gland to increase the T/S but not the non-potentiated thyroid growth.

The first workers to show a definite thyroidal effect due to hypothalamic lesions without treatment with goitrogens was Ganong et al.

(1955). They were able to demonstrate a substantial decrease in the I^{131} uptake and a histological picture indicative of a resting thyroid 3 weeks after lesioning the hypothalamus at the anterior end of the median eminence and anterior to it. When the lesions were caudal to this region or just under the anterior commissure there was no thyroidal inhibition although lesions in the caudal region of the median eminence produced gonadal atrophy. Thus, hypothalamic lesions caused not only inhibition of thyroidal growth and maintenance but also inhibition of iodide metabolism.

Since the previous work generally employed a very limited array of assays for thyroid function, it was perhaps felt by D'Angelo and Traum (1956) that a wider range of assays should be employed. They lesioned rats in the anterior hypothalamus, usually sparing the paraventricular nuclei and the supraoptic anterior hypothalamus, and 4-7 weeks thereafter started the rats on a diet containing PTU which was continued for 20 days. They also found the goiter inhibition due to lesions and in addition found that the lesions failed to inhibit the massive discharge of TSH seen after inhibition of the thyroid with a goitrogen. Further, in hypothalamic-lesioned rats which were not fed PTU they discovered that the half-life of thyroidal iodine was lengthened from 1.7 days for intact animals to 3.5 days for the lesioned ones. However, the finding that the half-life was much shorter in lesioned than in hypophysectomized rats indicated that a considerable amount of TSH was secreted even by animals on a regular diet.

Analysis of the TSH content of the anterior pituitary of the lesioned rat revealed that while the concentration was normal the total

store was less because the gland had suffered a 40% reduction in weight. Furthermore, when PTU was fed to these rats the concentration of TSH decreased also.

The preceding experiments pointed up some interesting facts which may be summarized as follows: 1) lesions in the PTU-fed rat blocked the goitrogenic response but not the discharge of TSH; 2) rats on a standard diet responded to lesioning by showing thyroidal histology indicative of a reduced level of circulating TSH; 3) pituitary concentration of TSH, although normal in lesioned rats on a regular diet, was reduced by a diet containing PTU; 4) the biological half-life of thyroidal I^{131} was doubled in lesioned animals but did not approach the level seen after hypophysectomy. These observations suggest that the lesioned rat secretes less TSH than the normal control but considerable more than the hypophysectomized control and that its secretion rate can be elevated by feeding PTU.

TSH secretion can also be inhibited in lesioned animals by exogenous T_4. Lesioned rats have a 20-hour I^{131} uptake of about 50% of normal and 10 ug T4/da for 15 days markedly inhibit the 20-hour uptake in both control and lesioned but more so in lesioned animals (Courrier et al., 1956).

Likewise, lesioned rats can respond to decreased levels of circulating thyroid hormone (produced by partial thyroidectomy) by increasing their T/S. The T/S in the lesioned rat, nonetheless, was significantly lower than in the control rat (Reichlin, 1957).

D'Angelo (1958) observed the changes in the pituitary TSH concentration following various manipulations and found that T_4 (or T_3)

markedly and equally suppressed the TSH concentration of the anterior pituitary of both intact and lesioned rats (T_3 was about 5 times more potent than T_4 in suppressing pituitary TSH concentration). On the other hand circulating levels of TSH were significantly reduced in the lesioned animals and the lesions didn't prevent the anterior pituitary from re-accumulating TSH after goitrogen withdrawal. Thus, the maintenance of abundant TSH reserve in the adenohypophysis, its depletion after thyroid hormone treatment, and rebound after goitrogen withdrawal prompted D'Angelo to conclude that the rat pituitary possesses a good deal of autonomous TSH function, that the thyroid hormone can act directly on the adenohypophysis independently of the hypothalamus, and that the pituitary thyroid servomechanism is systemically controlled by the circulating level of thyroid hormone and is not under neural domination.

The parallelism between the circulating levels of TSH (as judged by thyroid function) and the pituitary concentration of TSH shown by D'Angelo (1958) were also reported by Florsheim (1958) who further showed that lesions which block the goitrogenic response to PTU also depressed the 24-hour iodide uptake, thyroid hormone discharge rate, and the plasma PBI level. In fact, the only phase of iodine metabolism not affected by the lesions was the T/S. Florsheim found the most effective lesions in the hypothalamus to be those which involved the paraventricular nucleus. Furthermore, he showed that while some lesions did not block goiter formation they did inhibit other thyroid functions. In these "partially effective" lesions the pituitary stores of TSH, while less than normal, were higher than in the goiter-blocked lesioned rats. He concluded that the most sensitive assays

for effectiveness of hypothalamic lesions on TSH secretion in descending order of sensitivity were: 24-hour iodine uptake, I^{131} discharge rate, and plasma PBI.

In contrast to the findings of D'Angelo and Traum (1956) and Florsheim (1958), Knigge and Bierman (1958) found that although chronic hypothalamic lesions in the hamster inhibited I^{131} uptake it did not alter the thyroid secretion rate.

The foregoing lesioning experiments were all chronic ones. Usually the assay of thyroidal function was performed at least two weeks after lesioning. Averill et al. (1961) showed, using large lesions, that I^{131} release rate was affected as early as six hours after lesioning the rat. They found that the release rate could be further reduced by T_4 injections and that, like Courrier et al. (1956), the lesioned animals required less T_4 than normal controls to get complete inhibition. These workers concluded that the reason for the reduction in thyroid function following hypothalamic lesions was because the lesions made the pituitary more sensitive to circulating levels of thyroid hormone.

The next paper of rather definitive nature appeared in 1963 (van der Werff ten Bosch and Swanson, 1963) when it was shown, as expected, that lesions in the basal midline of the anterior hypothalamus just posterior to the optic chiasma reduced thyroid weight to 75% of control value at both 2 and 4 weeks. However, when the rats were fed PTU it was found that the thyroid weights increased 278% after 2 weeks and 355% after 4 weeks in both lesioned and nonlesioned rats. To these investigators this meant that although the pituitaries of lesioned rats were secreting less TSH than those of intact ones (as shown by smaller

thyroids) they were able to increase their thyroid weights (i.e. increase their TSH secretion rates) the same percent as the controls. The lesioned rats, however, were starting from a lower basal thyroidal weight and at autopsy the thyroids were still smaller, about the same size or only slightly larger than the thyroids from rats on the regular diet therefore, it appeared that they had not hypertrophied at all. In recalculating some of the earlier reports on goiter-blocked rats they discovered that several of these papers presented data which showed (at 2 weeks or longer) thyroid hypertrophy due to PTU feeding.

It was concluded by van der Werff ten Bosch and Swanson (1963) that while hypothalamic lesions produced a lowered steady state of the thyroid-pituitary feed back system that the system still responded normally to alterations in the thyroid hormone output caused by PTU feeding. This interpretation is consistent with the finding that partial thyroidectomy in hypophysectomized, pituitary transplant-bearing rats resulted in augmentation of the iodide metabolism and hypertrophy of the thyroid remnant.

However, it must be pointed out that Moll et al. (1961) definitely observed a goiter-block due to methylthiouracil treatment for 2 weeks. Furthermore they found rather drastic reductions in the I^{131} uptake (10% compared with 75% for normal controls), and the biological half-life was lengthened from 6.2 to 16 days. Thus, whether or not the anterior pituitary is autonomous in regard to the feedback control the fact seems apparent that the basal activity of the pituitary is very dependent on the hypothalamus.

This conclusion is also supported by a small number of papers which

indicate that the formation of $MI^{131}T$ and $DI^{131}T$ are strongly inhibited by lesions in the anterior hypothalamus involving the paraventricular nucleus and formation of radioactive T_4 is much more strongly inhibited (Kovacs and Vertes, 1963; Blanquet, 1962). These results are indicative of lowered TSH stimulation on the thyroid as shown by hypophysectomy (Taurog et al., 1958).

Although it now appears certain that mammals with hypothalamic lesions can respond to alterations in the thyroid hormone level of the blood their ability to respond to changes in environmental temperature is less convencing. In 1960 Reichlin showed that the thyrotropic and temperature centers of the rat hypothalamus were in close proximity and apparently overlapped to a certain extent. In the same year D'Angelo (1960) reported that reduction in the biological half-life of thyroidal radioiodide normally seen after cold exposure was abolished by lesioning the hypothalamus. At room temperature these rats showed the same serum TSH level, I^{131} release rate and thyroidal histology as sham operated controls (conflicting with D'Angelo and Traum, 1956, and Florsheim, 1958; but in agreement with Knigge and Bierman, 1958) but after cold exposure of sham-operated animals the biological half-life of I^{131} was reduced to 35 hours from 52 hours; the lesioned animals showed a reduction to 50 hours from 52. However, both serum and pituitary TSH levels decreased in lesioned animals. They concluded that this experiment showed the almost absolute dependency of the pituitary on the hypothalamus for augmented TSH secretion in response to cold while at room temperature these lesions permitted adequate trophic hormone secretion to maintain a normal secretion rate.

On the contrary van Beugen and van der Werff ten Bosch (1961) found that the biological half-life of thyroidal I^{131} was doubled by hypothalamic lesions (in the basal region behind the optic chiasma) at room temperature but exposure of the rats to cold for 30 days halved the biological half-life of I^{131} in both the controls and the lesioned animals. That hypothalamic lesions reduce the I^{131} uptake by about 50% was also confirmed by these workers. They concluded that the hypothalamus is required for maintenance of normal thyroid gland activity but not for thyroid response to a lowered environmental temperature.

The response to cold in the guinea pig was studied by Yamada et al. (1965) and was found to be very rapid; the PBI was significantly elevated within 2 hours after exposure. The PBI reached a peak by 4 hours and was maintained at this level for the duration of the experiment (48 hours). Either thyroid hormone or hypothalamic lesions prevented the increase in TSH release due to cold (Yamada et al., 1965). Since it was also found that thyroidectomized guinea pigs maintained on exogenous T_4 showed a significant decrease in plasma PBI within 2 hours after cold exposure it was suggested that cold accelerated the peripheral degradation of thyroid hormone. Thus the feedback inhibition on TSH production would be reduced, more TSH would be released, and the thyroid response should be enhanced. This may account for some of the conflicting evidence for the effects of lesions on the thyroidal cold response.

Andersen and his associates (1962) found that local cooling (called central cooling) of the preoptic region and the anterior hypothalamus in calm goats induced shivering only at ambient temperatures below 18° C or when local cooling was applied to the peripheral cold receptors.

Vasoconstriction was obtained after central cooling at all ambient temperatures tested.

These investigators also found that central cooling at an ambient temperature above that necessary to produce shivering resulted in a marked hyperthermia which lasted as long as the central cooling (as long as 7 days). They felt that this pointed to an increased metabolism of non-shivering origin and since it had been shown by Knigge and Bierman (1958) that cold exposure activated thyroid hormone release via the hypothalamic-pituitary axis, Andersson's group decided to investigate whether local cooling of the preoptic region could cause release of the thyroid hormone. The PBI increased rapidly after the onset of central cooling, reached a peak in six hours and remained at this level for as long as central cooling was continued (24 hours). After central cooling was stopped the PBI slowly returned to normal. Central cooling stimulated the thyroid to a greater extent than did cold exposure (4° C). In the latter instance the PBI rose only slightly and they were unable to detect any drop in brain temperature. However, when both low environmental temperature and ruminal cooling were combined the brain temperature dropped and the plasma PBI increased (Andersson et al., 1962b).

Conversely, local warming of the preoptic region resulted in a slight lowering of the PBI even with ruminal cooling which results in an elevated PBI in the absence of central warming. When the central warming was stopped in goats with ruminal cooling the brain temperature dropped quite low and the PBI rose (Andersson et al., 1962a).

In the foregoing experiments rather severe degrees of central

cooling were generally used. In an attempt to study the effects of graded central cooling they found that either very moderate (35° C) or deep (25° C) cooling of the preoptic region resulted in an increased plasma PBI while only moderate cooling of the anterior hypothalamus produced such an effect; deep cooling of the anterior hypothalamus produced no response at all (Andersson et al., 1965).

Lesioning the median eminence permanently blocked the thyroidal response to central cooling (Andersson et al., 1963).

Andersson and associates suggested that since a moderate lowering of the temperature of the preoptic-anterior hypothalamic region was sufficient to elicit a full thyroidal response that it was justifiable to conclude that the thyroidal response to central cooling reflected a physiologic cold defense mechanism. This mechanism was already operating at a moderate hypothermia since warming the region lowered the thyroidal response further. They did not show whether the response to central cooling was due to the removal of an inhibition of TSH release normally exerted by "warmth detectors" or to stimulation of central "hypothermia detectors" or both. That peripheral cold receptors were not required, however, was shown (Andersson et al., 1965).

Although the previous discussion leaves one with the conclusion that the hypothalamus or preoptic area, when cooled, enhanced TSH secretion even in the absence of peripheral cold detector activation one is still left with the question of whether the secretion of TSH can be altered by changes in environmental temperature independently of the hypothalamus. As already pointed out conflicting reports have been issued concerning the effects of hypothalamic lesions on thyroidal

response to cold. D'Angelo (1960), Knigge and Bierman (1958), Yamada et al. (1965), and de Jong and Moll (1965) claim that hypothalamic lesioning blocks the thyroidal response to cold exposure while van Beugen and van der Werff ten Bosch (1961) claim that, while hypothalamic lesions reduce the biological half-life of thyroidal iodine, exposure of lesioned animals to cold reduces their thyroidal iodine half-life just as much as in intact animals.

In two experiments, one acute and one chronic, de Jong and Moll (1965) showed that when rats were placed in the cold for a few days and then lesioned that the biological half-life of thyroidal radioiodine was 2.2 times longer than it was in cold-exposed, sham-operated rats. At room temperature the lesioned rats had thyroidal I^{131} half-lives 2 times those of the sham-operated controls. It was found that the thyroidal iodine half-life of the cold-exposed, sham-operated animal was 62% of its value at room temperature while the cold-exposed, lesioned animal had a thyroidal I^{131} half-life of 82% of its value at room temperature. (All lesions were in the paraventricular nuclei; half-lives of thyroidal radioiodine were measured, commencing five days after exposure to cold and two days after lesioning, for 7 days.)

In the chronic experiment the rats were kept at room temperature for 23 days after lesioning and were then transferred to the cold. They received I^{131} two days later and commencing 3 days after injection of I^{131} the biological half-life of thyroidal radioiodine was determined. In this experiment the sham-operated rats showed a half-life of thyroidal radioiodine which was 70% of that shown by the rats at room temperature while the lesioned animals showed no change. Further-

more, the same lesions inhibited the thyroidal radioiodide release at both room temperature and in the cold environment.

These authors interpret this to mean that the same neural mechanism is involved in the hypothalamic drive on TSH secretion at normal and cold temperature conditions.

It would seem, in view of the demonstrations that exposure to cold lowers the circulating levels of PBI (Yamada et al., 1965) and that the pituitary thyroid feedback mechanism operates independently of the hypothalamus, that exposure to cold would result in the reduction of the thyroidal radioiodide biological half-life in lesioned animals. This would explain the results of the acute experiments of de Jong and Moll (1965) as well as the chronic ones of van Beugen and van der Werff ten Bosch (1961) but not the chronic ones of de Jong and Moll (1965).

The observation that T_4 can prevent the increased release of thyroidal radioiodide normally seen after cold exposure (Yamada et al., 1965) lends support to this hypothesis. However, since it takes more T_4 to inhibit the release rate in cold temperature-exposed than in room temperature-exposed animals, this observation may merely mean that additional T_4 can further inhibit the increased stimulus for TSH secretion due to a neurally mediated cold stimulus.

In regard to the area of the hypothalamus which influences TSH secretion, de Jong and Moll (1965) made a very careful study of the area around the paraventricular nucleus and found that only those lesions which involved the midline at the level of the paraventricular nucleus caused an inhibition on the thyroidal radioiodide release rate at room temperature and blocked the thyroidal response to cold. When

the lesions were just inferior to the paraventricular nucleus they failed to inhibit thyroidal radioiodide release at room temperature although the response to cold was blocked. Lesions slightly anterior to the paraventricular nucleus slightly inhibited the cold response but did not affect the thyroid at room temperature. Lesions superior, lateral or posterior to the paraventricular nucleus had no effect on the thyroid activity. They reasoned that this indicated that the paraventricular nucleus is largely independent of influences from all other parts of the CNS. In this connection Halasz and Pupp (1965b) severed all nervous pathways leading to the "hypophysiotropic area" and found that these animals apparently had normal thyroid structure and function 3 to 5 weeks after sectioning (as shown by thyroid hypertrophy due to methylthiouracil feeding). This was an especially poor assay since it has been definitely shown that lesion-bearing rats can respond to goitrogens by producing goiters of equal size to those of controls after about 40 days of goitrogen treatment. Similarly treated lesion-bearing rats showed thyroidal hypertrophy after 28 days on goitrogen (deJong and Moll, 1965). Furthermore, such sectioning does not prevent the diffusion of neurohumors into the island. Therefore, it is doubtful that this last mentioned experiment is of much significance.

However, these workers may have laid the foundation of a good technique and if permamency and completeness of separation could be insured in such a preparation a valuable tool for use in determining whether or not peripheral cold inflow is needed to elicit the thyroidal cold response would be available.

From the few lesioning studies made in the bird where thyroid func-

tion was studied it was shown that lesion in the wall of the preoptic recess and the lateral walls of the third ventricle of the duck resulted in a slight but significant reduction in thyroid weight and epithelial height (Assenmacher, 1958). Lesions in the nucleus praeopticus paraventricularis magnocellularis ventralis (nomenclature after van Tienhoven and Juhasz, 1962) resulted, after two weeks, in rather drastic reduction in the thyroid weights in White Leghorn hens but in no obvious morphological alterations in the follicular epithelium (Egg and Chiasson, 1963). The reabsorption lacunae appeared to be absent in the lesioned birds.

The only report on the effects of hypothalamis lesioning in the avian class on iodine metabolism was from the Japanese quail. Lesions in the nucleus ventrolateralis (van Tienhoven and Juhasz, 1962) of this species decreases the disappearance rate of exogenous radioactive T_4 from the blood (McFarland et al., 1966).

The few reports of the effects of destroying parts of the brain outside of the hypothalamus on TSH production have largely been negative. Thus, removal of the entire brain rostral to the superior colliculi, leaving a hypothalamic island attached to the pituitary, in force-fed rats ten days post-operatively caused no effect on thyroidal weight or I^{131} uptake. Such "hypothalamic island" rats also showed goiter formation of equal magnitude with similarly fed controls (Matsuda et al., 1963).

Of course this experiment raises the question of whether both a facilitatory and inhibitory center might not have been lost which would leave the hypothalamic center unaltered. That this is possible

was shown when either transaction of the fronto-orbital connections or destruction of the thalamus in rats markedly inhibited I^{131} uptake and release from the thyroid (Kovacs et al., 1960).

The habernular nucleus has been the target for destruction in at least three separate experiments. In two of these experiments habenular destruction produced no noticable effects on thyroidal iodine metabolism (Yamada, 1961; Matsuda, 1963). In one experiment, however, Mess (1964) noticed that exposure to moderate cold (14° C) elicited an elevated T/S in intact rats; lesioning the habenular nucleus exaggerated this response. However, in extreme cold (0-4° C) the T/S was elevated in neither lesioned nor intact rats. The meaning of this observation is obscure but it does implicate the habenular nucleus in the cold defense mechanism.

Effects of amygdala destruction on thyroid function has been found to be ineffective (Yamada and Greer, 1960; Kovacs et al., 1965a).

Brain Stimulation Studies

The first evidence that hypothalamic stimulation could result in an increased thyrotropin secretion by the pituitary was presented in 1949 by Colfer who found an increased epithelial cell height in both rats and rabbits after stimulation for 4 one-hour periods over two days. The response was not seen in hypophysectomized animals. The stimulation also caused increased adrenal corticoid secretion as shown by a lymphopenia and an increased urinary excretion of 17-keto-steroids. No optimal hypothalamic site was found. Greer and Riggle (1957 [quoted from Blanquet, 1962, p. 247]) failed to obtain a thyroidal weight increase in rats stimulated in their hypothalami for

5 minutes every half hour for three weeks.

It must be pointed out that thyroid weight appears to be the least sensitive of all assays for TSH. Using a more sensitive assay for TSH secretion rate, Harris and Woods (1956) found that stimulation of the hypothalamus resulted in inhibition of thyroidal iodide release in 20 of 34 experiments and no change in the other 14 experiments. However, if they adrenalectomized the animals and maintained them on a constant dose of cortisone then stimulation of the hypothalamus resulted in a very marked increase in the release rate of thyroidal radioiodide. In some cases the plasma PBI level increased to as much as 35 times normal. This observation was interesting since it showed that TSH secretion could be enhanced by hypothalamic stimulation even in the presence of an elevated thyroid hormone level.

The exact position of the stimulating electrodes was not known but it was probably in the area of the median eminence; direct stimulation of the pituitary produced no change in the thyroidal response. When the electrodes were implanted in the anterior median eminence near the supraoptical hypophysial tract, enhanced thyroidal activity, after stimulation, was obtained even in animals with intact adrenals (Harris and Woods, 1958).

In an acute experiment stimulation of the hypothalamus near the supraoptic-hypophysial tract produced an accelerated iodine release from the thyroid of rabbits within 15 minutes after commencing the stimulation. The response reached a peak within 1-2 hours and was maintained at this level during several hours of stimulation. If the electrodes were placed elsewhere in the hypothalamus the stimulation

caused no change in the thyroidal secretion rate (Campbell et al., 1959). Injection of TSH produced the same results with the same latency period (Campbell et al., 1960).

In the dog, stimulation of either the anterior hypothalamus or the median eminence resulted in an elevated plasma PBI (in thyroidal venous blood) in intact but not in hypophysectomized, pituitary-autotransplanted ones. Stimulation in the lateral or posterior hypothalamus, preoptic area, hypophysis, thalamus, amygdala, mid-brain or reticular formation) produced no change in the PBI in intact animals (Shizume et al., 1962a).

D'Angelo and Snyder (1963) found that the plasma level of TSH (stasis tadpole assay) was elevated (approximately 3.5 times normal) by stimulation of the anterior hypothalamus, suprachiasmatic nucleus, preoptic nucleus, arcuate nucleus or the median eminence of the rat for 20 minutes a day for 4 days. There was no detectable change in the plasma TSH level 1-2 hours after a single 20-minute stimulation period. The enhanced TSH-secretion response was blocked by previously placed lesions in the same area of the hypothalamus. Thyroid weights were not significantly altered in any of the experiments. The anterior pituitary stores of TSH were markedly lowered in the chronic (4 day) experiments and it gave evidence of basophilic activation (D'Angelo et al., 1964).

Vertes et al. (1965) found the effective site for enhancement of thyrotropin secretion following electrical stimulation to be located dorsally in the anterior hypothalamus (usually just in front of the paraventricular nucleus at the level of the first one-third or at the

lateral sides of this nucleus in the intact rat). When the frontal area of the dorsomedial nucleus was stimulated a decrease in thyroidal I^{131} uptake was noted; however, stimulation of this area after adrenalectomy resulted in an increased thyroidal activity.

The complications in the stimulation studies induced by the adrenals has been somewhat clarified. Woodbury et al. (1951) found that the increased PBI normally seen after TSH treatment was inhibited when both adrenocorticotropic hormone (ACTH) and TSH were injected simultaneously into the hypophysectomized rat. They reported that the effects seen after TSH plus cortisone treatment were equivocal. (Their data showed that the increase due to TSH was halved by simultaneous cortisone treatment.) Thus, it appeared that either ACTH or cortisone acted directly on the thyroid to inhibit its production of the thyroid hormones. The following year it was reported that TSH plus cortisone treatment definitely inhibited the uptake of radioiodide by the thyroid in hypophysectomized rats (Verzar and Vidovic, 1952) and it was concluded that the corticoids inhibited the thyroid directly.

On the other hand, using the thyroidal radioiodine release rate in rabbits, Brown-Grant et al. (1954a) showed that both emotional stress and physical trauma induced a prompt inhibition of iodine release for 1-2 days and neither denervation of the thyroid gland nor adrenalectomy prevented the inhibition of release due to the stress. These observations suggested that the inhibition was mediated from the central nervous system through alteration of the secretion of TSH from the anterior pituitary and not indirectly via the adrenals nor directly via nerves

to the thyroid.

When either ACTH or cortisone was given to intact rabbits the radioiodine release rate was inhibited. However, although cortisone given to adrenalectomized rabbits inhibited the thyroidal release rate it was ineffective in hypophysectomized animals in altering the response of the thyroid to exogenous TSH (Brown-Grant et al., 1954b). Further, ACTH decreased the release rate in normal but not in adrenalectomized rabbits and corticosterone inhibited the rate in both rats and rabbits (Brown-Grant, 1956).

Thus it appears that the suppression effect of adrenal corticoids and ACTH is on the production of TSH by the pituitary and not directly on the thyroid. In those stimulation experiments where the adrenals were interfering with TSH secretion apparently the ACTH "center" of the hypothalamus was also being stimulated, this caused ACTH to be secreted by the adenohypophysis, the ACTH stimulated the adrenal cortex to produce steroids which acted back on the pituitary and inhibited the production of TSH therefrom (Harris and Woods, 1958). Conversely, when the adrenals were removed the ACTH effect was abolished and only the TSH response was seen.

The effects of electrical stimulation of brain areas other than the hypothalamus and infundibulum are very scanty. There is some evidence that stimulation of the hippocampal formation in the dog activates the pituitary-thyroid system as measured by an increased PBI in thyroid venous blood and an increased TSH content of the jugular venous blood. The response was prevented by hypophysectomy (Shizume et al., 1962b). Inhibition of thyroidal I^{131} uptake has also been

reported to follow stimulation of the mesencephalic reticular formation. On the other hand, lesioning this area enhances I^{131} uptake by the thyroid.

About the only conclusion that can be drawn from these experiments is that apparently brain areas other than the hypothalamus can influence the TSH response, presumably through mediation of the hypothalamus.

Hypothalamic Extract Studies

As has been shown, the production of TSH by the anterior pituitary is at least partially under the control of the anterior hypothalamus which, anatomically, is in a position to receive stimuli from a vast network of afferents from the rhinencephalon, midbrain, and cerebral cortex. These connections probably influence the hypothalamic area responsible for TSH secretory control. In addition, the hypothalamic nuclei appear to be able, at least in the case of temperature, to be influenced directly.

In any event it is necessary for the information to be relayed from the hypothalamus to the adenohypophysis and since this last mentioned structure is for all practical purposes devoid of nerve fibers then one must look elsewhere for some transmission mechanism. The mechanism appears to be some neurohumor which is conveyed to the pars distalis, probably via the portal vessels. However, Assenmacher (1958) indicated that some pathway other than the portal vessels may be involved since sectioning the median eminence altered thyroid morphology in the duck while sectioning the portal veins without involving the pars tubero-median eminence connection failed to alter thyroid mor-

phology.

The name given to the neurohumoral transmitter substance responsible for TSH secretion is thyrotropin-releasing factor (TRF). One of the first to attempt to show such a substance was Florsheim and his associates who, in 1957, were unable to reinitiate TSH production by mouse pituitaries in tissue cultures when incubated with hypothalamic tissue despite the fact that both tissues grew well in the media.

Shibusawa et al. (1959a,b,c) reported that extracts from the hypothalamus, blood, cerebrospinal fluid, neurohypophysis, and urine of dogs stimulated the release of TSH from the pituitaries of intact rats and rats with hypothalamic lesions but not in hypophysectomized ones (as assayed by thyroid function). This extract was ineffective in the laboratory of Reichlin et al. (1963) and has been criticized by others (Guillemin, 1964).

In 1962 Guillemin et al. obtained a substance from the hypothalamus which stimulated a linear secretion rate of TSH in response to a log dose of the extract within certain limits. Its action was inhibited by the administration of large doses of T_4 and it was inactive in hypophysectomized recipients.

The nature of the substance was not known and some investigators suspected that it was a posterior pituitary hormone or a synaptic mediator such as epinephrine. Although many and varied reports appeared concerning the effectiveness of these agents most of the reports could be criticized on the basis of the amount used and the route by which they were administered (see Guillemin, 1964).

The slow intravenous infusion for two hours into rabbits of an

acetic acid extract of bovine median eminence, TSH, vasopressin, epinephrine, norepinephrine, bovine anterior hypothalamic extract, oxytocin, insuline, glucagon, ACTH or 3'5'-adenison monophosphate was carried out. Only the first three substances were effective in elevating the release of thyroidal radioiodine. The response to vasopressin was indistinguishable from that following TSH with regard to latency, time of the peak of the reaction and the duration of the response. However, it was inactive in the hypophysectomized animal and hence couldn't have had its effect through the hypophysis (Schindler and Harris, 1963; Garcia et al., 1964).

Schreiber and his associates (Schreiber and Kmentova, 1963; Schreiber et al., 1963) found a non-protein extract, obtained from bovine hypothalamic tissue by electrophoresis and gel filtration, which when injected into the anterior eye chamber of a hypophysectomized rat containing an intraocular autotransplant of the adenophypophysis increased the release rate of I^{131} from the thyroid gland. The same extract also stimulated the release of TSH from the pars distalis of the rat in vitro and increased thyroidal activity in the guinea pig in vivo. The substance was not TSH. On the other hand, Reichlin and Boshans (1963) obtained no stimulatory substance in extracts from any part of the hypothalamus except the ventral part and from the median eminence. They concluded that their active principal was TSH since it was inactivated by anti-TSH and was effective in hypophysectomized animals.

Others, however, have confirmed the presence of a non-protein, non-posterior pituitary peptide, peptide-like substance from the hypothalamus (of ovine, bovine, porcine and human origins [Bowers et al., 1965]) which increased thyroid activity in vivo (Ducommun et al.,

1965; Bowers et al., 1965) and in vitro (Vertes and Kovacs, 1965; Bowers et al., 1965; Guillemin et al., 1963).

These observations coupled with the fact that a TRF substance of hypothalamic origin has been highly purified, being active in vivo in doses of less than 0.1 µg and having no LH or ACTH releasing action (Guillemin et al., 1965a,b), argues in favor of the presence of a neurohumor of hypothalamic origin which is capable of selectively enhancing TSH secretion (and/or synthesis). Chemically and biologically the highly purified TRF is different from oxytocin, vasopressin, or melanocyte stimulating hormone and leuteotrophin releasing factor.

CHAPTER III

MATERIALS AND METHODS

Experimental Animals

Adult White Leghorn cocks between 7 and 24 months of age were used. The birds were raised on regular chicken mash in 30 x 15 ft. floor pens (about 50 cocks/pen) at the Oklahoma State University Poultry Farm. The cocks were brought into the laboratory animal room on the day before they were to be lesioned and were kept in this room, 3-4 animals per cage, until sacrifice. The air-conditioned room had a northern exposure and lighting was of natural duration. Water and feed were available ad libitum.

On the day the birds were to be lesioned they were weighed, numbered and moved to the laboratory. They were anesthetized by intravenous injection of sodium pentobarbital (65 mg/Kg of body weight) and their heads were fixed in a small animal headholder in a stereotaxic instrument*. The headholder was placed in the instrument backwards to minimize interference of the comb with the electrode.

The feathers were clipped from the scalp, the scalp was sponged with 70% alcohol, and a midline incision was made through the skin from the base of the comb posteriorly for about 2.5 cm. After the skin

*Stereotaxic Instrument, Model U, with a small animal headholder, Baltimore Instrument Company, Inc., Baltimore, Md.

was retracted laterally a small spirit level was placed on the frontal and parietal bones and the beak holder was adjusted so that the frontal and parietal bones were level. The periosteum was then scraped free from the skull in the area where the electrodes were to be introduced. The electrode was moved into the predetermined, lateral and antero-posterior position, lowered to the skull surface and the point of contact was marked on the skull. This was done for the left and the right sides. The electrode was then moved aside and holes, about 1.5 mm in diameter, were drilled through the cranium at the marked sites with a dental drill* and bur**. Except in the few instances when the holes were made in the midline, bleeding was not a serious problem and was either minimal or could be easily controlled by packing the hole with cotton for a few minutes.

The electrode was again moved into position over the holes in the skull and then lowered to a predetermined depth. The electrode was constructed of number 24 nichrome wire which had been coated twice with epoxylite insulator***. The terminal end was scraped bare for about 0.5 mm. One lead from a radiofrequency lesion maker**** was attached to the electrode holder and the other to the headholder; the two holders were insulated from each other. An ammeter was connected to the monitor circuit of the lesion maker and the rheostat of the

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*Dental Drill, Series CC, Foredom Electric Company, Inc., Bethel, Conn.

**Dental Bur, Round, Number 6, S. S. White Dental Manufacturing Co., Philadelphia, Pa.

***Epoxylite Insulator, Number 6001, Epoxylite Corporation, South El Monte, Calif.

****Radiofrequency Lesion Maker, Model LM-3, Grass Instrument Co., Quincy, Mass.

latter was adjusted, during the first two or three seconds of current, to give a reading on the monitor circuit of about 2 milliampers. The current was applied for 20 seconds. The electrode was then raised, moved to the other side of the cranium, lowered to the same depth, and again a current of the same magnitude and duration was applied. Although a cleaned electrode was used for each bird, the same electrode was used on both sides of the same bird without cleaning between uses.

The electrode was removed, the skull mopped with clean cotton and the skin incision sutured together with cotton thread. The cocks were then returned to the animal room where they remained until sacrifice.

Sham-operated controls were treated in the same manner as the operated birds except that no current was applied to the inserted electrodes. In most experiments unoperated controls were also used. Their housing, water and feed were the same as for the lesioned and sham-lesioned birds.

Usually nine days after lesioning, all birds of an experiment were injected intraperitoneally with 5 microcuries of carrier-free NaI^{131}* in 0.5 ml of water. Twenty-four hours later a heparinized blood sample was drawn after which the cocks were killed by decapitation. The thyroids, testes and pituitaries were removed, cleaned of adherent connective tissue and weighed. The dead body weights were also determined. The anterior pituitary of each lesioned bird was removed by removing the bone in the roof of the mouth and extracting the pituitary with forceps. This sometimes resulted in fragmented anterior pituitaries

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*NaI^{131} was obtained from ISO/SERVE Division of Cambridge Nuclear Corporation, Cambridge, Mass. and was used without further purification.

which, although they were used for TSH assays were not used in pituitary weight studies.

Immediately after removal the thyroids were placed into test tubes in an ice bath; the pituitaries were wrapped in parafilm and frozen for assay for TSH (1 week to 6 months later). The length and height of each comb were measured. The heads were skinned and placed into 10% formalin for fixation of the brain. The blood was centrifuged and a plasma sample was taken for radioiodine determination.

The stereotaxic coordinates used for positioning the electrodes were determined from preliminary experiments. The heads of adult White Leghorn cocks were fixed in the headholder of the stereotaxic instrument, they were leveled, and a series of midline lesions were made. The coordinates for each lesion were recorded. The chickens were then killed and the heads were fixed in formalin. The brains were then removed, sectioned, and stained. The sites of the lesions and the stereotaxic coordinates used to produce the lesions were compared and adjustments were made in the coordinates to correspond with other hypothalamic nuclei which were to be lesioned.

The adjusted coordinates were then used to position the electrodes in the hypothalami of a second series of cocks in an attempt to destroy predetermined nuclei selectively.

The precision of lesion placement in this study was not particularly high, probably due to the difficulties encountered in leveling the heads.

Organ Size

The weights of the thyroid and anterior pituitary were expressed as mg per 100 g of body weight (mg%). The testis weight was expressed as g per 100 g of body weight (g%). The product of the length times the height (in cm) of the comb divided by 2 was called the comb factor.

Twenty-four-hour Radioiodine Uptake

The radioactivities of the thyroids and an aliquot of the injected dose of I^{131} were determined using a well-type scintillation detector with a scaler*; the 24-hour radioiodine uptake was calculated as the counts per minute for both thyroids/the counts per minute of the injection dose, multiplied by 100.

Analysis of Intrathyroidal Iodine Metabolism

The freshly removed thyroids, after having been counted, were homogenized in an all-glass tissue grinder which contained 2 ml of cold buffer (0.04 M $NaHCO_3$ - 0.11 M NaCl, pH 8.2) containing 25 mg thiouracil/100 ml of buffer. Homogenization was carried out in an ice bath. An aliquot of the homogenate was removed and frozen for subsequent chromatographic analysis.

To the remaining homogenate were added: one ml of the above buffer (without the thiouracil) containing 30 mg Viocase** and 12 mg

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*Baird Atomic Scintillation Detector, Model 810, with Multi-scaler II, Model 132.

**Viocase, Viobin Corporation, Monticello, Ill.

Pronase*, 0.03 ml of 0.1 M $MnCl_2$, and two or three drops of toluene. The tubes were stoppered and incubated, without shaking, at 37° C for 16-18 hours.

After digestion of the thyroid homogenates, aliquots (about 0.3 ml) of the hydrolyzed as well as unhydrolyzed homogenates were applied to strips of Whatman number 3 filter paper (1.5 x 20 inches). The filter paper strips were developed by ascending chromatography in glass tanks in a solvent system composed 5 parts of n-butanol, 1 part of ethanol (95%), and 2 parts of NH_4OH, (0.5-0.25 N). Strips containing either T_4 + DIT + I^{127} or T_3 + MIT + I^{127} were also developed simultaneously (in the same tank) with the filter paper strips containing the thyroid homogenates.

The radioactive areas of the chromatograms containing the thyroid homogenates were located using a chromotagram scanner** and identified with the aid of the carrier chromatograms (which had been stained with ninhydrin reagent, 0.1%, and palladium chloride solution, 0,2%).

The radioactive areas of the chromatograms were then cut out and counted in the well-type scintillation detector. Each component was expressed as the percent of the total radioactivity of the chromatogram found as a single component.

Bioassay of TSH

The TSH content of the anterior pituitaries was determined using the bioassay method of Bates and Cornfield (1957) with but few modi-

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*Pronase, B grade, Calbiochem, Los Angeles, Calif.
**Scanogram II, Chromatogram Scanner, Model RSC-160, with Ratemeter, Model 432A, Baird Atomic, Inc., Cambridge, Mass.

fications.

The pituitaries were homogenized in a known volume of water which was kept at a minimum; usually 1.5 ml of water was used for each pituitary. In order to have the highest possible amount of pituitary per assay chick, only a single dilution of the pituitary homogenate was tested. However, in a preliminary experiment pituitaries of roosters (Oklabars) were pooled and various doses of the pituitary homogenate between 0.57 and 2.3 mg of anterior pituitary per assay chick gave, when injected into the assay animal, a linear response which was within the area of linearity of the standard curve.

Day-old White Leghorn cockerels of mixed strains, obtained from a commercial hatchery*, were used as the assay birds. Upon arrival at the laboratory the cockerels were injected intraperitoneally with approximately 3 microcuries of carrier-free I^{131} in 0.2 ml of water and placed in a brooder under continuous light and with water ad libitum. Twenty-four hours later the chickens were divided into groups of five and were individually marked. Their thyroids were counted by holding the deep neck region over a scintillation detector. The conventional lead shield of the detector was replaced with a 2-inch thick lead shield which had a conical shaped center hole.

Two separate counts were made of each chick at each time and if they differed more than 10% from each other additional counts were made until the counts were more consistent. The counts for a single chick were remarkably constant and the necessity for additional counts was infrequent.

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*Stillwater Hatchery, Inc., Stillwater, Oklahoma.

Immediately after counting the thyroids of each chick, the chick was injected subcutaneously on the inner aspect of one thigh with 0.2 ml of a 0.035 M NaOH solution containing 8 mg of T_4 and 0.5 mg PTU and on the other side with 0.2 ml of a standard TSH* solution containing 0, 1, 2, 4, 8, 16 or 32 mU/TSH or 0.2 ml of the pituitary homogenate. Thus, five chicks constituted one point on the standard curve for TSH and five chicks constituted one point for the test homogenate.

The chicks were returned to the brooder, allowed feed and water ad libitum, and 24 hours later were again counted. The second count was corrected for physical decay. The percent of radioactivity remaining 24 hours after the injection of the TSH standard or the pituitary homogenate was calculated by dividing the second count by the first count and multiplying the quotient by 100. This value was referred to as the 24-hour relative percent remaining (RPR).

The average RPR's for the chicks receiving the TSH standards were plotted on the arithmetic axis (ordinate) against the injected doses on the logarithmic axis (abscissa) of semilogarithmic paper. The standard curve was linear between doses of 2 and 8 mU, usually linear between 2 and 16 mU, but rarely so between 0 and 2 mU. The distressing result was that the RPR of those chicks receiving 2 mU of TSH was often greater than that of those receiving 0 to 1 mU.

The average RPR for the chicks receiving the test dose of anterior pituitary homogenate was compared with the standard curve and the quantity of TSH received by each chick was read from the abscissa. By

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*Thyrotropin, U.S.P. Reference Standard, Board of Trustees of The United States Pharmacopeial Convention, Inc.

dividing this value by the number of mg of anterior pituitary injected the mU TSH/mg anterior pituitary was obtained and when this was multiplied by the anterior pituitary weight the total quantity of TSH in the anterior pituitary was calculated.

Because of exceptionally low radioiodine uptakes by the chicks during the warmer months the chicks could be used for assay purposes only during the cooler months (November through March). Consequently, the pituitaries of those cocks killed during the summer months were frozen and assayed during the cooler months.

Even during the cool months radioiodine uptake was somewhat erratic. Furthermore, the RPR's within a group of chicks varied considerably. After preliminary trials it was decided to select the assay chicks by counting about 25 of them individually 20 hours after I^{131} injection, determining the mean and the standard deviation from the mean of the counts, and selecting only those chicks which had iodine uptakes within one standard deviation of the mean. This procedure failed to improve appreciably the assay and was abandoned. However, in all assays a few chicks were rejected for use as assay animals because their 24-hour uptake of radioiodine was extremely low.

Brain Sectioning and Staining

The heads of the lesioned cocks were fixed in 10% formalin, usually for 4-5 days. After fixation the brains were removed by chipping away the surrounding bone and removing the dura mater. The brain was sectioned transversly at the middle of the cerebellum with a razor blade. The central cut end was dipped into water and then placed, cut

edge down, on the platform of a dry ice-acetone containing chamber which was attached to a sliding knife, frozen-section microtone*. Powdered dry ice was placed around the brain for about five minutes to freeze the tissue. The brain was then sectioned at 70 microns; every fourth section was saved. Sections to be saved were lifted from the microtome blade on a small wet paint brush and placed in distilled water from which they were floated onto a glass slide (which had previously been coated with a 2% gelatin solution and dried).

Several sections were placed on each glass slide and allowed to dry for several hours at room temperature. After the sections were dry they were treated as described below for staining and mounting. The slides were placed in 50-70% ethanol for about 5 minutes, a procedure which appeared to aid in affixing the sections to the glass slide. The sections were then defatted by placing them in dioxane for 3 minutes and then washing them by repetitive dippings in distilled water. After the sections were defatted they were stained by placing them in a solution of thionin** (0.5 g of stain in 100 ml of water to which 2 drops of acetic acid were added) for 2 to 3 minutes. Differentiation was accomplished by dipping the slides into a mixture of dioxane-absolute ethanol (50:50, V/V) after which they were dehydrated by dipping the slides several times in one change of dioxane and 4 changes of xylene. The slides were mounted from the last xylene treatment using Permount*** as the mounting medium.

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*Clinical Microtome, Model 70-a, Lipshaw Manufacturing Company, Detroit, Mich.

**Thionin, Fisher Certified Reagent, Fisher Scientific Company, Fair Lawn, N. J.

***Permount, Fisher Scientific Company, Fair Lawn, N. J.

The stained and mounted sections were studied under low magnification to confirm and locate the lesions. Operated birds which showed no brain damage were not used.

CHAPTER IV

RESULTS AND DISCUSSION

Preliminary Considerations

The lesioning studies were carried out over a period of seven months (February through August). Small groups of birds were treated at any one time; usually 6 to 10 cocks were lesioned, 3 were sham-lesioned, and 3 were treated the same as the lesioned and sham-lesioned birds in every respect except that they did not undergo surgery. This last group served as the normal controls (N.C.). Seventy-five cocks were lesioned, 26 were sham-lesioned and 21 served as normal controls.

Of the 75 birds which were lesioned, 43 survived the 10-14 day interval between lesioning and sacrifice. Of these 43 surviving birds 10 showed no brain damage when their brains were subjected to histological examination. Of the remaining 33 birds which showed brain damage, 15 were found to have lesions in the anterior hypothalamus and 18 in the posterior hypothalamus. The anterior hypothalamus was taken, somewhat arbitrarily, to be that part of the hypothalamus anterior to a transverse plane running through the pallial commissure and the posterior one-third of the decussatio supraoptica while the posterior hypothalamus was taken to be that part caudal to this plane.

The intended target for all lesions was the anterior hypothalamus,

especially either the nucleus paraventricularis magnocellularis (PVM) or the nucleus praeopticus paraventricularis magnocellularis (PPM). However, interference of the comb often made leveling of the frontal and parietal bones difficult or impossible. This probably accounts in large part for the lack of consistency in lesioning the desired hypothalamic nuclei. Over one-half of the surviving lesioned birds showed brain damage in the posterior hypothalamus, possibly because the mortality rate was higher for the birds receiving lesions in the anterior hypothalamus. Although only 10 brains from the birds which died before sacrifice were sectioned, 8 of the 10 showed lesions in the anterior hypothalamus.

Since the birds were processed in small, experimental groups over a rather long period of time and since in some experiments there were no anteriorly lesioned birds and in others no posteriorly lesioned ones, it was deemed necessary to make more than simple comparisons between all anteriorly lesioned or all posteriorly lesioned birds and all sham-operated or all normal control ones. Therefore, in this study the following comparisons were made: 1) all anteriorly or all posteriorly lesioned birds were compared with all sham-operated or all normal control birds (designated as Group All); 2) anteriorly (designated as Group A) or posteriorly (designated as Group B) lesioned birds were compared with only those controls (either sham-operated or normal controls) from the same experiment from which the lesioned birds came; and 3) birds which showed rather discrete lesions localized in the nucleus PVM (designed as Group C) or the nucleus PPM (designated as Group D) were compared with the controls of the

experiments from which these lesioned birds came. Table I shows the numbers of the lesioned birds included in each group and the control birds with which they were compared.

Effects of Hypothalamic Lesions on Body Weight

From Table II and Figure 1 it may be seen that the body weights of all the birds were less at the time of sacrifice than at the time of lesioning. This was due in part to the fact that the birds were bled before and weighed after they were killed. Assuming that the lost blood represented about 5% of the body weight, it appeared that the controls (sham-operated and normal) weighed about the same, the anteriorly lesioned birds weighed more and the posteriorly lesioned birds weighed less at time of killing than at time of lesioning. However, only the posteriorly lesioned birds showed a significant change in body weight from time of lesioning to sacrifice. Not only was the weight loss of the posteriorly lesioned birds significantly greater but their body weights were significantly smaller than the control groups (both at times of lesioning and sacrifice).

Thus it appeared that lesioning the posterior hypothalamus of chickens caused a weight loss greater than that caused by lesioning the anterior hypothalamus or by sham-lesioning. The cause of the weight loss is not known. Records were not kept on the water or feed consumption of individual birds or on their urinary or fecal excretion. However, a casual observation was made that the lesioned birds as a whole produced a fluid feces while the control cocks did not. Further, many of the lesioned birds appeared to be dehydrated and emaciated.

TABLE I

GROUPING OF COCKS TO SHOW COMPARISONS BETWEEN EXPERIMENTAL AND CONTROL BIRDS

Group Desig-nation	Area Lesioned	Identification Number of Birds Used in This Study		
		Lesioned	Sham-Operated	Normal Control
A-1	Anterior hypothalamus	0-1,2,6,8,9, 12,35,36,37, 39,40,64,67, 70,76	S-1,2,3,4,5, 6,7,23,24, 25,31,32,33 34,35,40,41 42	---
A-2	Anterior hypothalamus	0-1,2,6,8,9, 12,35,36,37, 39,40	---	NC-1,2,3,4,5, 6,7,8,23,24, 25
B-1	Posterior hypothalamus	0-14,21,24,25, 30,34,38,41, 43,44,47,50, 52,53,57,59, 69,73	S-8,10,20,21, 22,23,24,25, 26,27,28,29, 30,31,32,40, 41	---
B-2	Posterior hypothalamus	0-14,21,24, 25,30,34,38, 41,43,44,47, 50,52,53,57, 59	---	NC-9,10,11,20, 21,22,23,24, 25,26,27,28, 29,30
C	PVM nucleus	0-35,37,39, 40	S-23,24,25	NC-23,24,25
D	PPM nucleus	0-2,36,39,70	S-1,2,23,24, 25,40,41,42	NC-1,2,3,23, 24,25

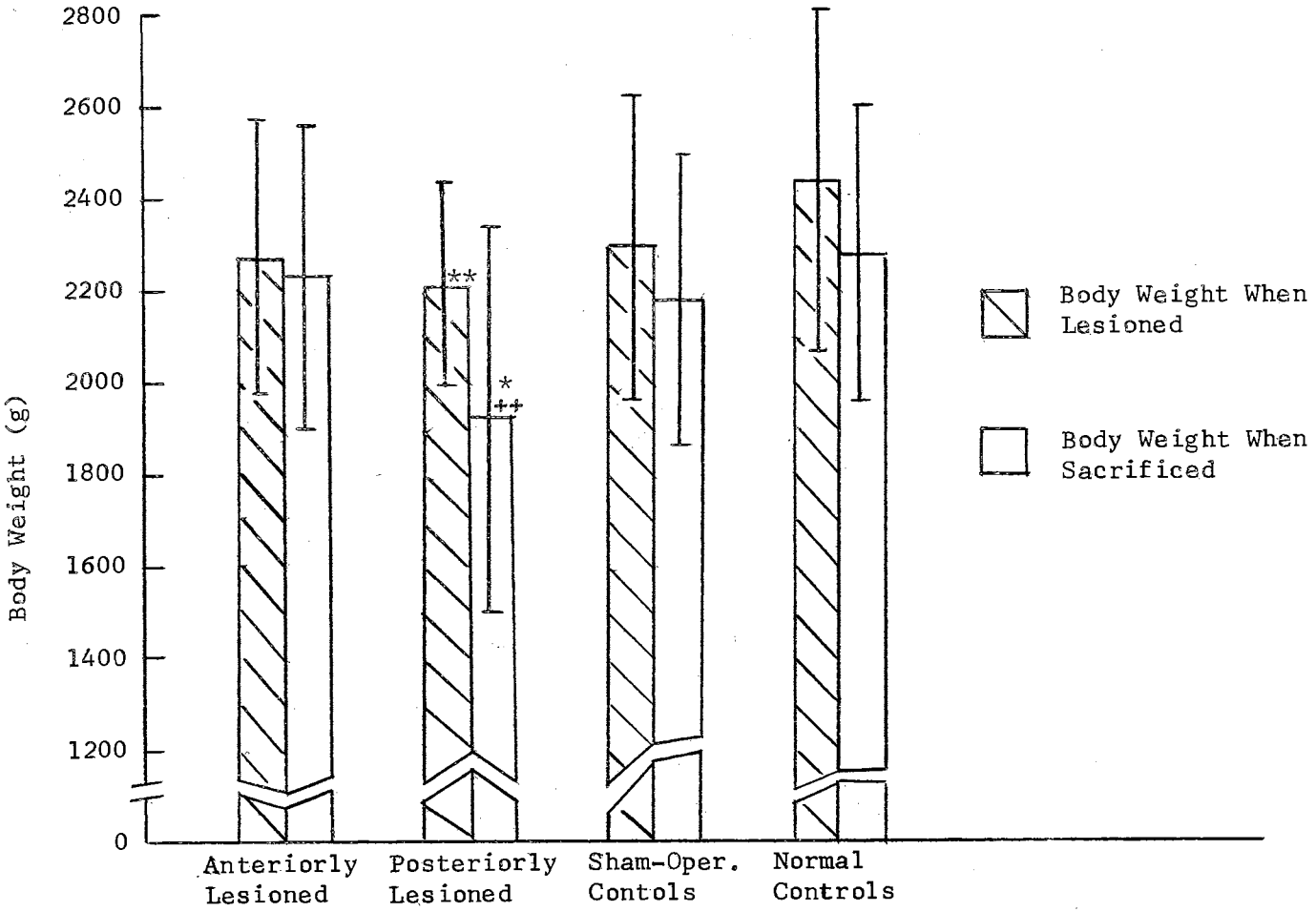

Figure 1. Body Weight of Hypothalamic-Lesioned and Control Cocks at Time When Operated Birds Were Lesioned and at Time of Sacrifice. $^{*}P<0.05$, $^{+}P<0.10$ Lesioned birds compared with sham-operated controls. $^{**}P<0.05$, $^{++}P<0.10$ Lesioned birds compared with normal controls.

TABLE II

BODY WEIGHTS OF ALL HYPOTHALAMIC-LESIONED AND ALL CONTROL COCKS AT TIME OF LESIONING AND 10-14 DAYS AFTER LESIONING

Treatment	Body Weight When Lesioned (g)	Body Weight When Killed (g)	% Change in Mean Body Wt.	$P <$ [b]
Ant. lesion	2277 ± 296[a]	2234 ± 328	- 1.9	N.S.
Post. lesion	2204 ± 224**	1925 ± 418***	- 12.6	0.025
Sham-operated	2298 ± 335	2180 ± 319	- 5.1	0.20
N.C.	2442 ± 374	2274 ± 322	- 6.9	N.S.

*$P < 0.05$, lesioned birds compared with sham-operated controls.
**$P < 0.05$, lesioned birds compared with normal controls.
[a]Number represents mean ± standard deviation from the mean.
[b]Comparison of body weights between time of lesioning and time of killing.

Effects of Hypothalamic Lesions on Thyroid Weight

The effects of hypothalamic lesions on thyroidal weight in mg are shown in Table III and Figure 2 and in mg% in Table III and Figure 3. It may be seen that the only group to show any significant difference between lesioned and control thyroidal weights in mg were those birds lesioned in the PVM nucleus (Group C). This group had thyroids which were significantly larger than those of the normal controls and non-significantly larger than those of the sham-operated controls.

When the thyroid weights were expressed on the basis of mg% the significance of the thyroidal weight in the birds lesioned in the PVM nucleus disappeared although they were still larger than those for either control group. Similar observations were also made for those cocks bearing lesions in the PPM nucleus although the differences were

TABLE III

THYROID WEIGHT, 24-HOUR IODINE^{131} UPTAKE, PER CENT OF MIT + DIT, PER CENT OF $T_4 + T_3$, AND $T_4 + T_3$/MIT + DIT RATIOS OF HYPOTHALAMIC-LESIONED AND CONTROL COCKS 24 HOURS AFTER INJECTION OF I^{131} AND 10-14 DAYS AFTER LESIONING

Group No.	Treatment	Thy. wt. (mg)	Thy. wt. (mg%)	24-Hr. I^{131} Uptake	MIT + DIT	$T_4 + T_3$	$\frac{T_4 + T_3}{MIT + DIT}$
All	Ant. Lesion	162 ± 40[a]	7.5 ± 2.1*	8.4 ± 5.5	86.1 ± 4.2	6.5 ± 3.3	7.7 ± 4.3
	Post. Lesion	140 ± 40	7.4 ± 2.0*	10.5 ± 4.7	85.5 ± 3.8	8.7 ± 3.7**	10.4 ± 5.0**
	Sham-Operated	141 ± 45	6.3 ± 1.4	10.2 ± 4.6	86.2 ± 3.8	7.5 ± 3.7	8.9 ± 4.7
	N.C.	160 ± 34	7.0 ± 1.4	8.5 ± 5.0	87.4 ± 4.6	5.7 ± 3.9	6.7 ± 4.9
A-1	Ant. Lesion	162 ± 40	7.5 ± 2.1*	8.4 ± 5.5	86.1 ± 4.2	6.5 ± 3.3	7.7 ± 4.3
	Sham-Operated	144 ± 47	6.3 ± 1.4	10.0 ± 4.9	86.7 ± 3.9	7.4 ± 3.8	8.8 ± 4.8
A-2	Ant. Lesion	162 ± 44	7.4 ± 2.2	6.0 ± 4.3	85.6 ± 4.8	6.3 ± 3.7	7.5 ± 5.0
	N.C.	159 ± 42	6.4 ± 1.6	5.4 ± 0.9	86.6 ± 5.9	5.5 ± 4.5	6.6 ± 5.8
B-1	Post. Lesion	140 ± 40	7.5 ± 2.0*	10.5 ± 4.7	85.5 ± 3.8	8.7 ± 3.7	10.4 ± 5.0
	Sham-Operated	134 ± 42	6.0 ± 1.3	10.3 ± 4.4	84.6 ± 3.6	8.7 ± 3.4	10.5 ± 4.3
B-2	Post. Lesion	146 ± 38	7.6 ± 2.0	10.8 ± 5.1	85.4 ± 3.8	8.9 ± 3.8	10.6 ± 5.2
	N.C.	151 ± 32	7.0 ± 1.4	10.4 ± 5.4	85.9 ± 4.7	7.1 ± 3.8	8.4 ± 4.8
C	PVM	174 ± 49**	8.3 ± 2.6	7.6 ± 6.5	82.6 ± 4.6	9.1 ± 3.0	11.2 ± 4.5
	Sham-Operated	161 ± 56	6.6 ± 1.5	5.9 ± 0.6	80.8 ± 3.0	11.1 ± 2.5	13.8 ± 3.5
	N.C.	114 ± 7.2	5.5 ± 1.0	5.9 ± 0.8	79.9 ± 2.6	10.1 ± 3.1	12.7 ± 4.3
D	PPM	170 ± 40	6.8 ± 1.2	6.5 ± 5.8	80.7 ± 4.1	10.3 ± 2.9	12.9 ± 3.7
	Sham-Operated	148 ± 53	6.6 ± 1.6	9.2 ± 5.2	84.3 ± 4.3	9.3 ± 2.6	11.2 ± 3.7
	N.C.	144 ± 35	5.9 ± 0.7	5.5 ± 0.7	79.9 ± 2.6	10.1 ± 3.1	12.7 ± 4.3

*$P < 0.05$, Lesioned birds compared with sham-operated controls.
**$P < 0.05$, Lesioned birds compared with normal controls.
[a]Number represents the mean ± standard deviation from the mean.

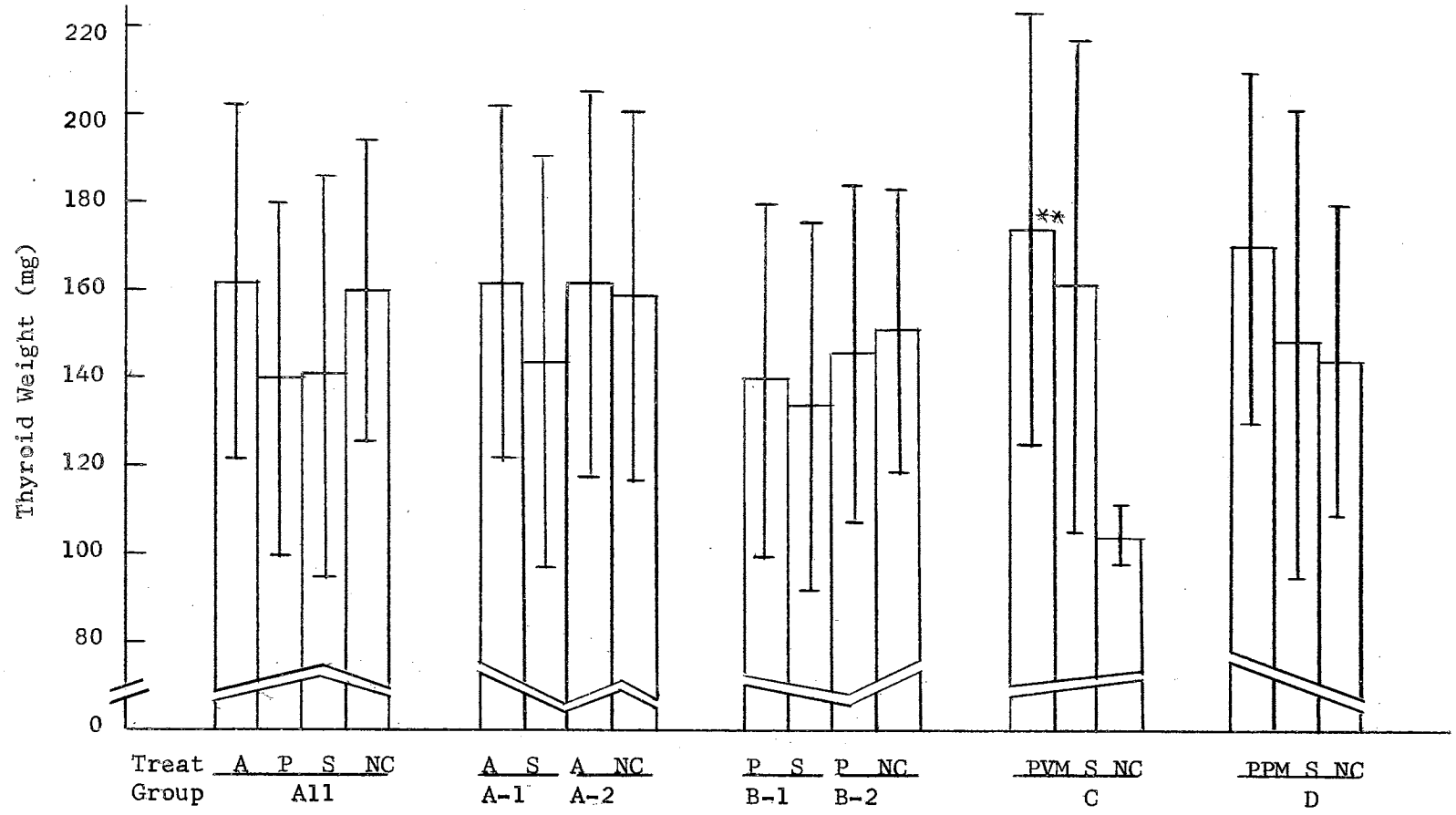

Figure 2. Thyroid Weights in mg of Hypothalamic-Lesioned and Control Cocks 10-14 Days After Lesioning. Treatments: A, anterior hypothalamic-lesioned; P, posterior hypothalamic-lesioned; S, sham-operated controls; NC, normal controls; PVM, lesions in the nucleus paraventricularis magnocellularis; and PPM, lesions in the nucleus praeopticus paraventricularis magnocellularis. Vertical bars represent ± one standard deviation from the mean. For explanation of group designations see the text and Table I. $^{*}P<0.05$, $^{+}P<0.10$ Lesioned birds compared with sham-operated controls. $^{**}P<0.05$, $^{++}P<0.10$ Lesioned birds compared with the normal controls.

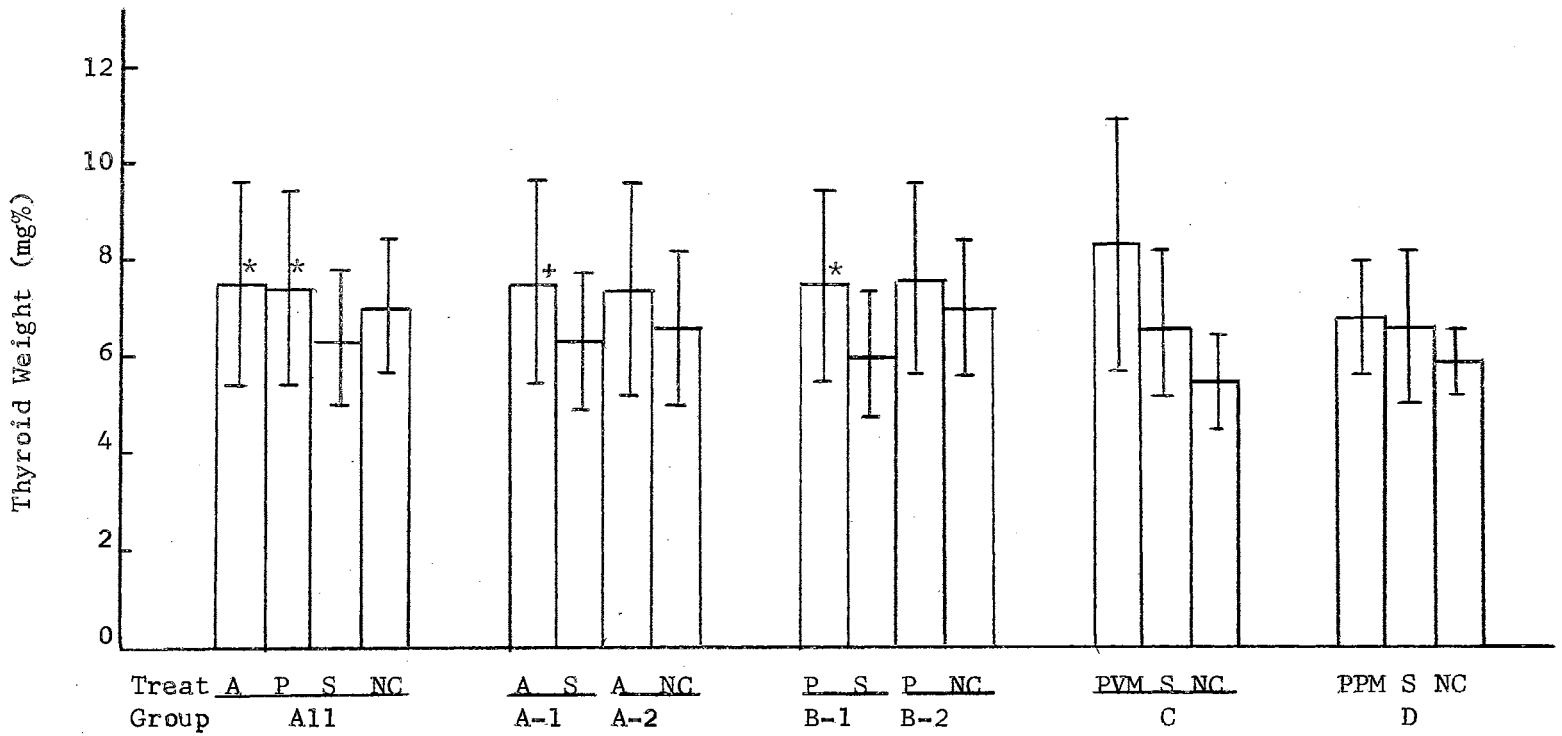

Figure 3. Thyroid Weights in mg% of Hypothalamic-Lesioned and Control Cocks 10-14 Days After Lesioning. For explanation of symbols see Figure 2.

not so great. In fact, when the results were expressed on the basis of mg% all lesioned groups showed mean thyroidal weights greater than their respective controls (see Figure 3).

Lesioning the anterior hypothalamus in mammals (Moll et al., 1961; Ganong et al., 1955) and in chickens (Egg and Chiasson, 1963) significantly reduced thyroid weights when compared with control animals even when the animals were not receiving a goitrogen. The observations made in the present study do not agree with the above observations and may have one of three explanations. In the case of the posteriorly lesioned birds it was noted that they lost a greater amount of body weight after lesioning than did the other groups and it may be that the body-weight loss was greater than the thyroidal weight loss and therefore the glands were relatively larger than those in the cocks which did not lose so much weight. This explanation would not account for the observations concerning thyroidal weights seen in the anteriorly lesioned birds, however, since they appeared actually to have gained weight.

A second explanation for the disparity may be found in the observations made by Harris and Woods (1956) in which it was found that stimulation of certain brain areas resulted in the inhibition of thyroidal iodine-release rate in the rabbit; when the animals were adrenalectomized, however, stimulation of the same area resulted in an enhanced thyroidal radioiodine-release rate. Thus it is possible that in the current study the "corticotropin" center of the hypothalamus was inhibited by the hypothalamic lesions. This would have reduced the production of ACTH and concomitantly the production of the corticoids and would have resulted in the release of TSH inhibition due to the corti-

coids. This possibility is rather conjectural since several investigators have failed to demonstrate any dependency of the corticoid content of the adrenals in the bird on the hypothalamus (Egg and Chiasson, 1963) or on the pituitary (Miller, 1961; Dulin, 1955). However, Resko et al. (1964) found that although hypophysectomy in the bird did not reduce the corticosterone level in adrenal venous effluent to nearly so low a level as in the mammals it did significantly lower this level. Furthermore, they found that both hypophysectomy alone or hypophysectomy with adenohypophysial autotransplantation to the kidney resulted in equal suppression (64% of normal) of corticosterone secretion from the adrenal. When the median eminence was damaged during hypophysectomy, however, the corticosterone secretion rate was further reduced. These findings support the hypothesis that the adrenals of the chicken do depend, in considerable measure, upon the pituitary for corticosterone secretion. Apparently the adrenals can also respond to some direct stimulus from the median eminence since destruction of this structure in hypophysectomized birds caused a further reduction (50% of hypophysectomized level) in the corticosterone secretion rate.

Finally, it may very well be that the lesions in this study had no effect on the thyroid weights since in only Group C (when compared with normal controls but not sham-operated controls), Figure 2, and Groups All and B-1, Figure 3, (compared with sham-operated controls) were the differences significant ($P < 0.05$). In Group C the number of lesioned birds was extremely small (4 lesioned birds) and in Group All the birds of the lesioned and control groups were not all from the same experiments.

The avian thyroid, unlike the avian adrenal, is very dependent upon the pituitary for maintenance and function (Ma, 1963; Hurst, 1963). However, its dependency upon the hypothalamus is less certain since Ma (1963) showed that the anterior pituitary when autotransplanted to the kidney maintained thyroid structure and function at nearly normal levels. Thus, if the production of TSH by the avian pituitary is dependent upon the hypothalamus, apparently the pituitary can be influenced by the hypothalamus via the systemic circulation and is not dependent on the hypothalamic-hypophysial portal system. This may be the explanation for the findings of Assenmacher (1958) who showed that while sectioning of the portal vessels in the duck failed to inhibit thyroidal weight, sectioning of the connections between the pars distalis and the infundibulum did.

The results of the work done by Shirley and Nalbandov (1956) also cast doubt on the hypothesis of a hypothalamic control of the avian pituitary. These investigators failed to show any inhibition of thyroidal weight by sectioning the pituitary stalk. However, Egg and Chiasson (1963) found a significant reduction in thyroid weight two weeks after lesioning the lateral division of the nucleus PPM of White Leghorn hens.

It may also be noted that many investigators failed to obtain a reduction in thyroid weights in mammals by lesioning the hypothalamus even though such lesions blocked the goitrogenic response to PTU (Greer, 1951; Bogdanove and Halmi, 1953; D'Angelo and Traum, 1956; Bogdanove et al., 1955; de Jong and Moll, 1965). The results of the present study are in agreement with these observations in mammals.

However, it must be pointed out that no birds in this study which were lesioned in the same hypothalamic area as those in the experiments of Egg anc Chiasson (1963) survived to time of autopsy.

Effects of Hypothalamic Lesions on Iodine Metabolism by the Thyroid

The per cent of the injected dose of I^{131} taken up by the thyroid of the White Leghorn cocks of this study was not significantly altered by lesions in any of the areas of the hypothalamus tested as shown by Table III and Figure 4. Work done in mammals showed that lesions in the anterior hypothalamus, especially those lesions involving the nucleus PVM, inhibited the uptake of I^{131} (Reichlin, 1960; Moll et al., 1961; Kovacs et al., 1960; Ganong et al., 1955; Courrier et al., 1956; Averill et al., 1961). Although the effects of hypothalamic lesions on radioiodine uptake in the avian species have not been reported, it has been reported that sectioning of the hypophysial portal veins in the duck resulted in a significant decrease in radioiodine release-rate even though such a technique failed to decrease the thyroidal weight (Assenmacher, 1958; Assenmacher and Tixier-Vidal, 1959).

On the other hand, Ma (1963) found only a slight decrease (17% decrease) in the 24 hour I^{131} uptake in hypophysectomized cockerels bearing pituitary autotransplants in the kidney while the uptake was greatly reduced (78% decrease) by hypophysectomy alone.

Taurog et al. (1958) conducted a very extensive series of experiments which clearly showed that hypophysectomy in the rat not only reduced the uptake of radioiodine by the thyroid to a few per cent of

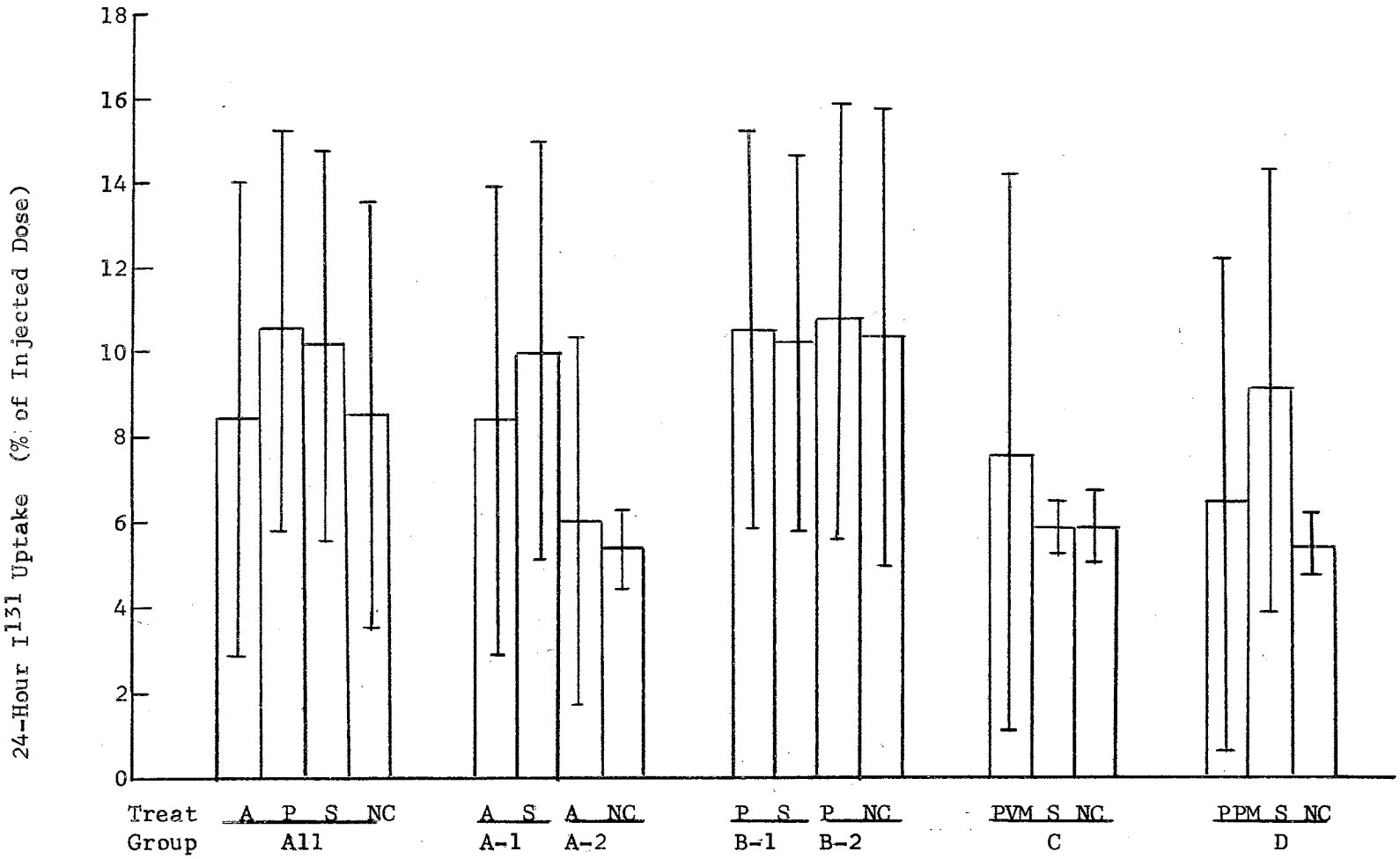

Figure 4. Per Cent of Injected Dose of I^{131} Taken up by both Thyroids in 24 Hours (24-Hour I^{131} Uptake) by Hypothalamic-Lesioned and Control Cocks 10-14 Days After Lesioning. For explanation of symbols see Figure 2.

normal but also that the iodine taken up by the thyroid was distributed differently in the hypophysectomized animal. The thyroglobulin of the hypophysectomized rat contained only a very small percentage of the concentrated iodine as radioactive thyronines, a normal percentage as $DI^{131}T$ and a larger percentage as $MI^{131}T$. They concluded that the various phases of thyroidal iodine metabolism were unequally affected by hypophysectomy; the later phases were inhibited more than the earlier phases.

The same conclusions were reached from studies on the thyroids of hypophysectomized, White Leghorn cockerels (Hurst, 1963) except that, while the above relationships were apparent within 10 hours after injection of I^{131} into the rat, they were not apparent in the White Leghorn cockerel until after 24 hours. At this time both DIT and the thyronines represented a smaller and MIT and iodide a larger percentage of the concentrated iodine in the thyroids of hypophysectomized than that in normal control chickens. The inorganic iodide was present at a higher percentage in the thyroids of hypophysectomized than that in control cockerels from the earliest time interval studied (3 hours after I^{131} injection), was still higher at 24 hours, but by 48 hours it was the same in the two groups. In the rat this was accomplished within 10 hours after I^{131} injection.

Tixier-Vidal and Assenmacher (1962) reported that hypophysectomy in the male duck did not affect iodotyrosine formation but that it did markedly inhibit the synthesis of the iodothyronines. Also, the uptake of I^{131} by these hypophysectomized ducks was strongly inhibited (less than 12% of the controls). It might be pointed out that this

last mentioned study utilized only 10 hypophysectomized animals distributed over 6 intervals of time and if their data are plotted for the percentages of the iodinated amino acids in the thyroid the curves are erratic.

It was anticipated, in the present study, that if lesions in the hypothalamus interfered with the release of TSH from the pars distalis that they would also mimic the effects of hypophysectomy on not only iodine uptake but also iodinated amino acid formation by the thyroid. From Table III and Figures 5, 6, and 7 it may be seen that the lesions in this study produced neither significant alterations in the per cent of concentrated iodine present either as the tyrosines or the thyronines nor in the ratio of the thyronines to the tyrosines except in one isolated case. When all of the birds bearing lesions in the posterior hypothalamus were compared with all normal controls it was noted that the lesioned birds had a significantly greater percentage of thyronines and a significantly larger iodothyronine to iodotyrosine ratio.

This observation is no doubt caused by some factor other than the lesions, such as season, (since the lesioned and control birds were not necessarily from the same experiments) because when the posteriorly lesioned birds were compared with only those controls from the same experiments the differences disappeared. Furthermore, at no time were any of the differences between the lesioned and sham-operated birds significant so far as percentages of iodinated amino acids are concerned.

That this test for effectiveness of hypothalamic lesions on

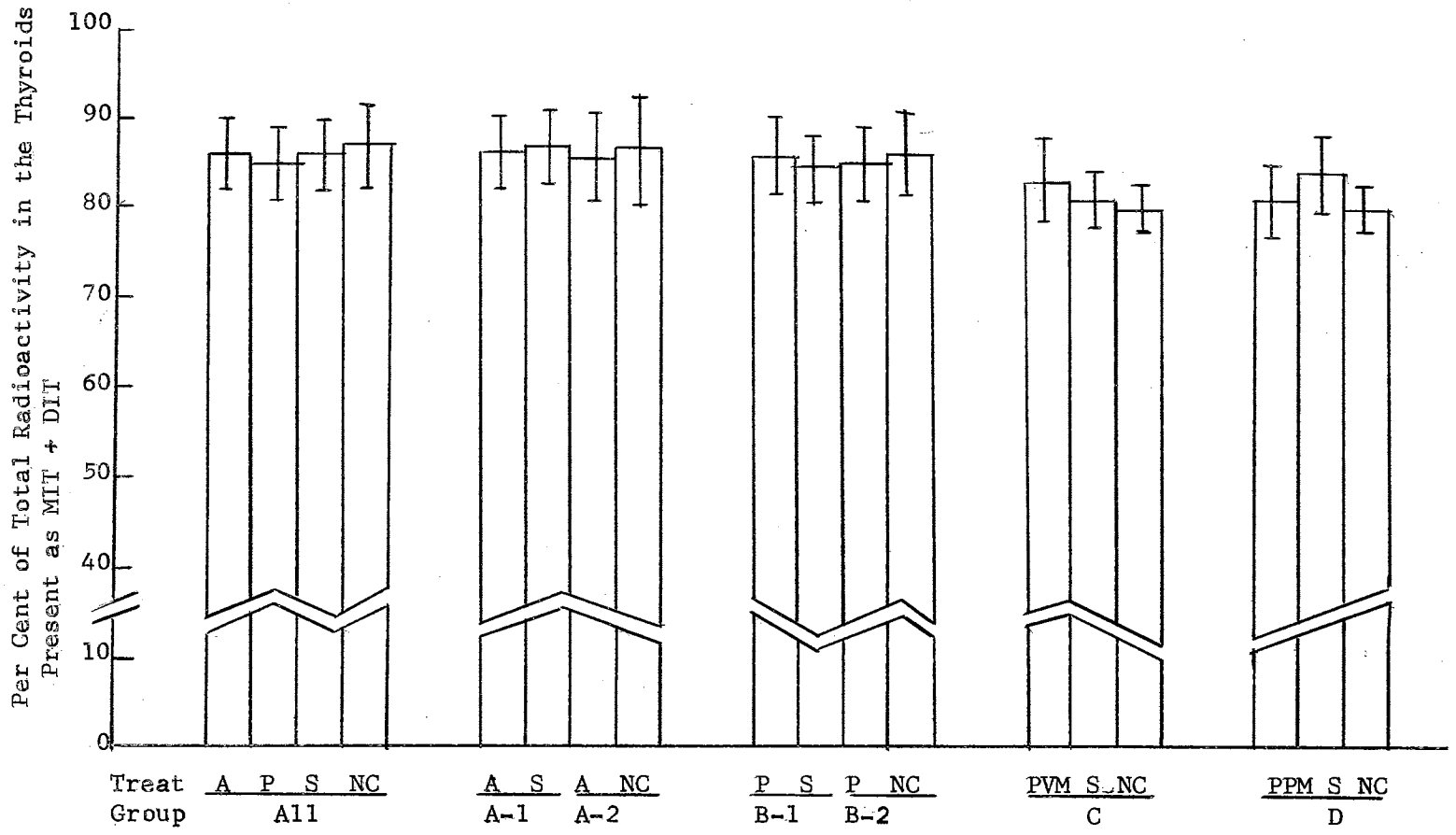

Figure 5. Per Cent of Total Thyroidal Radioactivity Present as MIT + DIT in Hypothalamic-Lesioned and Control Cocks 24 Hours After a Single Injection of I^{131} and 10-14 Days After Lesioning. For explanation of symbols see Figure 2.

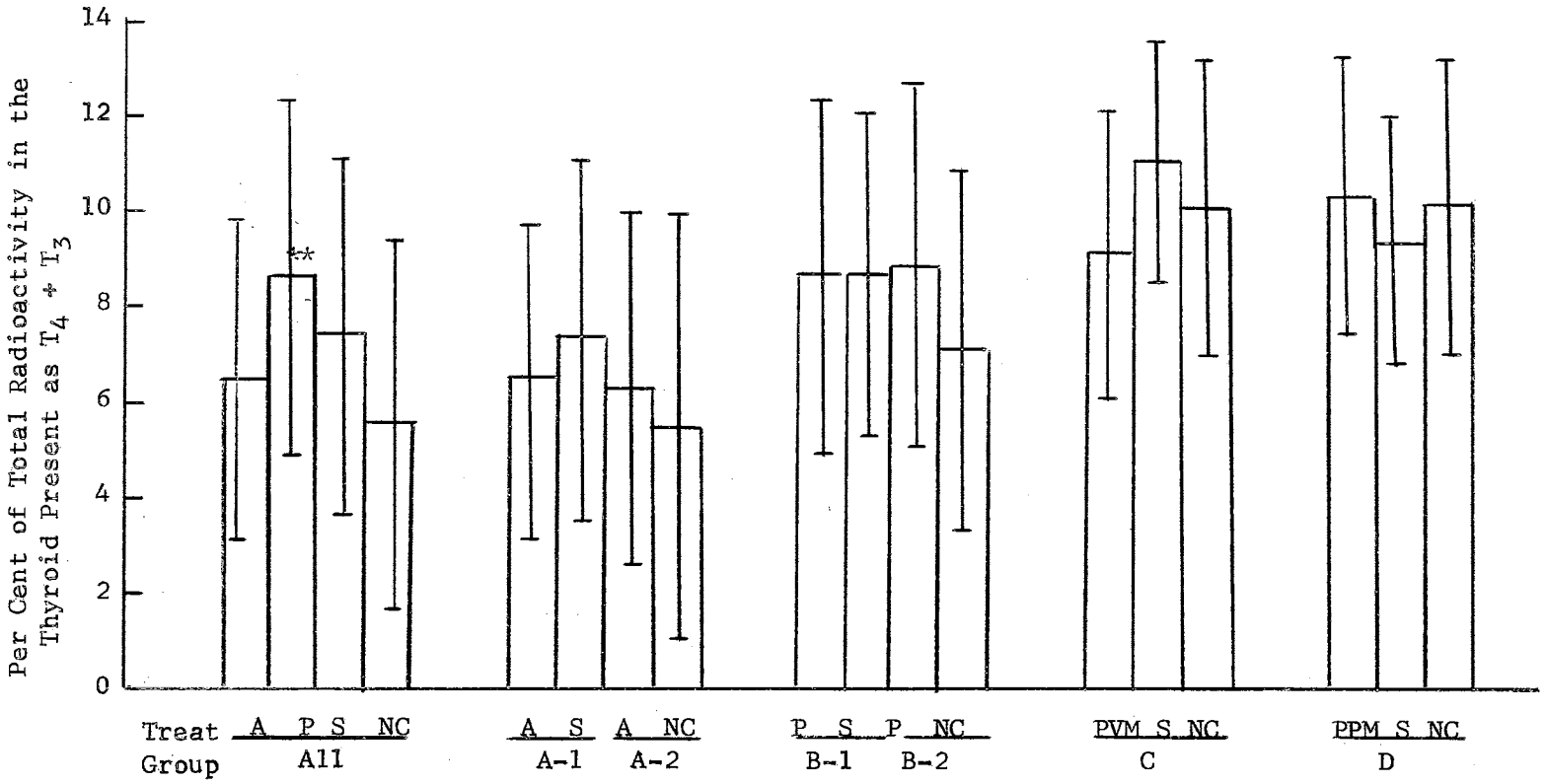

Figure 6. Per Cent of Total Thyroidal Radioactivity Present as T_3 + T_4 in Hypothalamic-Lesioned and Control Cocks 24 Hours After a Single Injection of I^{131} and 10-14 Days After Lesioning. For explanation of symbols see Figure 2.

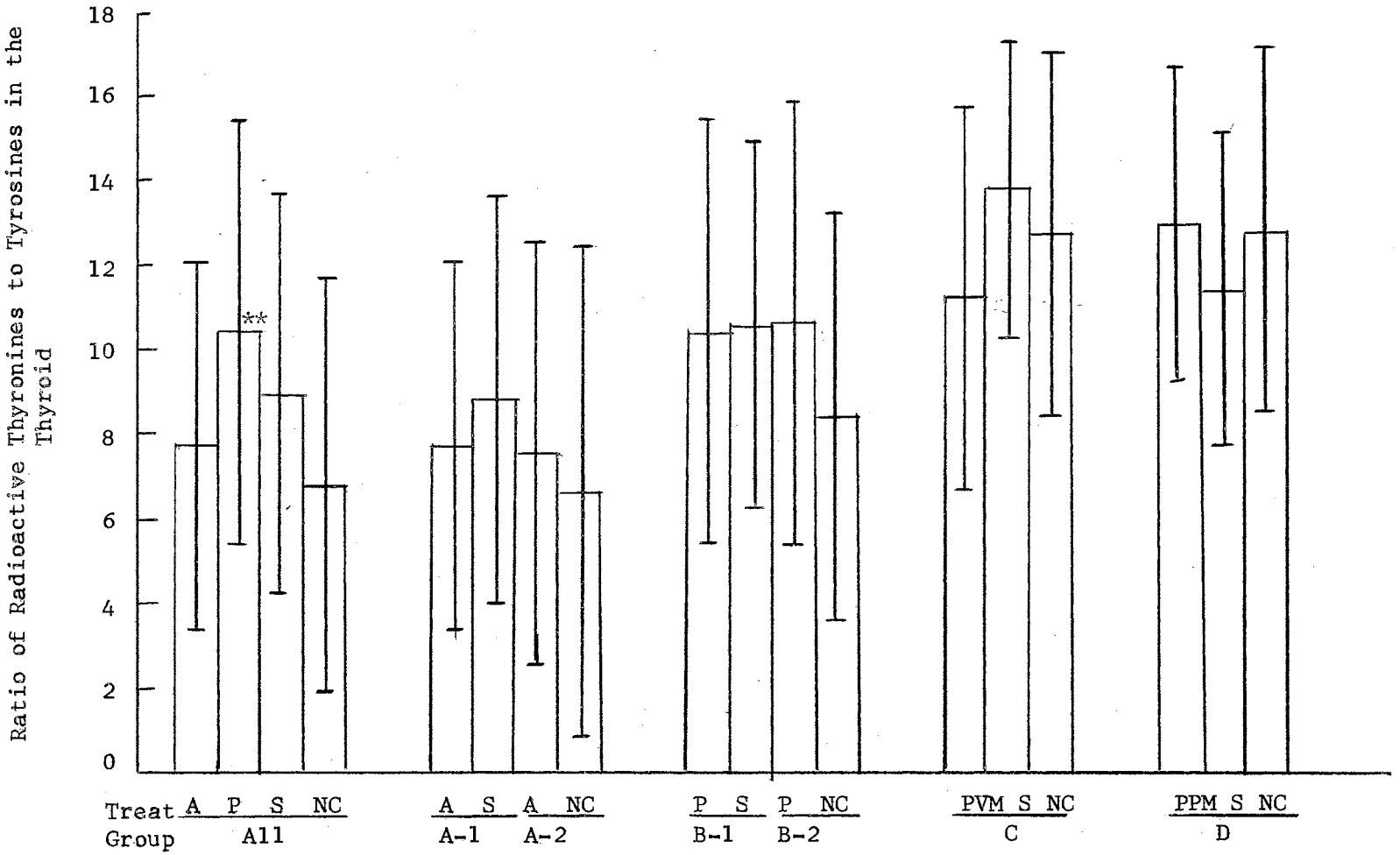

Figure 7. Ratio of Radioactive Iodothyronines to Radioactive Iodotyrosines in Hypothalamic-Lesioned and Control Cocks 24 Hours After a Single Injection of I^{131} and 10-14 Days After Lesioning. For explanation of symbols see Figure 2.

thyroidal physiology was rational is indicated by the work of Kovacs and Vertes (1963). These investigators showed that hypothalamic lesions damaging the PVM nucleus of the rat strongly inhibited the formation of MIT and DIT but much more strongly inhibited the formation of T_4.

Thus it appears from the present study that the hypothalamic areas investigated in this study either had no effect on the secretion of TSH from the anterior hypothalamus or, if they did, the effect was not of sufficient magnitude to cause detectable alterations in the iodinated amino acid composition of the thyroid or the uptake of iodine 24 hours after injection of I^{131}.

Effects of Hypothalamic Lesions on the Anterior Pituitary Weight and Content of TSH

Table IV and Figure 8 show the results of hypothalamic lesioning on the anterior pituitary weight in mg. The only group to show a statistically significant change in anterior pituitary weight was Group C. This group showed the anterior pituitary weight (in mg) to be smaller in the lesioned birds than in either control group.

When the anterior pituitary weights were expressed in mg% (Figure 9) the statistically significant difference was no longer evident; however, Group C still showed a trend toward a reduced anterior pituitary weight. The only other group to show such a trend was Group A.

Thus it appears that lesions in the anterior portion of the hypothalamus produced a trend toward reduced anterior pituitary weight in the White Leghorn chicken; possibly the PVM nucleus is more intimately

TABLE IV

ANTERIOR PITUITARY WEIGHT AND TSH CONTENT OF HYPOTHALAMIC-LESIONED AND CONTROL COCKS 10-14 DAYS AFTER LESIONING

Group No.	Treatment	Ant. Pituitary Weight (mg)	Ant. Pituitary Weight (mg%)	TSH/mg Ant. Pituitary (mU)	Total TSH/Ant. Pituitary (mU)
All	Ant. Lesion	8.2 ± 2.9**[a]	0.35 ± 0.16**+	3.5 ± 2.5	24.2 ± 16.9+
	Post. Lesion	9.4 ± 2.5	0.49 ± 0.28	6.6 ± 4.8*‡	59.8 ± 58.1++
	Sham-Operated	9.5 ± 3.1	0.44 ± 0.39	4.2 ± 2.7	38.0 ± 22.5
	N.C.	10.5 ± 2.5	0.46 ± 0.32	3.2 ± 3.0	34.1 ± 35.6
A-1	Ant. Lesion	8.2 ± 2.9	0.35 ± 0.16	3.5 ± 2.5	24.2 ± 16.9+
	Sham-Operated	8.6 ± 3.0	0.39 ± 0.11	4.8 ± 3.2	39.5 ± 24.8
A-2	Ant. Lesion	7.9 ± 2.5	0.30 ± 0.11	3.2 ± 2.0**	20.0 ± 10.4
	N.C.	9.9 ± 2.5	0.39 ± 0.12	1.7 ± 1.0	16.5 ± 9.4
B-1	Post. Lesion	9.4 ± 2.6	0.49 ± 0.15	6.6 ± 4.8+	59.8 ± 58.1
	Sham-Operated	9.8 ± 2.8	0.44 ± 0.13	4.1 ± 2.1	38.7 ± 22.9
B-2	Post. Lesion	9.6 ± 2.2	0.50 ± 0.16	6.3 ± 4.8	56.0 ± 55.9
	N.C.	10.9 ± 2.5	0.51 ± 0.12	3.9 ± 3.6	43.2 ± 41.7
C	PVM Lesion	6.5 ± 0.7*++	0.31 ± 0.06+	2.0 ± 0.6++	13.2 ± 4.5
	Sham-Operated	9.7 ± 1.0	0.41 ± 0.02	3.9 ± 3.0	39.7 ± 31.8
	N.C.	9.9 ± 3.0	0.48 ± 0.16	1.2 ± 0.02	11.7 ± 3.7
D	PPM Lesion	7.7 ± 2.2	0.36 ± 0.18++	5.6 ± 3.7**	39.3 ± 25.6
	Sham-Operated	8.3 ± 2.2	0.37 ± 0.09	5.0 ± 3.6	41.8 ± 28.3
	N.C.	9.9 ± 2.1	0.42 ± 0.13	1.1 ± 0.3	11.3+± 4.6

*$P < 0.05$, +$P < 0.10$ lesioned birds compared with sham-operated controls.
**$P < 0.05$, ++$P < 0.10$ lesioned birds compared with normal controls.
[a]Number represents mean ± standard deviation from the mean.

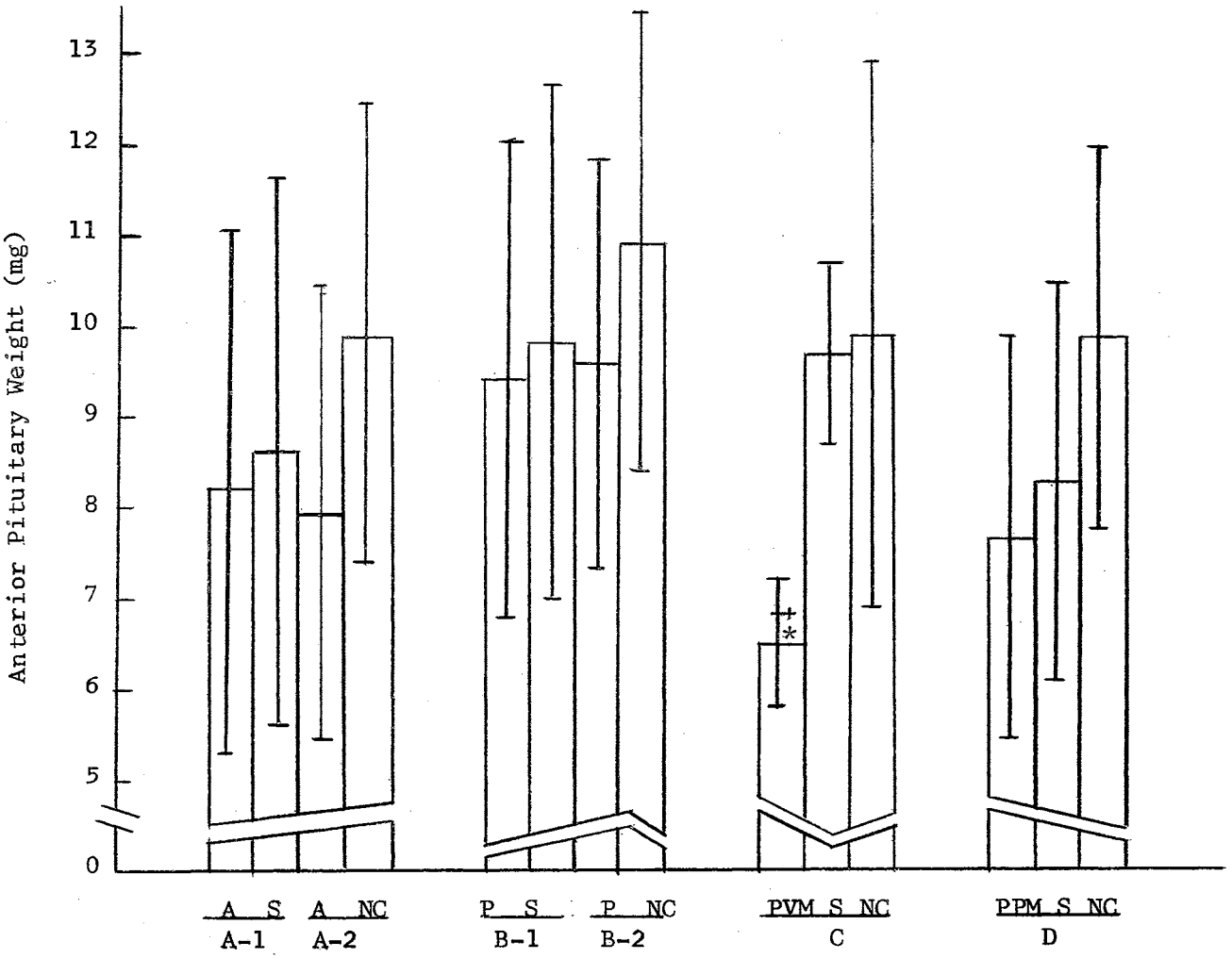

Figure 8. Anterior Pituitary Weight in mg for Hypothalamic-Lesioned and Control Cocks 10-14 Days After Lesioning. For explanation of symbols see Figure 2.

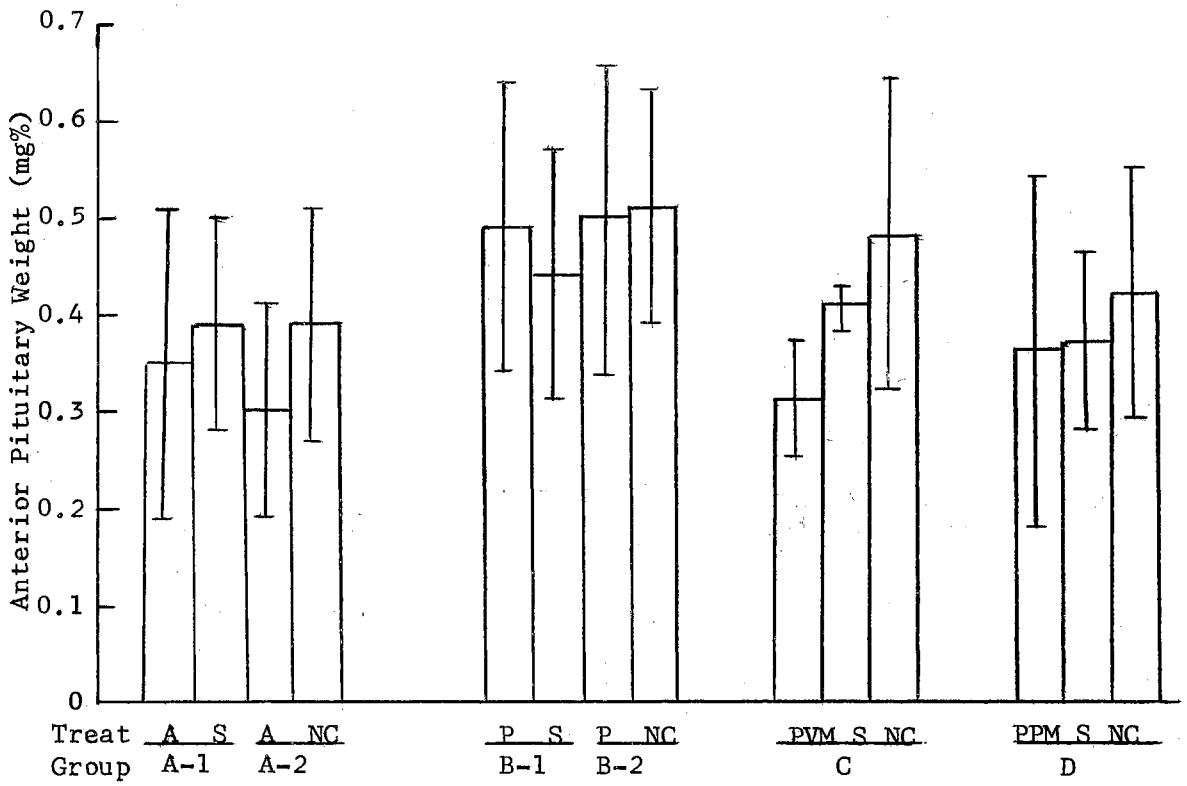

Figure 9. Anterior Pituitary Weights in mg% for Hypothalamic-Lesioned and Control Cocks 10-14 days after Lesioning. For explanation of symbols see Figure 2.

associated with this phenomena than other hypothalamic areas.

D'Angelo and Traum (1956) and D'Angelo (1958) have reported that lesions in the ventral anterior hypothalamus of the rat resulted in a reduction in the anterior pituitary weight of about 40%. Similarly, Florsheim (1958) found that lesions involving areas of the hypothalamus caudal to the PVM nucleus in the rat produced no change in the thyroid weight while lesions involving this nucleus did.

Table IV and Figures 10 and 11 show the effects of hypothalamic lesions on the TSH content of the pars distalis. The standard deviations from the means were very large for the pituitary level of TSH. Whether this is a true representation of the variability of the TSH

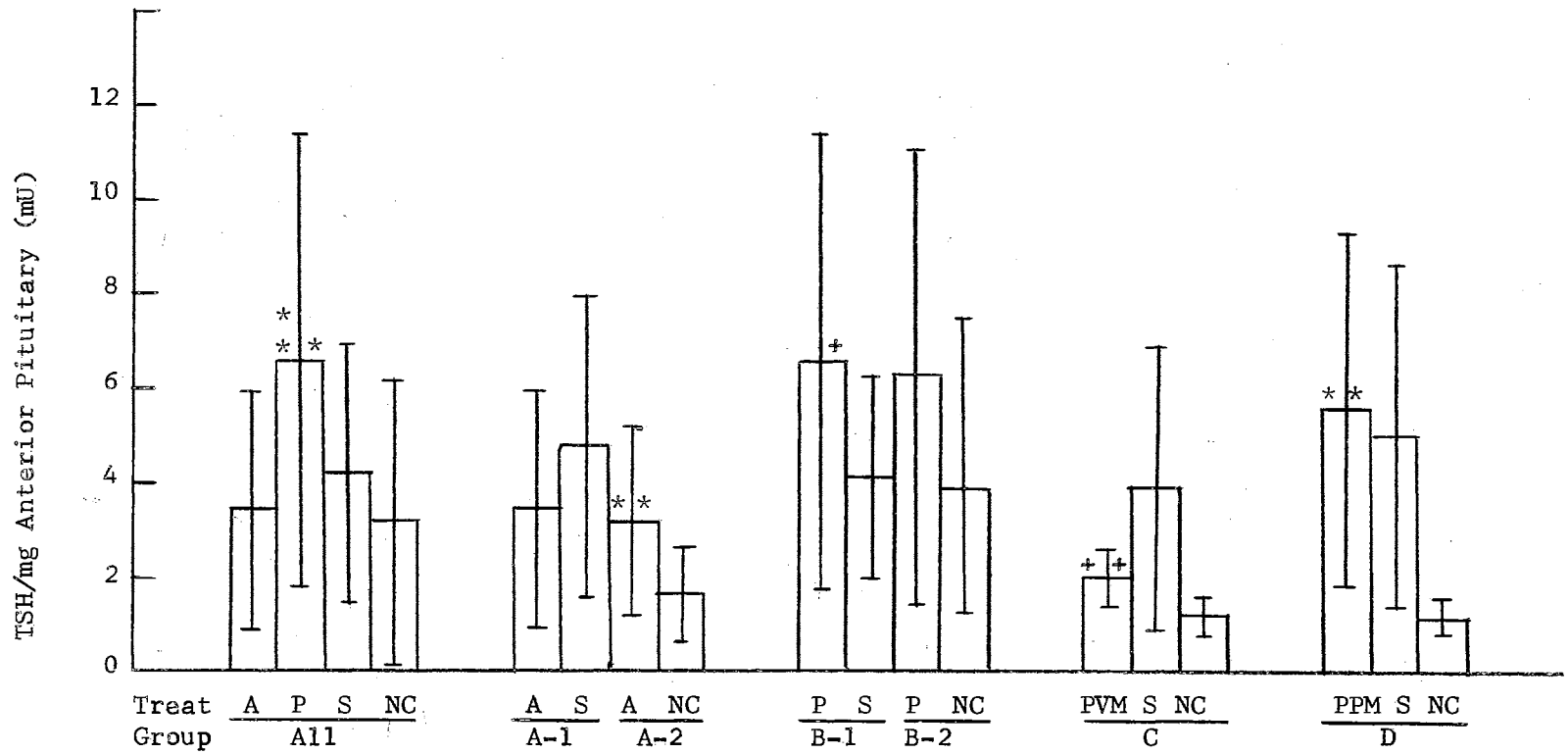

Figure 10. Concentration of TSH in the Anterior Pituitary of Hypothalamic-Lesioned and Control Cocks (mU TSH/mg Anterior Pituitary, wet Weight) 10-14 Days after Lesioning. For explanation of symbols see Figure 2.

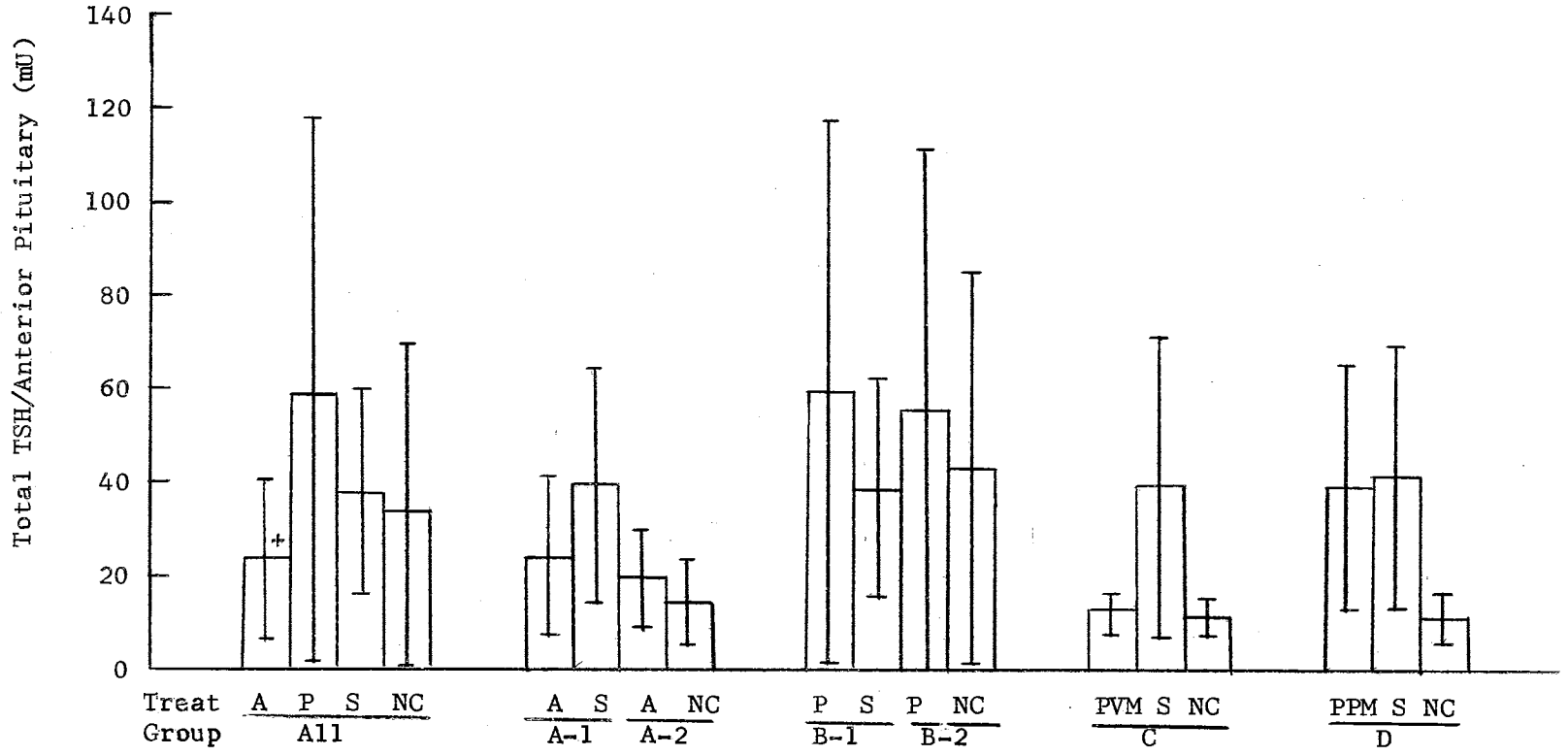

Figure 11. Total Amount of TSH in the Anterior Pituitary of Hypothalamic-Lesioned and Control Cocks (mU TSH/Anterior Pituitary) 10-14 Days After Lesioning. For explanation of symbols see Figure 2.

content of the pituitaries or a variability of the assay technique or a combination of both is not known. Nevertheless, certain trends were observed and they seemed to be the reverse of those seen from the limited information available for mammals.

In this study (see Figure 10) it was found that lesions in the anterior hypothalamus and in particular in the nucleus PPM resulted in an increase in the concentration of TSH in the anterior pituitary as compared with normal controls.

When these same birds were compared with the sham-operated controls, however, there was no significant difference in the concentration of TSH in the pars distalis and in the case of Group A the sham-operated controls showed a higher concentration and in the case of Group D the sham-operated controls showed a lower concentration of TSH in the pars distalis than did the lesioned birds.

If both the concentration and total stores of TSH in the anterior pituitary are observed (Figures 10 and 11) it is seen that the TSH levels seem to be considerably lower in all groups (except Group B) for the normal controls than for either lesioned or sham-operated controls. An explanation for this observation is not evident; possibly the surgical manipulations of the lesioned and sham-lesioned animals affected the level of TSH in the pars distalis in a positive manner.

It may be more meaningful, therefore, to compare the levels of TSH in the anterior pituitary of the lesioned birds with only those from the sham-operated birds. When this was done the results became more plausible and the data suggested that lesions in the anterior hypothalamus, especially the PVM, reduced (non-significantly); lesions

in the nucleus PPM produced no change; and lesions in the posterior hypothalamus resulted in an increase in the concentration of TSH in the anterior hypothalamus. The total stores of TSH in the pituitary followed the same trend.

D'Angelo (1958) and D'Angelo and Traum (1956) found that anterior hypothalamic lesions involving the PVM nucleus of the rat, while not interfering with the concentration of TSH in the pars distalis, reduced the total stores of the hormone in that gland. Florsheim (1958) found that the thyroid function of rats lesioned in the anterior hypothalamus was inhibited and that the level of TSH in the pituitary paralleled the thyroid activity. These observations suggested that anterior hypothalamic lesions interfered with the level of TSH in the anterior pituitary of the mammal.

On the other hand, both the concentration and total stores of TSH in the anterior pituitary of the posteriorly lesioned birds (Group B-1, Figures 10 and 11) was greater than in the sham-operated controls. This may possibly be explained on the basis of interference of the lesions with ACTH secretion as discussed in the section on the effects of hypothalamic lesions on thyroidal weight.

Although the posterior hypothalamic lesions resulted in both an increased TSH content in the pars distalis and an increase in thyroid weight (both biologically significant at the 0.10 level) these same lesions did not enhance thyroidal iodine metabolism. Since the 24-hour iodine uptake was considered by Florsheim (1958) to be the most sensitive assay for effect of hypothalamic lesions in the mammal, it appeared in the present study in the bird that the posterior hypo-

thalamic lesions enhanced primarily the synthesis and not the secretion of TSH.

That hypothalamic damage in the chicken might result in an exaggerated TSH alteration in the bird indirectly via the adrenals is indicated by the findings that the florescent material (assumed to be corticosterone) in the venous plasma of chickens remained high after hypophysectomy (about 60% of control values). However, if the median eminence was damaged in the hypophysectomized chicken the florescent material was significantly decreased to only about 50% of the hypophysectomized levels (Resko et al., 1964).

That the finding of an increased level of TSH in the anterior pituitary without an increased secretion rate of TSH (as indicated by the failure to show an enhanced 24-hour iodine uptake) was feasible was recently shown by van Rees (1966) and Salaman (1964) in the mammal. These investigators showed that thyroidectomy was accompanied by a fall in the TSH level of the pituitary and a rise in the blood levels of this hormone and when T_4 was then given to these animals the pituitary level of TSH rose very precipitously while the blood level declined.

Thus, contrary to the earlier hypothesis that pituitary and blood levels of TSH paralleled each other, it now appears that these two levels may vary independently of each other.

Effects of Hypothalamic Lesions on Gonadotropic Hormone Secretion by the Anterior Pituitary

From Table V and Figures 12 and 13 it may be seen that posterior hypothalamic lesions significantly reduced the testicular weight in the

TABLE V

TESTIS WEIGHTS AND COMB FACTORS OF HYPOTHALAMIC-LESIONED AND CONTROL COCKS

Group No.	Treatment	Testis Weight (g)	Testis Weight (g%) Based on Body Weight When Sacrificed	Testis Weight (g%) Based on Body Weight When Lesioned	Comb Factor
A-1	Ant. Lesion	15.7 ± 9.9[a]	0.59 ± 0.40	0.68 ± 0.41	59.3 ± 14.4
	Sham-Operated	15.2 ± 13.2	0.66 ± 0.59	0.62 ± 0.48	55.2 ± 13.9
A-2	Ant. Lesion	19.9 ± 7.9	0.79 ± 0.35	0.85 ± 0.33	57.3 ± 15.4
	N.C.	21.9 ± 8.5	0.84 ± 0.31	0.87 ± 0.35	55.9 ± 9.9
B-1	Post. Lesion	9.4 ± 12.2^{+}	0.51 ± 0.53	0.43 ± 0.45^{+}	45.0 ± 9.0
	Sham-Operated	17.9 ± 15.7	0.85 ± 0.78	0.80 ± 0.71	45.1 ± 11.5
B-2	Post. Lesion	10.4 ± 9.7**	0.56 ± 0.55	0.47 ± 0.46^{++}	42.2 ± 9.0**
	N.C.	20.2 ± 13.5	0.95 ± 0.68	0.82 ± 0.56	52.3 ± 15.7
All	Ant. Lesion	15.7 ± 9.9^{++}	0.59 ± 0.40^{++}	0.68 ± 0.41	59.2 ± 14.4^{+}
	Post. Lesion	9.4 ± 12.2**	0.51 ± 0.54***$^{+}$	0.43 ± 0.45***	45.0 ± 9.0**
	Sham-Operated	18.0 ± 14.0	0.87 ± 0.69	0.81 ± 0.62	50.8 ± 14.4
	N.C.	22.2 ± 11.5	0.97 ± 0.59	0.89 ± 0.48	54.9 ± 13.8

[a]Number represents mean ± standard deviation from the mean.

*$P < 0.05$, $^{+}P < 0.10$ lesioned birds compared with sham-operated controls.

**$P < 0.05$, $^{++}P < 0.10$ lesioned birds compared with normal controls.

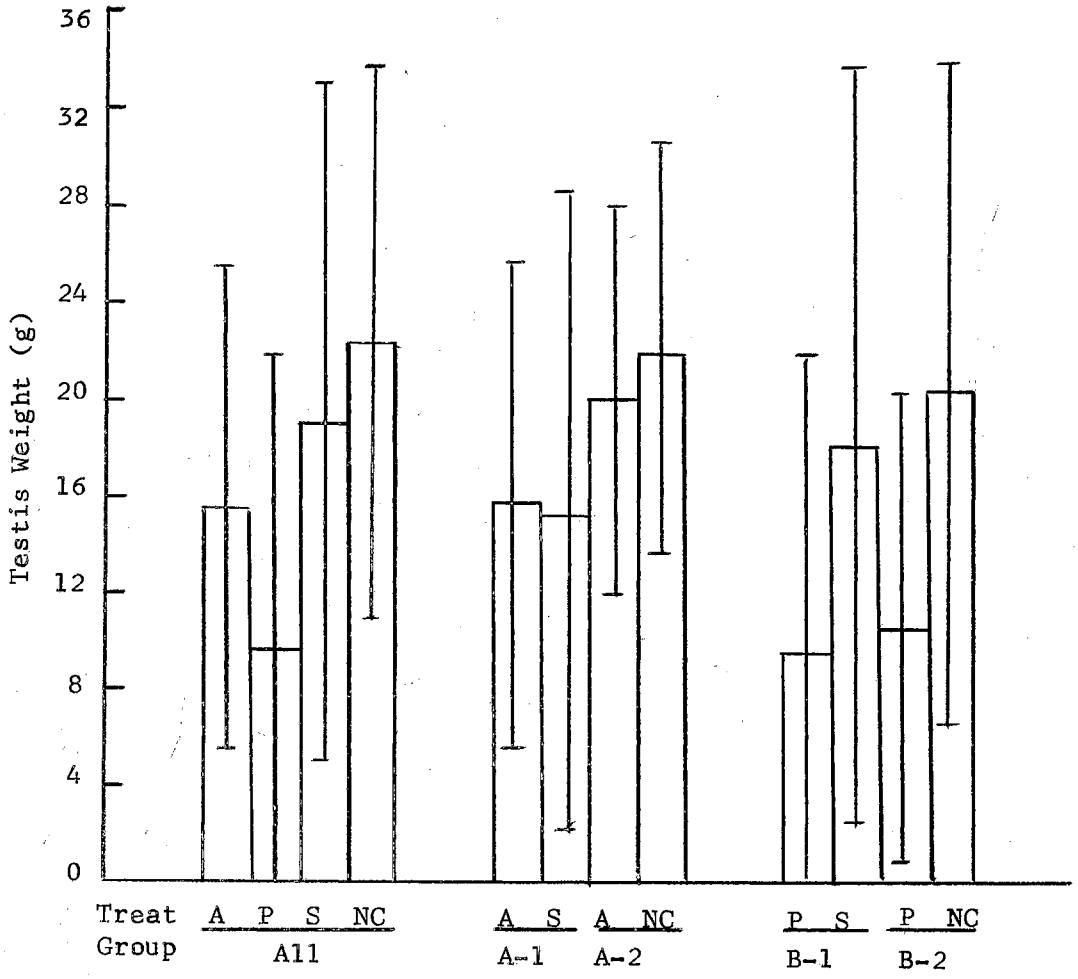

Figure 12. Testis Weights in Grams in Hypothalamic-Lesioned and Control Cocks 10-14 Days After Lesioning. For explanation of symbols see Figure 2.

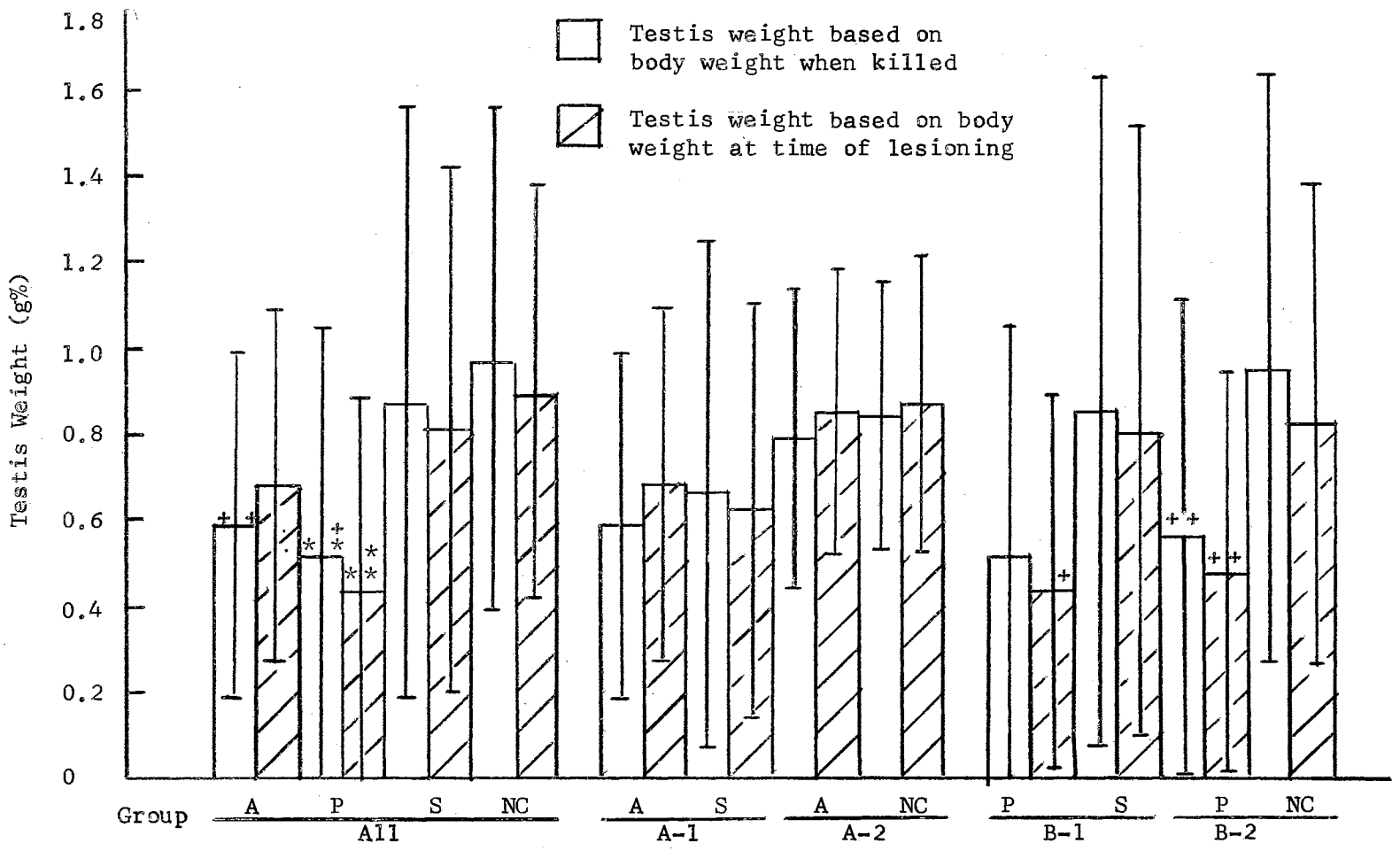

Figure 13. Testis Weights in g% for Hypothalamic-Lesioned and Control Cocks at Time of Lesioning and 10-14 Days After Lesioning. For explanation of symbols see Figure 2.

White Leghorn chicken. This same trend was not observed following anteriorly placed lesions. These findings are somewhat consistent with the findings of others in regard to the effects of hypothalamic lesions on gonadal function. Pituitary grafts in the hypophysectomized drake (Benoit, 1962) and hypophysectomized cockerel (Ma, 1963); pituitary stalk sectioning in the domestic hen (Shirley and Nalbandov, 1956) and drake (Benoit, 1962); and lesions in the area of paraventricular or supraoptic regions of the duck (Benoit, 1962), in the area of the ventral part of the nucleus PVM of the hen (Ralph, 1959), and in the areas of the nucleus PVM, median eminence or pars tuberalis in the hen (Egg and Chiasson, 1963) brought about gonadal and comb atrophy.

Thus there seemed to be no doubt that the hypothalamus was necessary, along with the hypophysis, for normal gonadal function. However, the location of the lesions which interfered with gonadotropic hormone (GTH) release in this study did not correspond to the effective lesion sites of the lesion studies presented above.

There are several possible explanations for the differences seen between this study and the ones referred to above. First, the other studies utilized either the male duck or the domestic hen and the possibility exists that the rooster differs from these other animals in respect to the area of the hypothalamus involved in controlling the release of the gonadotropic hormones. Second, Ralph (1959) reported that regardless of where the hypothalamic lesions were located in the hen, egg laying ceased for 10-14 days following the lesioning. This indicated that the lesions interfered with the release of GTH. This would explain the inhibition on testiscular weight produced by the

posteriorly placed lesions but not the lack of the response following anterior lesions. Third, damage to the median eminence (Benoit, 1962; Egg and Chiasson, 1963) usually resulted in the reduction of gonad size in the bird as apparently did damage to the arcuate nucleus as stated by Yasuda (reported by Farner and Oksche, 1962).

Lisk (1964), in a review article, claimed that destruction of the median eminence resulted in gonadal atrophy in all mammalian species studied up to the time of his review. Furthermore, in the rat the implantation of crystalline estradiol into the arcuate nucleus prevented ovulation in the intact rat and the appearance of signet ring cells in the adenohypophysis of the castrated rat for several months (Lisk, 1964).

These observations tend to support the findings in this experiment which suggest that damage to the posterior hypothalamus or median eminence resulted in the atrophy of the avian testis.

Only posteriorly placed hypothalamic lesions were effective in altering comb size as shown in Table V. This effect was only demonstrable in comparisons between the posteriorly lesioned birds and the normal controls. There was no difference between the comb size of the lesioned and sham-operated birds. Since the body weights of the posteriorly lesioned cocks were significantly smaller than either those of the normal controls or sham-operated controls and since the comb size was not expressed on the basis of body size, this observation may mean that the size of the comb was not interfered with by the hypothalamic lesions.

As in the case of testicular weight so in the case of the size of

the comb it was expected that anteriorly placed hypothalamic lesions would result in comb regression. That this did not occur in the present study may mean that the 10-day period between time of lesioning and time of autopsy was insufficient to allow detectable atrophy of the comb or it may mean that the lesions in the areas of the anterior hypothalamus investigated in this study did not interfere with comb maintenance.

CHAPTER V

SUMMARY AND CONCLUSIONS

Adult male White Leghorn chickens were bilaterally lesioned in various parts of the hypothalamus. Ten to 14 days after lesioning and 24 hours after a single injection of carrier-free I^{131} the animals were killed and the thyroids, pituitaries, and testes were removed and weighed; the combs were also measured.

The pituitaries were assayed for their content of TSH by an *in vivo* bioassay. The 24-hour I^{131} uptake was determined by comparing the amount of I^{131} in the thyroid at time of necropsy with the amount injected. The distribution of radioiodine among the various iodinated amino acids of the thyroid was determined by homogenizing the thyroids and subjecting aliquots of the homogenate to paper chromatography in a butanol-ethanol-ammonium hydroxide solvent system. The identities of the iodinated amino acids were determined with the aid of carrier chromatograms and the quantities of the amino acids were found by counting the radioactivity of the various components. The quantitative results were expressed as the per cent of each homogenate represented by an individual component.

The lesioned birds were compared with normal controls and with sham-operated controls. Location and size of the lesions were confirmed at necropsy by histologic examination.

The results of this study showed that lesions in those areas of the anterior hypothalamus studied had no significant effect on body weights, thyroid weights, anterior pituitary weights, testis weights, nor comb factor. However, the concentration of TSH in the anterior pituitary was significantly greater in the lesioned birds as compared with the normal controls but smaller (non-significantly) than that in the sham-operated controls.

When only those chickens bearing anteriorly placed lesions which included the nucleus PVM were studied it was found that the thyroids (in mg) were significantly larger than the normal controls and non-significantly larger than the sham-operated controls. This same lesioned group had smaller pituitaries than either control group and an elevated TSH concentration in the anterior pituitary when compared with normal controls. Lesions in the nucleus PVM produced no significant change in thyroidal iodine metabolism. However, the mean thyroidal 24-hour iodine uptake appeared to be elevated over control values by the lesions. On the other hand the iodothyronine to iodotyrosine ratio was non-significantly smaller.

Lesions in the anterior hypothalamus which included the nucleus PPM caused no significant alteration in any aspect of thyroidal physiology or morphology studied. These lesions did result in an elevation in the concentration of TSH in the anterior pituitary when compared with the normal controls. Lesions in this area of the hypothalamus had no effect on the size of the pituitary.

Birds bearing lesions in the posterior hypothalamus showed a significant loss in body weight. This group of lesioned birds also

had significantly larger thyroids (in mg%) than did the sham-operated controls and non-significantly larger thyroids than did the normal controls. This may not have been an actual thyroidal enlargement but, instead, a reflection of the significantly greater weight loss suffered by the birds bearing posterior lesions. This is especially suggested by the findings that the thyroidal iodine metabolism of these birds was not altered.

The anterior pituitary weights were the same in the posteriorly lesioned and control groups. However, the concentration and total stores of TSH in the anterior pituitaries of the posteriorly lesioned birds showed a trend toward being enhanced (non-significantly).

The gonadotropic hormone secretion rate appeared to have been inhibited by the posterior lesions as reflected by a reduction in testis weights (in both mg and mg%). The comb size was not reduced by the posterior hypothalamic lesions in comparison with those of sham-operated controls; when compared with normal controls the comb size was significantly reduced. However, this may again reflect the fact that the posteriorly lesioned birds were smaller than the controls.

It was concluded that the anterior hypothalamic lesions employed in this study did not appreciably interfere with, or enhance, the secretion rate of TSH in the White Leghorn cocks as ascertained by various phases of thyroidal iodine metabolism. The lesions did appear to augment slightly, but non-significantly, the thyroidal weight.

The effects of the anteriorly placed lesions on the content of TSH in the anterior pituitary were inconsistent; when compared with sham-operated controls the concentration of TSH was non-significantly

lower and when compared with normal controls the concentration was significantly higher.

Lesions in the posterior hypothalamus, likewise, failed to alter TSH secretion as measured by thyroidal size or iodine metabolism. However, these same lesions resulted in a non-significantly ($P < 0.1$) elevated concentration of TSH in the anterior pituitary as compared with either control group.

The secretion rate of the gonadotropic hormones was not affected by the anteriorly placed hypothalamic lesions in this study; posteriorly placed lesions inhibited gonadotropic hormone secretion rate as judged by a reduction in testis weight. The effects of hypothalamic lesions on the comb size were inconclusive.

The average concentration of TSH in the anterior pituitary of the intact White Leghorn cock for the period of February through July was found to be 3.2 ± 3.0 mU(USP)/mg (wet weight) of anterior pituitary tissue and the total stores of TSH in the gland, during the same period of time, was 34.1 ± 35.5 mU/gland.

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APPENDIX

BODY, THYROID, AND TESTIS WEIGHTS; 24-HOUR IODINE UPTAKE; COMB FACTOR; AND DATE KILLED FOR HYPOTHALAMIC-LESIONED BIRDS

Cock Number	Date Killed Mo/Day	Body wt. When Lesion.	Body wt. When Killed	Thy. Wt. mg	Thy. Wt. mg%	24-Hr I^{131} uptake	Testis Wt. g.	Testis Wt. g%	Comb Factor
0-1	2/15	2640	2520	192	7.62	3.04	27.9	1.11	62.4
0-2	2/15	2420	2096	118	5.65	2.35	22.8	1.09	47.9
0-6	2/22	2630		193		4.51	20.9		49.0
0-8	2/22	2492		168		6.48	30.1		67.5
0-9	2/22	2200		107		7.74	24.1		82.5
0-12	3/1	2394	2164	103	5.68		25.1	1.16	69.8
0-14	3/8	2318	1927	139	7.21	9.93	20.9	1.08	
0-21	5/6	2080	1360	121	8.90		19.2	1.42	45.5
0-24	5/6	2060	1850	108	5.84		37.5	2.03	50.6
0-25	5/6	2096	2060	235	11.41		7.8	0.38	28.8
0-30	5/6	1860	1260	153	12.14		11.6	0.92	43.8
0-34	6/10	2540	2500	177	7.08	5.35	21.6	0.86	64.0
0-35	6/10	2180	2360	143	6.06	5.16	13.4	0.57	67.5
0-36	6/10	2646	2860	200	6.99	5.13	10.8	0.36	39.0
0-37	6/10	2476	1828	226	12.36	17.2	8.0	0.44	68.0
0-38	6/10	2450	2572	138	5.35	4.55	5.6	0.22	45.5
0-39	6/10	2040	2424	204	8.42	3.5	25.9	1.07	36.0
0-40	6/10	1780	2000	124	6.20	4.4	10.0	0.50	40.3
0-41	6/10	2445	2440	208	8.52	5.47	6.8	0.28	45.5
0-43	7/5	1980	1775	151	8.51	8.96	2.0	0.12	39.0
0-44	7/5	2140	2278	136	5.97	9.71	6.4	0.28	43.8
0-47	7/5	2306	2145	182	8.46	9.25	7.6	0.35	48.8
0-50	7/5	2648	1802	141	7.82	11.14	2.4	0.13	45.5
0-52	7/12	2206	1934	133	6.88	18.10	4.8	0.25	61.6
0-53	7/12	2186	1345	85	6.32	18.91	2.1	0.16	42.2
0-57	7/12	2226	1947	119	6.11	18.50	5.0	0.26	34.5
0-59	7/12	1980	2118	112	5.29	9.65	4.7	0.22	39.0
0-64	8/2	2104	1990	157	7.86	11.2	4.3	0.22	57.2
0-67	8/2	1800	1982	126	6.38	13.7	7.0	0.35	57.4
0-69	8/2	1858	1194	80	6.70	9.0	2.1	0.18	52.7
0-70	8/25	2416	2672	160	5.99	15.09	2.9	0.11	80.0
0-73	8/25	2290	2146	108	5.03	8.55	1.7	0.08	34.5
0-76	8/25	1938	1915	202	10.55	17.76	2.3	0.12	64.9

BODY, THYROID, AND TESTIS WEIGHTS; 24-HOUR IODINE UPTAKE; COMB FACTORS; AND DATE KILLED FOR SHAM-OPERATED BIRDS

Cock Number	Date Killed Mo/Day	Body wt. When Lesion.	Body wt. When Killed	Thy. Wt. mg	Thy. Wt. mg%	24-Hr I^{131} Uptake	Testis Wt. g.	Testis Wt. g%	Comb Factor
S-1	2/15	2210	2245	146	6.50	3.80	21.3	0.95	62.4
S-2	2/15	2706	2530	232	9.17	6.94	23.9	0.94	52.8
S-3	2/22	2750		183		3.93	28.2		68.9
S-4	2/22	2688		177		11.48	8.6		85.5
S-5	3/1	3000	2651	205	7.73		32.2	1.21	64.0
S-6	3/1	2380	2341	163	6.96		20.6	0.88	62.0
S-7	3/1	2160	1812	81	4.47		16.9	0.93	64.0
S-8	3/8	2462	2265	174	7.68	10.32	29.9	1.32	
S-10	3/8	2338	2174	169	7.77	6.31	31.6	1.45	
S-20	5/6	2238	2106	142	6.74		42.5	2.02	54.4
S-21	5/6	1885	1822	119	6.53		49.4	2.66	33.0
S-22	5/6	1900	1746	92	5.27		27.4	1.57	37.5
S-23	6/10	2080	2728	225	8.25	6.42	2.0	0.07	30.0
S-24	6/10	2624	2188	133	6.08	5.36	51.0	2,33	58.0
S-25	6/10	2174	2320	124	5.34	5.92	11.6	0.50	48.8
S-26	7/5	2800	2590	190	7.34	13.46	14.3	0.55	33.0
S-27	7/5	2308	2222	78	3.51	3.49	11.0	0.50	47.1
S-28	7/12	2744	2794	183	6.55	11.99	24.6	0.88	40.5
S-29	7/12	2270	2314	142	6.11	15.16	11.2	0.48	27.5
S-30	7/12	1702	1810	82	4.53	13.47	9.1	0.50	60.0
S-31	8/2	1838	2005	104	5.19	8.84	3.4	0.17	34.5
S-32	8/2	2096	2074	106	5.11	10.02	5.5	0.27	43.8
S-34	8/2	2095	2150	156	7.26	17.29	4.7	0.22	48.2
S-35	8/2	1880	1856	96	5.17	15.20	13.0	0.70	63.8
S-40	8/25	2072	2072	87	4.20	15.66	7.1	0.34	41.1
S-41	8/25	2388	2166	125	5.77	17.55	6.4	0,30	46.1
S-42	8/25	2262	1530	112	7.32	12.09	2.0	0.13	63.8

BODY, THYROID, AND TESTIS WEIGHTS; 24-HOUR IODINE UPTAKE; COMB FACTOR; AND DATE KILLED FOR NORMAL CONTROL BIRDS

Cock Number	Date Killed Mo/Day	Body wt. When Others Lesion.	Body wt. When Killed	Thy. Wt. mg	Thy. Wt. mg%	24-Hr I^{131} Uptake	Testis Wt. g.	Testis Wt. g%	Comb Factor
C-1	2/15	2940	2765	176	6.37	5.06	30.0	1.08	58.1
C-2	2/15	3080	3060	188	6.14	4.91	20.8	0.68	57.2
C-3	2/15	2720	2540	156	6.14	5.05	24.6	0.97	58.6
C-4	2/22	3020		145		5.58	31.3		45.5
C-5	2/22	2750		140		6.11	15.2		78.4
C-6	2/22	2700		212		3.71	32.6		56.0
C-7	3/1	2670	2365	232	9.81		25.6	1.08	60.0
C-8	3/1	2300	2100				26.7	1.27	
C-9	3/8	2520	2219	116	5.23	8.0	30.7	1.38	
C-10	3/8	2444	2300	203	8.83	9.2	24.5	1.07	
C-11	3/8	2028	2029	155	7.64	8.8	23.8	1.17	
C-20	5/6	2004	1952	149	7.63		38.5	1.97	56.0
C-21	5/6		2020	165	8.16		44.3	2.19	63.8
C-22	5/6	2200	2040	146	7.16		38.0	1.86	45.5
C-23	6/10	2000	2024	120	5.93	6.86	11.0	0.54	54.4
C-24	6/10	2420	2414	106	4.39	5.38	16.6	0.69	49.0
C-25	6/10	1770	1852	116	6.26	5.51	6.9	0.37	42.0
C-26	7/5	2368	2056	130	6.35	8.04	1.0	0.05	21.4
C-27	7/5	2764	2687	148	5.51	7.22	12.6	0.47	70.1
C-28	7/12	2326	2390	193	8.08	16.22	18.0	0.75	80.8
C-29	7/12	2360	2426	195	8.04	19.07	7.1	0.29	47.2
C-30	7/12	1890	1960	171	8.72	20.10	9.4	0.48	45.0

DISTRIBUTION OF RADIOIODINE IN THE THYROID AND THE THYRONINE TO TYROSINE RATIOS OF HYPOTHALAMIC-LESIONED BIRDS 24 HOURS* AFTER A SINGLE INJECTION OF I^{131} AND 10-14 DAYS AFTER LESIONING

Cock Number	% of Total Thyroidal Radioactivity								Thyronine: Tyrosine
	Hydrolyzed Thyroid						Unhydrolyzed Thyroid		
	Origin	DIT	MIT	I	T_4	T_3	Origin	I	
0-6	9.4	42.8	44.8	1.2	1.2	0.8	99.9	0.1	2.28
0-8	2.1	28.4	59.2	4.3	2.4	0.6	99.9	0.1	3.42
0-9	2.9	31.1	60.7	2.3	1.7	0.3	99.3	0.7	2.18
0-12	1.0	45	46	3.0	4.7	0.2	99.1	0.9	5.38
0-14	2.73	42.1	44.8	8.2	1.4	0.8	99.2	0.8	2.53
0-21	1.42	70.8		22.75	5.0		83.4	16.6	7.06
0-24	1.11	53.5	39.9	2.48	3.1		99.1	0.9	3.32
0-25	1.40	52.1	39.5	3.07	3.9		99.1	0.9	4.26
0-30	0.18	33.0	46.0	20.53	0.3		98.7	1.3	0.38
0-34	1.7	45.3	44.1	3.1	4.5	1.3	98.1	1.9	6.49
0-35	1.2	50.6	33.6	3.7	6.7	1.1	98.5	1.5	9.26
0-36	1.9	56.8	25.6	5.6	6.8	1.3	98.8	1.2	9.83
0-37	1.0	30.9	55.7	3.1	6.3	1.4	98.5	1.5	8.89
0-38	3.4	49.3	34.3	3.0	6.4	0.9	98.7	1.3	8.73
0-39	1.2	46.1	29.9	3.2	4.7	8.9	98.3	1.7	17.89
0-40	1.6	42.8	40.7	4.0	6.0	1.2	98.7	1.3	8.62
0-41	1.6	51.1	24.8	3.7	6.4	10.9	99.1	0.9	22.79
0-43	1.0	54.0	32.8	3.8	5.1	3.2	99.5	0.5	9.56
0-44	1.6	67.1	19.4	2.0	6.9	3.1	99.8	0.2	11.56
0-47	1.1	59.6	30.1	2.6	5.5	1.7	99.6	0.4	8.08
0-50	0.8	52.5	33.9	2.4	6.4	0.8	99.6	0.4	8.33
0-52	1.5	39.3	42.7	3.0	12.3	1.2	99.0	1.0	16,46
0-53	1.7	41.0	42.0	5.1	9.6	1.2	98.3	1.7	13.01
0-57	0.9	48.7	37.7	2.5	8.8	1.3	99.2	0.8	11.69
0-59	0.7	38.9	49.4	3.7	6.6	0.8	99.2	0.8	8.38
0-64	2.0	50.2	36.9	2.2	8.1	0.5	99.3	0.7	9.87
0-67	1.6	51.3	38.1	5.3	3.4	0.3	99.4	0.6	4.14
0-69	1.7	36.2	53.5	3.5	4.9	0.2	99.2	0.8	5.68
0-70	1.1	47.3	36.4	5.8	5.5	3.8	98.5	1.5	11.11
0-73	1.1	44.3	38.2	6.3	8.8	1.3	99.2	0.8	12.24
0-76	1.5	45.5	43.3	3.3	5.5	0.9	99.5	0.5	7.21

*Values in the rectangle represent distribution of thyroidal radioactivity three hours after injection of I^{131}.

DISTRIBUTION OF RADIOIODINE IN THE THYROID AND THE THYRONINE TO TYROSINE RATIOS OF SHAM-OPERATED CONTROL BIRDS 24 HOURS* AFTER A SINGLE INJECTION OF I^{131} AND 10-14 DAYS AFTER SHAM LESIONING

Cock Number	% of Total Thyroidal Radioactivity								Thyronine: Tyrosine
	Hydrolyzed Thyroid						Unhydrolyzed Thyroid		
	Origin	DIT	MIT	I	T_4	T_3	Origin	I	
S-3	1.6	43.5	47.5	2.4	0.9	1.6	99.6	0.4	2.78
S-4	6.4	40.1	50.1	1.6	0.6	0.2	99.3	0.7	0.89
S-5	1.2	37	55	4.6	2.5	0	98.8	1.2	2.72
S-6	1.2	22	68	5.2	3.0	0.8	99.0	1.0	4.22
S-7	0.9	51	37	4.3	6.8	0.4	99.2	0.8	8.18
S-8	1.9	47.3	41.4	6.4	2.5	0.6	99.2	0.8	3.49
S-10	3.1	46.8	42.0	5.7	1.8	0.6	99.3	0.7	2.70
S-20	0.71	46.0	49.8	1.9	1.5		98.8	1.2	1.56
S-21	0.70	50.2	43.0	3.0	3.1		98.8	1.2	3.33
S-22	1.25	44.4	45.6	4.8	4.0		98.6	1.4	4.44
S-23	2.0	36.8	43.4	3.9	9.3	1.5	98.7	1.3	13.47
S-24	1.7	41.4	36.9	5.4	11.7	2.0	99.1	0.9	17.52
S-25	1.4	47.0	37.1	4.5	7.7	1.1	98.9	1.1	10.46
S-26	0.9	54.7	33.1	4.6	5.6	1.0	99.6	0.4	7.52
S-27	1.7	57.0	27.9	3.4	7.7	2.2	99.1	0.9	11.66
S-28	1.2	38.4	43.9	7.3	7.1	2.0	99.4	0.6	11.06
S-29	0.8	48.3	34.7	6.5	9.1	1.9	99.1	0.9	13.25
S-30	1.3	45.4	35.5	5.7	11.2	0.9	99.1	0.9	14.96
S-31	1.8	51.9	36.5	2.6	6.4	1.6	99.6	0.4	9.05
S-32	1.7	42.3	40.0	3.3	11.2	1.5	99.7	0.3	15.43
S-34	1.6	56.2	31.6	2.5	7.5	0.7	99.8	0.2	9.34
S-35	1.6	42.9	42.4	2.6	9.4	1.0	99.7	0.3	12.19
S-40	1.7	48.9	37.1	4.5	7.2	0.7	99.8	0.2	9.19
S-41	0.9	47.0	41.8	3.9	5.7	0.6	99.9	0.1	7.07
S-42	0.6	45.9	42.5	2.8	7.3	0.9	99.8	0.2	9.28

*Values in the rectangle represent distribution of thyroidal radioactivity three hours after injection of I^{131}.

DISTRIBUTION OF RADIOIODINE IN THE THYROID AND THE THYRONINE TO TYROSINE RATIOS OF NORMAL CONTROL BIRDS 24 HOURS* AFTER A SINGLE INJECTION OF I^{131} AND 10-14 DAYS AFTER OTHER BIRDS LESIONED

Cock Number	% of Total Thyroidal Radioactivity								Thyronine: Tyrosine
	Hydrolyzed Thyroid						Unhydrolyzed Thyroid		
	Origin	DIT	MIT	I	T_4	T_3	Origin	I	
C-4	4.9	33.8	53.7	3.3	1.2	0.8	99.8	0.2	2.28
C-5	1.6	26.0	66.8	3.7	0.8	0.3	97.8	2.2	1.18
C-6	3.3	52.0	40.0	2.1	0.3	0.3	99.6	0.4	0.65
C-7	1.3	50	41	3.8	3.6	0.5	98.9	1.1	4.50
C-8	0.8	53	37	3.4	5.2	0.5	98.8	1.2	6.33
C-9	1.4	36.4	52.9	6.0	2.7	0.5	99.0	1.0	3.58
C-10	2.5	45.0	44.4	5.7	1.9	0.5	99.2	0.8	2.81
C-11	2.3	51.0	39.4	5.1	1.6	0.5	99.2	0.8	2.32
C-20	1.0	52.3	40.2	4.4	2.0		99.2	0.8	3.03
C-21	1.1	40.9	52.4	3.2	2.3		99.8	0.2	2.68
C-22	1.5	50.8	41.0	4.7	1.9		99.5	0.5	2.61
C-23	1.6	46.6	35.2	3.5	8.4	1.2	99.1	0.9	11.74
C-24	1.7	47.9	29.1	2.9	8.7	4.7	98.8	1.2	17.40
C-25	5.3	46.5	34.4	2.7	6.1	1.2	99.1	0.9	9.02
C-26	1.1	57.7	27.7	2.9	9.3	1.2	99.4	0.6	12.30
C-27	2.3	59.7	26.4	2.2	6.9	2.5	99.6	0.4	10.92
C-28	1.4	47.8	44.3	1.8	3.9	0.7	99.5	0.5	4.99
C-29	1.3	43.2	41.8	3.9	8.5	1.3	99.6	0.4	11.53
C-30	1.0	55.2	36.0	2.3	4.7	0.7	99.3	0.7	5.92

*Values in the rectangle represent distribution of thyroidal radioactivity three hours after the injection of I^{131}.

ANTERIOR PITUITARY WEIGHT, AMOUNT OF ANTERIOR PITUITARY INJECTED INTO EACH ASSAY CHICK, AVERAGE RPR FOR EACH GROUP OF ASSAY CHICKS, TSH IN THE ANTERIOR PITUITARY AND DATE OF TSH ASSAY FOR HYPOTHALAMIC-LESIONED BIRDS

Cock Number	Ant. Pit. Wt. mg	Ant. Pit. Wt. mg%	Ant.Pit. Inj./ Assay Chick mg	RPR	TSH/ mg Ant. Pit. mU	Total TSH/ Ant. Pit. mU	Date Assay Commenced
0-1	5.0	0.20	0.80	55	5.38	26.9	2/15/65
0-2	10.0	0.48	1.60	52	3.00	30.0	2/15/65
0-6	5.0(F)*		0.77	58	3.50	17.5	2/22/65
0-8	12.0		1.85	61	1.19	14.3	2/22/65
0-9	3.0(F)		0.46	64	3.91	11.7	2/22/65
0-12	10.4	0.18	1.16	63	2.24	23.3	3/1/65
0-14	8.8(F)		1.11	46	6.58	58.2	3/8/65
0-21							
0-24	12.2	0.66	1.63	30	18.40	225.4	12/6/65
0-25	10.2	0.50	1.37	45	5.84	59.9	12/6/65
0-30	9.4	0.75	1.26	37	12.70	129.5	12/6/65
0-34	7.5	0.30	1.00	41	11.40	85.5	12/13/65
0-35	6.6	0.28	0.88	80	1.14	7.5	12/13/65
0-36	5.5(F)		0.73	52	7.95	43.7	12/13/65
0-37	6.4	0.35	0.86	67	2.67	17.2	12/13/65
0-38	3.8(F)		0.51	69	3.92	14.9	12/13/65
0-39	5.6	0.23	0.74	74	2.03	11.3	12/13/65
0-40	7.4	0.37	0.99	68	2.22	16.5	12/13/65
0-41	7.0	0.29	0.93	52	6.23	43.3	12/13/65
0-43	3.2(F)		0.47	58	9.78	34.2	12/27/65
0-44	12.6	0.55	1.68	61	2.26	28.5	12/27/65
0-47	9.6	0.45	1.29	66	2.25	21.6	12/27/65
0-50	11.8	0.65	1.58	65	2.03	24.1	12/27/65
0-52	10.4	0.54	1.39	66	2.01	21.0	1/3/66
0-53	4.0(F)		0.53	72	3.58	14.1	1/3/66
0-57	10.0	0.51	1.33	56	4.21	42.1	1/3/66
0-59	5.4	0.25	0.71	72	2.68	36.9	1/3/66
0-64	4.7	0.24					
0-67	8.8	0.45	1.18	76	1.61	14.25	1/17/66
0-69	4.6	0.39	0.61	69	5.25	24.2	1/17/66
0-70	7.6(F)		1.0	55	9.40	71.5	1/17/66
0-73	11.5	0.54	1.5	45	13.05	150.0	1/17/66
0-76	13.3	0.69	1.8	65	2.42	32.2	1/17/66

*(F) anterior pituitary fragmented while being removed.

ANTERIOR PITUITARY WEIGHT, AMOUNT OF ANTERIOR PITUITARY INJECTED INTO EACH ASSAY CHICK, AVERAGE RPR FOR EACH GROUP OF ASSAY CHICKS, TSH IN THE ANTERIOR PITUITARY AND DATE OF TSH ASSAY FOR SHAM-OPERATED CONTROLS

Cock Number	Ant. Pit. Wt. mg	Ant. Pit. Wt. mg%	Ant.Pit. Inj./ Assay Chick mg	RPR	TSH/ mg Ant. Pit. mU	Total TSH/ Ant. Pit. mU	Date Assay Commenced
S-1	9.0	0.40	1.44	62	2.08	18.7	2/15/65
S-2	6.5	0.26	1.04	80	1.25	18.1	2/15/65
S-3							illegible
S-4	10.0		1.54	53	2.33	23.3	2/22/65
S-5	16.7	0.63	1.86	49	2.58	43.1	3/1/65
S-6	11.4	0.49	1.27	37	6.30	71.8	3/1/65
S-7	6.9	0.38	0.77	81	1.95	13.5	3/1/65
S-8	13.4	0.59	1.68	51	3.57	47.8	3/8/65
S-10	13.4	0.62	1.67	40	5.62	75.0	3/8/65
S-20	12.6	0.60	1.68	50	3.33	39.7	12/6/65
S-21	7.8	0.43	1.03	58	2.72	19.5	12/6/65
S-22	11.4	0.65	1.52	48	4.21	47.3	12/6/65
S-23	10.2	0.37	1.36	53	3.97	40.5	12/13/65
S-24	10.4	0.48	1.39	44	6.83	71.0	12/13/65
S-25	8.6	0.37	1.14	80	0.88	7.5	12/13/65
S-26	11.6	0.45	1.55	54	3.61	42.1	12/27/65
S-27	8.8	0.40	1.17	67	2.31	20.3	12/27/65
S-28	14.0	0.50	1.87	75	0.80	11.2	1/3/66
S-29	6.2	0.27	0.83	69	2.77	17.2	1/3/66
S-30	11.0	0.61	1.47	58	3.26	35.9	1/3/66
S-31	5.0	0.25	0.67	64	7.01	35.2	1/17/66
S-32	6.0	0.29	0.80	70	3.73	22.4	1/17/66
S-34	9.2	0.43	1.23	72	2.11	19.4	1/17/66
S-35	6.5	0.35	0.87	55	10.92	71.0	1/17/66
S-40	10.5	0.51	1.40	52	8.58	90.1	1/17/66
S-41	6.2	0.29	0.83	64	5.67	35.2	1/17/66
S-42	4.8	0.31	0.64	60	10.20	49.0	1/17/66

ANTERIOR PITUITARY WEIGHT, AMOUNT OF ANTERIOR PITUITARY INJECTED INTO EACH ASSAY CHICK, AVERAGE RPR FOR EACH GROUP OF ASSAY CHICKS, TSH IN THE ANTERIOR PITUITARY AND DATE OF TSH ASSAY FOR NORMAL CONTROL BIRDS

Cock Number	Ant. Pit. Wt. mg	Ant. Pit. Wt. mg%	Ant.Pit. Inj./ Assay Chick mg	RPR	TSH/ mg Ant. Pit. mU	Total TSH/ Ant. Pit. mU	Date Assay Commenced
C-1	8.5	0.31	1.36	83	0.81	6.9	2/15/65
C-2	10.0	0.33	1.70	81	0.75	7.5	2/15/65
C-3	11.0	0.43	1.76	63	1.65	18.2	2/15/65
C-4	12.8		1.97	58	1.37	17.5	2/22/65
C-5	10.0		1.54	44	3.90	39.0	2/22/65
C-6	12.8		1.97	51	2.03	26.0	2/22/65
C-7	8.8	0.37	0.98	75	1.63	14.3	3/1/65
C-8	5.2	0.25	0.58	71	3.28	17.1	3/1/65
C-9	14.8	0.67	1.86	53	2.95	43.8	3/8/65
C-10	13.7	0.60	1.71	65	1.99	27.3	3/8/65
C-11	11.8	0.58	1.48	67	2.09	24.8	3/8/65
C-20	11.6	0.59	1.55	35	12.90	142.2	12/6/65
C-21	12.7	0.63	1.69	37	9.47	120.3	12/6/65
C-22	10.4	0.51	1.39	47	5.04	50.9	12/6/65
C-23	6.4	0.32	0.86	80	1.16	7.5	12/13/65
C-24	11.3	0.47	1.51	70	1.26	14.2	12/13/65
C-25	12.0	0.65	1.60	71	1.12	13.4	12/13/65
C-26	11.6	0.56	1.54	49	4.87	56.5	12/27/65
C-27	12.0	0.45	1.60	72	1.31	15.7	12/27/65
C-28	9.9	0.41	1.32	50	6.36	63.0	1/3/66
C-29	7.4	0.30	0.98	71	2.04	15.0	1/3/66
C-30	6.8	0.35	0.91	77	1.43	9.7	1/3/66

THE RPR's FOR TSH STANDARD INJECTED 24 HOURS PREVIOUSLY

Date Assay Commenced Mo/da/yr.	Slope of Curve b*=	Dose of TSH Injected (mU) 0	1	2	4	8	16	32
2/15/65	-15.6	85		72	62	34	26	
2/22/65	-11.0		74	73	50	40	29	
3/1/65	-15.6		74	82	57	30	22	
3/8/65	-17.3		89	93	53	46	26	
12/6/65	- 7.9	50	56	59	56	44	35	30
12/13/65	-11.0	61	71	68	59	48	35	
12/27/65	-12.0		64	73	60	48	59	
1/3/66	-10.2	67	68	72	56	56	38	
1/17/66	- 9.3	72	70	73	70	58	46	46

*Sample regression coefficient.

VITA

Jerry Glenwood Hurst

Candidate for the Degree of

Doctor of Philosophy

Thesis: ROLE OF THE HYPOTHALAMUS IN THE CONTROL OF THYROTROPIN PRODUCTION BY THE ANTERIOR PITUITARY OF CHICKENS.

Major Field: Physiology

Biographical Sketch:

Personal Data: Born December 8, 1932, at Belleview, Missouri, the son of Oscar and Ethel P. Hurst.

Education: Attended elementary school at Belleview, Missouri, and high schools at Caledonia, Missouri, Alton, Illinois, Ironton, Missouri, and Ft. Worth, Texas, and graduated from Robert Lee Paschal High School, Ft. Worth, Texas, in 1950; attended Texas Wesleyan College for one year; received the Bachelor of Arts degree from Hardin-Simmons University, in 1954, with a major in biology; completed the requirements for the degree of Master of Science at Oklahoma State University in May, 1963.

Professional Experience: Employed as a laboratory instructor in Zoology at Hardin-Simmons University during 1953 and 1954; taught biology, chemistry and general science at Buckner Academy, Dallas, Texas, from 1954 to 1956; served as a Commissioned Officer in the United States Navy from 1956 to 1959; taught junior high school in Pampa, Texas, during the Academic year 1959-1960; served as graduate assistant from 1961 to 1962 and appointed Assistant Professor in 1964, in the Department of Physiology and Pharmacology, of the Oklahoma State University, Stillwater, Oklahoma.