THE HISTOLOGY OF THE ANTERIOR PITUITARY, THYROID AND ADRENAL OF THYROID-STIMULATED PUREBRED ENGLISH BULLDOGS

Thesis for the Degree of M. S.

MICHIGAN STATE COLLEGE

Esther M. Smith

1951

THESIS

This is to certify that the

thesis entitled

The Histology of the Anterior Pituitary, Thyroid and Adrenal of Thyroid-stimulated Purebred English Bulldogs

presented by

Esther M. Smith

has been accepted towards fulfillment of the requirements for

M.S. degree in Anatomy

Lois Calhoun
Major professor

Date May 21, 1951

O-169

ıS

This is to certify that the

thesis entitled

The Histology of the Anterior Pituitary, Thyroid and Adrenal of Thyroid-stimulated Purebred English Bulldogs

presented by

Esther M. Smith

has been accepted towards fulfillment of the requirements for

M.S. degree in Anatomy

Lois Calhoun
Major professor

Date May 21, 1951

O-169

THE HISTOLOGY OF THE ANTERIOR PITUITARY, THYROID AND ADRENAL OF THYROID-STIMULATED PUREBRED ENGLISH BULLDOGS

By

Esther M. Smith

A Thesis

Submitted to the School of Graduate Studies of
Michigan State College of Agriculture and
Applied Science in partial fulfillment
of the requirements for the degree
of

MASTER OF SCIENCE

Department of Anatomy

1951

THESIS

TABLE OF CONTENTS

ACKNOWLEDGMENTS

The author wishes to express her sincere appreciation to Dr. M. Lois Calhoun under whose inspirational guidance and unfailing confidence, this work was accomplished. Also to acknowledge indebtedness to Dr. Paul Reineke of the Department of Physiology for making these animals available to the author, and for the many hours of consultation.

Many thanks are extended to Dr. Pauline Paul, Department of Home Economics for her help with the statistical analysis of the data; to Dr. Charles Titkemeyer who did the post mortems on these animals; to Dr. Frank Thorp, Jr. and Dr. Frederick Smithcors for their helpful suggestions and assistance.

The writer wishes to thank Dean Armstrong, Department of Conservation, for the excellent drafting job on the charts which made subsequent photography of them a relatively simple matter.

INTRODUCTION

This problem was undertaken as a continuation of a study which investigated the effect of exogenous thyroid upon the growth rate of purebred English bulldog puppies (Borgman and Reineke 1949).

Since histologists seldom have an opportunity to study tissues obtained from purebred animals, and inasmuch as the microscopic anatomy of the endocrine organs varies with the different breeds of dogs, it is hoped that this work will be of value as a foundation for further study. According to Stockard (1941), no other mammalian species differs so widely in structural type as do the breeds of domestic dogs. The internal organs and functional reactions among different breeds show wide deviations from the known mongrel dog pattern. Gilmore (1940) reported that individual differences occurred within a group of several breeds of dogs and because of these diversities in glandular structure, the genetic make up of the dog played a role in the histology of the thyroid.

The information gathered from the control group should add to the literature on the normal histology of the dog.

REVIEW OF LITERATURE

Thyroid

It was some two thousand years ago that the thyroid gland was discovered to be a distinct anatomical entity. According to Hoskins (1941) the Greeks regarded it as a protective device to keep the throat warm while others thought it was used to round out the neck so as to make a beautiful contour. Hoskins further stated that the thyroid was discovered by Vesalius in 1545, but it was not until nearly a century later that Wharton gave it a name. The term is derived from a Greek word meaning "Shield-shaped".

It was as late as 1858 before any basic research was done on the thyroid. At that time, Schiff of Geneva began research on its function by removing the gland from experimental animals (Hoskins 1941). Another twenty years passed before clinicians began to notice symptoms of thyroid insufficiency in human patients.

Goiters were treated by radical operations after antiseptic surgery was introduced. The oral administration of thyroid first began in the latter part of the nineteenth century.

In 1659 Thomas Wharton first accurately described the development of the thyroid gland (Goldsmith 1949). He stated that the thyroid arises as a downgrowth of epithelium from the ventral wall of the pharynx between the first and second pharyngeal pouches. With the forward growth of the pharynx, the gland primordium appears to grow caudally, dividing into a right and left lobe. It remains attached to the pharynx by a stalk or neck, the thyroglossal duct. This thyroglossal duct connects the young thyroid with the posterior border of the developing tongue. The thyroglossal duct atrophies, but the site of origin remains as the foramen caecum in post uterine life.

According to Gudernatsch (1949), all glands now "ductless" once had an open secretory duct. The thyroid at one stage existed as a "duct gland", probably as a gland auxillary to metabolic function even after losing direct connection with the digestive tract.

The adult thyroid is composed of small sacs or follicles. These are considered to be the secretory units of the gland (Means 1948). Uhlenhuth (1923) reported that the cells of the follicles produced the hormone which was stored in the lumen. He considered the function of the thyroid from two different aspects; a colloid storage phase and a colloid release phase.

Bensley (1916) stated that the colloidal secretion was formed in the outer pole of the cell and excreted from it directly without passing by the indirect route through the follicular cavity. The indirect mode of secretion consisted of the condensation of the colloid into the form of droplets having a high content of solids.

Williams (1944) studied living thyroid cells and follicles and observed that each cell in a follicle had three surface regions; one in contact with the colloid, one in contact with or close to blood vessels and connective tissue, and one in contact with adjacent cells. Williams concluded that when hypertrophy or hyperplasia occurred, with loss of typical follicular structure, the colloid was found between the cells. As a result of this, a total or partial destruction of the cells occurred. The connective tissue and blood vessels grew in from the surface of the follicle until the necessary conditions of normal contact stated above were established. The factors which regulated follicular structure in the gland were as follows: (1) There was a lethal sensitivity of the thyroid cells to colloid in contact with any surfaces other that apical. (2) The thyroid cells must have their basal surfaces on or very near blood vessels. (3) The thyroid cells were supported by a connective tissue investment. (4) There was polarization of the cells with secretion toward the lumen. No evidence was obtained that there was secretion at the base of the cells. Williams concluded that colloid is released from the follicle solely by diffusion across the follicular wall.

According to Dvoskin (1948), Verson, using fresh tissue, was the first to describe the presence of colloid droplets within the thyroid epithelium. Recently a relationship has been established between the formation of intracellular colloid droplets and the thyrotrophic hormone.

DeRobertis (1941) demonstrated intracellular colloid particles in the thyroids of rats. Using the freezing-drying method of fixation, DeRobertis expressed the opinion that the intracellular colloid was distributed in the following forms: (1) In secretion droplets at the apical zone; (2) in large droplets lying in rows between the poles of the cell; (3) in pale-staining base vacuoles; (4) completely loading the cytoplasm, generally accompanied by pyknosis of the nucleus and desquamation into the lumen. DeRobertis postulated that the thyroid cell was able to secrete directly toward the basal pole, particularly when the gland was greatly activated in its secretory function. Upon analysing the activation of the cells of the thyroid, DeRobertis suggested that the thyroid cells produced an apocrine secretion, because the cytoplasm bulged into the lumen and was released by the rupture of the pedicle. After this phase, the cells stopped secretion toward the lumen, and secretion toward the base began, reabsorbing the colloid stored in the lumen.

Ultracentrifugation has shown that thyroglobulin has a molecular volume of 675,000 and therefore is a macromolecule and unable to pass through cell membranes (DeRobertis 1949).

DeRobertis (1949) studied the problem of the relation of colloid release to the proteolytic activity of this material. By extracting proteases by microdissection from single follicles, DeRobertis proved that the follicular colloid was digested by enzymatic action and the products were absorbed by the cells. It was also found that thyroid stimulating hormone increased the proteolytic activity, while iodine administered for a long period of time decreased it. DeRobertis (1949) postulated that the release of the secretion stored in the follicles takes place only after hydrolysis of the thyroglobulin inside the follicular cavity.

DeRobertis (1949) reported that the amount and disposition of the intracellular colloid varied with species, age, season, function and pathological state. In the case of hyperactive glands, all of the colloid particles of a cell were at the base, and the cell showed a complete inversion of polarity. It was further stated that this quantity and disposition of intracellular colloid was a sensitive index of the functional activity of the gland, and that these changes reflected the amount of circulating hormone. Therefore, DeRobertis expressed the opinion that this could be used as a means of thyrotropic hormone assay.

Dvoskin (1947a) tested different common fixatives for their ability to preserve the intracellular colloid droplets. He found that Carnoy's fluid resulted in the best histological fixation, but found variation in staining reaction following that fixative. Some non-staining vacuoles were seen in the stimulated glands, but whether these droplets represented colloid particles, as DeRobertis maintained, or whether they represented artifacts could only be ascertained by a comparison with the freezing-drying method described by DeRobertis (1941).

Dvoskin (1947b) experimented with the thyroids taken from two- to five-day old cockerels. The glands were incubated in vitro in a variety of media at different temperatures for various periods of time and under numerous atmospheric conditions. Chemical agents added to the media included I_2, NaI, thyroxine, thiouracil, thyrotrophic hormones, NaCN, KSCN and $KMNO_4$. Dvoskin found that under most conditions numerous large droplets of intracellular colloid formed in the absence of added thyrotrophic hormone. This droplet formation occurred almost entirely in the center of the gland.

The intrafollicular colloid of thyroids treated with common fixatives and stained with Mallory-Azan present a variety of colors, including blue, red or orange (DeRobertis 1941). These variations have been said to be due to iodine content, concentration of colloid, or fixation methods used. According to DeRobertis, the freezing-drying method always produced blue colloid throughout the sections stained with Mallory-Azan. It was concluded that these variations found in technics other than freezing-drying, were due to the method of fixation. With customary fixatives, clear vacuoles appeared, but in the freezing-drying method these did not occur. LeBlond (1949) stated that vacuoles and the blue-red-orange staining reaction of the colloid were artifacts, but had a definite meaning. Vacuoles were most numerous and colloid was predominantly basophilic in activated or stimulated glands, while in resting organs the colloid was acidophilic. LeBlond postulated that vacuoles and basophilia in the colloid corresponded to properties of colloid usually found in activated glands.

Nonidez (1932) reported the parafollicular cells to be a second epithelial component of the thyroid gland of the dog. These parafollicular cells were described by Nonidez as large epithelial cells with argyrophile granules. The cells lie in the interstitial spaces in close proximity to the follicular epithelium from which they arose. They are called parafollicular cells because the term does not suggest any functional interpretation. The argyrophile granules of the parafollicular cells differ from those contained in the cytoplasm of the follicular cells in that they are more numerous and have a greater affinity for silver nitrate.

It has been established that the thyroid status can be completely controlled by oral administration of synthetic materials. Varying degrees of hyperthyroidism can be produced by feeding iodinated casein, and a hypothyroid condition can be brought about with thiourea, thiouracil and their derivatives (Blaxter 1949).

According to Cortell (1944), working with guinea pigs, the effect of exogenous thyroxine upon the thyroid gland of the intact animal was to put the gland in a resting state. This was characterized by decrease in total size, diminution in height of thyroid epithelium and an accumulation of colloid.

Astwood (1949) reported evidence for direct effect of thyroxine in inhibiting the action of thyrotrophin upon the thyroid gland. Thyroxine decreased the effect of injected thyrotrophin in hypophysectomized rats. If extra thyroid hormone was administered, there was a compensatory atrophy of the thyroid gland.

Anterior Pituitary

The pituitary gland or hypophysis has been known since the time of Galen (200 A.D.). Vesalius named it through an erroneous theory of its function. It was thought that this gland secreted a fluid to lubricate the throat via minute channels coursing through the porous cribriform plate of the ethmoid bone. Later it came to be regarded as a vestige.

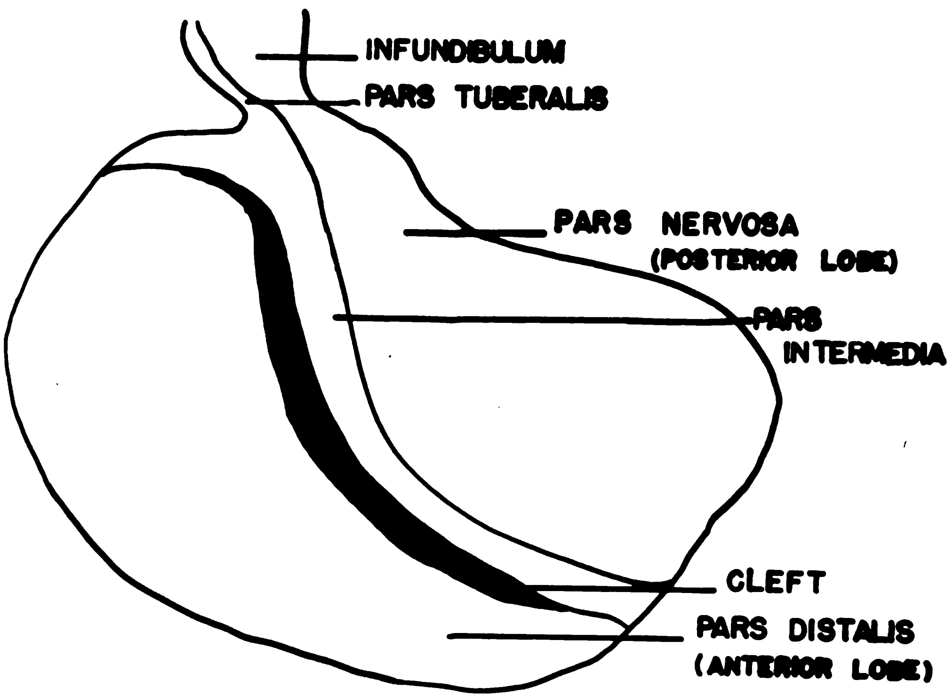
Gigantism was first associated with pituitary involvement during the middle of the 19th century, but it wasn't until 1886, that the French neurologist Pierre Marie finally determined the relationship of the pituitary to the disease. Since that time modern medicine has made great advances in correlating the relationship of the pituitary with the other endocrine organs and their respective dysfunctions (Hoskins 1941).

The first suggestion of the pituitary in the embryo is an outgrowth of Rathke's pouch which gradually extends toward the brain (Arey 1947). As the embryo grows, this pouch makes contact with a downgrowth from the brain, the infundibulum. Rathke's pouch ultimately gives rise to the anterior lobe or pars distalis, while the infundiblar part gives rise to the posterior lobe or pars nervosa. The pars intermedia is formed as the dorsal wall of Rathke's pouch surrounds the infundibulum. The pars nervosa is intimately coated by the intermedia and is easily detached from the pars distalis. The pars tuberalis does not completely surround the short infundibular stalk and the upper border of the pars distalis extends above the mid portion of the pars nervosa (Plate I). According to Stockard (1941), a common feature of the English bulldog is the persistence of a membranous connection between the anterior lobe and a depression in the basisphenoid bone and in rarer cases a complete foramen is present permitting the direct continuation between the glandular pituitary, Rathke's pouch and the oral epithelium. The gland lies in a recess in the sphenoid bone known as the sella turcica.

The anterior lobe consists of solid cords or nests of cells separated by capillary or sinusoidal blood vessels. The cells of the anterior hypophysis are usually classified in three groups; chromophobes, basophils, and acidophils. The quantitative value for each of the three types has been worked out for different species. The values vary with dysfunctions of the pituitary as well as other endocrine organs.

The morphology of the pituitary of the English bulldog differs markedly from that of breeds of long muzzled dogs (Stockard 1941). He reported the pars nervosa to be thickened, with its dorsal surfaces covered by a thin layer of the hypophyseal sac forming a double layer of the pars intermedia.

Plate I



DIAGRAMMATIC DRAWING OF HYPOPHYSIS CEREBRI

It has been found that the infundibular portions and the pars intermedia are larger in the human male than in the female, even though the pituitary as a whole is larger in the female, because of the enlarged pars distalis. Stockard (1941) found the pituitary of the English bulldog to have a small pars distalis and a very large pars intermedia and pars nervosa. On the basis of the human findings he classified the pituitary of the English bulldog as strongly of the male type. Stockard (1941), postulated that the masculine proportions of the bulldog pituitary may account for the fact that the female bulldog produces only small litters and displays defective maternal instincts. He further warned, however, that there was also frequent histopathology in the tuberalis, as well as low basophil counts, which might account for such masculine tendencies.

Stockard (1941) reported that the English bulldog pituitaries contained an abnormally small amount of secretory epithelium in the pars tuberalis and pars distalis. The acidophils were bright-staining and in the high proportion of thirty acidophils to one basophil. Stockard (1941) described the basophils as being frequently vacuolated, and in the form of the "signet ring" type of castrate cells.

In a study of the cyclic histological changes in the anterior pituitary of the female dog, Wolfe _et al_. (1933) described four cell types and designated them with Roman numerals. Type I cells were the acidophils or alpha cells, type II and type III were both of the basophilic type, but exhibited certain separate characteristics and the last group, type IV, were the chromophobes. The cells of type I were highest during anestrus, type II and III reached their peak at estrus and type IV were most numerous during the late lutein phase.

In differentiating the four cell types according to the erythrosin-Orange G-aniline blue method of Cleveland and Wolfe (1932), Wolfe *et al.* (1933) reported that the granules of type I cells stained sharply with Orange-G and the nucleus was light blue; the granules of type II cells stained red-orange. Type III cells were divided into the granular and non-granular. The granular cells appeared as dark blue cells filled with diffuse granules usually on the periphery of the cytoplasm. These cells were classified as type III because of these small clumps of granules and also due to the intact cell membrane and cytoplasm. Type IV cells were those which corresponded to the chromophobes. These cells arose as a result of the loss of granules and subsequent regression of the cells with granules. Wolfe *et al.* (1933) stated that all granular cells pass into cells of this type.

In 1938 Dawson and Friedgood discovered a fourth cell type in the anterior pituitary of female rabbits and cats with special affinity for azocarmine. These findings were followed by investigators finding similar cells in the anterior lobes of other species. They have been reported in the anterior hypophysis of the opossum, ferret, and monkey by Dawson and in the armadillo by Oldham (Hartmann *et al.* 1946).

Hartmann *et al.* (1946) confirmed the existence of a fourth cell type in the dog as described by Wolfe *et al.* in 1933. Hartmann *et al.* (1946) studied the relation of this cell type to the usual acidophils and basophils. They concluded that this fourth cell type, which they called the erythrosin cell because the granules stained selectively with erythrosin, cannot be viewed as a mere variant of another chromophilic type.

Griesbach and Purves (1945) using rats as their experimental animals, induced extreme thyroxine deficiency and found complete degranulation of acidophils with an increase in basophils. When a low dose of thyroxine was administered (2.0 ug/100g/day), basophil numbers decreased, revealing evidence of activation. When this dose was increased to 2.25 ug/100g/day the basophils were reduced in size and the granules stained purple. They concluded that the thyroxine requirement of a rat was equivalent to 2.25 ug/100g/day. The thyroxine requirement as it effects the pituitary function is the amount of thyroxine equivalent to the normal daily thyroid secretion in an animal receiving adequate amounts of iodine. Any slight reduction in normal thyroxine level causes an increase in numbers and cytological activity of the basophil cells of the pituitary, (Griesbach and Purves 1945). These same authors concluded that this activation of the basophilic cells is correlated with increased secretion of thyrotropin and therefore these basophil cells are a source of thyrotropic secretion.

Adrenal

The existence of the adrenal glands was first known to science in 1563 when they were described by Eustachius. It wasn't until 1855 that the functions of the adrenal glands were first described by Thomas Addison. This was followed by animal experiments conducted by Brown-Sequard in which he removed the adrenals from different animals and recorded the subsequent effects (Hoskins 1941).

In 1893 two British workers prepared extracts for use in treating patients and observed the effect upon the tone of the heart and arteries. This was the beginning of the work which led to the production of epinephrine or adrenalin.

According to Hoskins it was in 1905 that Elliot made some basic observation on the effects of the extract of adrenal medulla which later proved to be the basis for Cannon's "emergency theory".

The adrenal consists of two parts: a central portion, the medulla, and a surrounding outer portion, the cortex. According to Hoskins (1941), the two parts are of different embryological origin.

The cortex is derived from early mesoderm in the anterior part of the embryonic body cavity. The interrenal buds are the first suggestion of the future cortex. These cells coalesce into a larger structure.

The medulla arises from groups of cells of the abdominal plexus of ectodermal origin. These cells, called pheochromoblasts, invade the primitive interrenal structure, and take a position in the central part of the organ (Hoskins 1941).

The thyroid-pituitary-adrenal relationship is shown on plate II. It is clearly pictured that, in general, thyroxine stimulates the adrenal cortex.

Preston (1928), using mice, studied the effect of thyroxine on the adrenals. She reported the size of the cortex to be twice the normal size with the reticular zone showing greatest hypertrophy. Marked hyperemia was also reported by this author.

Herring (1917) administered small quantities of ox thyroid to female white rats and found an increase in the size and weight of the suprarenals. He stated that both the cortex and the medulla were enlarged but that the hypertrophy in the cortex was greater than in the medulla.

Deanesly (1931) submitted several mice to varying doses of thyroxine. She reported all adrenals to be increased in size fifty per cent with the cortex showing most of the enlargement. It was found that the fat disappeared first from the fascicular zone and had a tendency to accumulate around the medulla.

In 1938 Schmidt and Schmidt studied the variation in the structure of the adrenals produced by thyroxine and at various environmental temperatures. They found a large increase in the number of mitotic figures with the greatest amount in the fascicular zone in the animals kept at 20C.

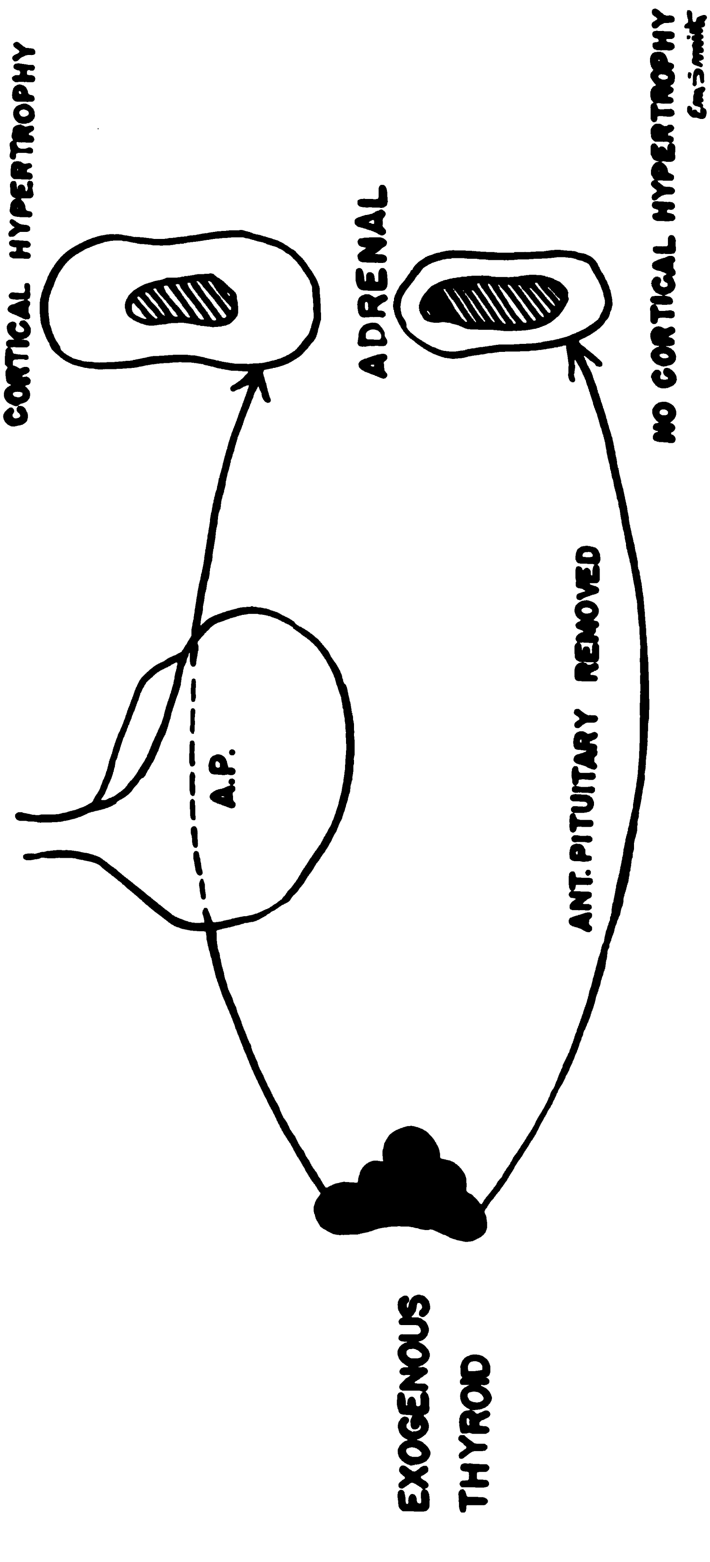
In an effort to find a method for estimating the increase in the adrenocortical secretion rate in hyperthyroid rats, Wallach and Reineke (1949) attempted to prevent the response of the adrenals to thyroxine by the simultaneous administration of adrenocortical hormone. The dosage necessary to accomplish this would be some indication of the increase in the secretion rate of the adrenals. They regarded this hypersecretion of the adrenals as strong evidence for an increased requirement for adrenocortical hormone. Because the adrenals were enlarged after thyroxine administration, more adrenocorticotrophic hormone was secreted (Wallach and Reineke 1949).

Hartman and Brownell (1949) reported that any increase in the thyroid hormone produced adrenal hypertrophy. They stated that there was cell increase in both number and size in all zones. The reticular zone sometimes doubled, and if removed regenerated. These same authors found over three times as many mitoses in thyroxin-treated mice. These mitotic figures occurred mostly in the fascicular zone.

The evidence of the precise relationship of the thyroid to either part of the adrenal is not complete. Hartman and Brownell (1949) stated that the thyroid was essential for the full effect of adrenocorticotrophic hormone since the influence the pituitary exerts on the adrenal was greatly diminished if the thyroid had been removed. (Plate III) Means (1948) claimed that there were indications of some sort of antagonism between the hormone of the adrenal cortex and the thyroid. Thorm, according to Means (1948), reported that thyrotoxic rats given adrenal-cortical extract had less body weight loss and less increase in cardiac weight.

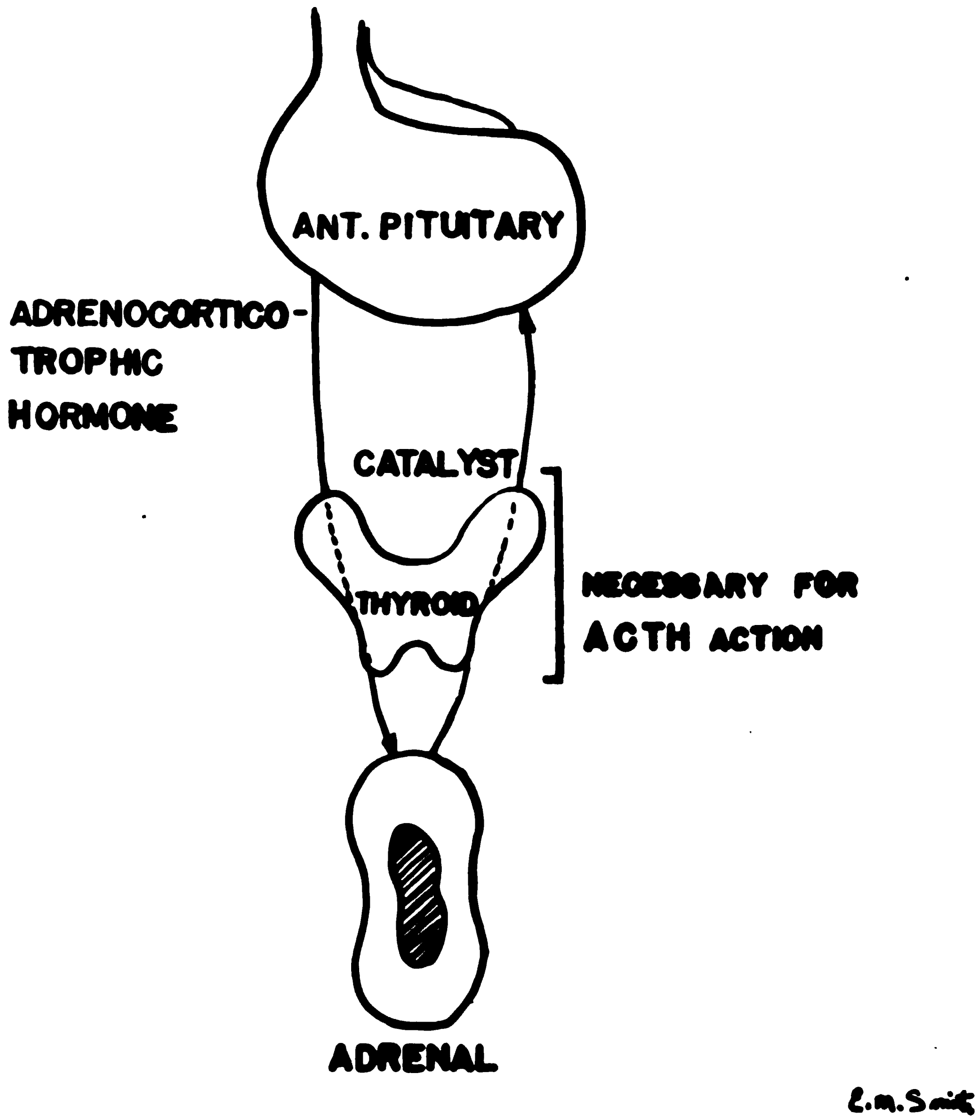
Hartman and Brownell (1949) stated that thyroxine acted through the pituitary since it was not effective after hypophysectomy. This phenomenon is not entirely understood or completely explained and has left many questions unanswered.

Plate II



PITUITARY NEEDED FOR ADRENAL HYPERTROPHY IN HYPERTHYROIDISM

Plate III



THYROID ACTS AS A CATALYST IN ACTH · ACTION

MATERIALS AND METHODS

The initial work of Borgman and Reineke (1949) was done in the Michigan State College Department of Physiology, as a part of the work for a Master's degree. A portion of the experimental detail is reviewed here as background material.

Seven English bulldog puppies were used in this experiment. There were four dogs in the control group: 1, 3, 5 and 6. The protamone group consisted of three dogs: 2, 4 and 7. Dogs 1, 2, 3 and 4 were males and 5, 6 and 7 were females. Animals 1 and 2 were sixty-four-day-old littermates, weaned fifteen days prior to the beginning of the experiment. Dogs 3, 4, 5, 6 and 7 were fifty-three-day old littermates, weaned two days previous to the initiation of the trial. Dog 3, placed on the same bitch as 1 and 2, received more nourishment than his littermates and grew faster. All animals were in good condition at the beginning of the experiment. Their ancestry indicated that they were a small type English bulldog.

The diet consisted of condensed milk, canned dog food, *"Pard" and dry meal, **"Gropup". The exogenous thyroid used for this investigation was protamone, the commercial name for the product supplied by Cerophyl Laboratories. It is a synthetically iodinated

---

*"Pard" - Swift and Company, Chicago, Illinois
**"Gropup" - Kellogg Company, Battle Creek, Michigan

casein. For the dogs in the experimental group, 4 gm. of Protamone were mixed to every 100 lbs. of dry feed. The three experimental dogs consumed a total of 30.269 lbs. of dry matter and 21.345 gm. of protamone. The trial lasted approximately 5 months.

On the 48th day of the trial, radioactivity studies were done. They indicated a lessened pickup of iodine by the thyroid glands of the protamone group. This was explained by the suppression of thyroid function by the exogenous thyroid.

For further details of the feeding experiments, the original paper by Borgman and Reineke (1949) may be consulted.

After the experimental period ended, the dogs were kept on a normal diet for approximately a month. They were sent to the Department of Anatomy for autopsy in July at the age of eight months. The dogs were anesthetized with sodium pentathol and bled out from the internal carotid artery. The thyroids, anterior pituitary and adrenals were secured from each dog.

The tissues were fixed in Bouin's fluid and dehydrated and infiltrated according to the butyl alcohol-paraffin mush method, Johnson **et al.** (1943).

The blocks were sectioned at 7 u and representative slides for each organ stained with hematoxylin-eosin. Other stains were used on certain sections, the stain depending on the structure under study. These stains will be discussed under the individual organs.

RESULTS AND DISCUSSION

Thyroid

Plate IV depicts the effect of protamone on the cytology of the thyroid. There is a general accumulation of colloid and decrease in the epithelial height. The thyroid-stimulating hormone which is produced by the anterior pituitary is inhibited by the protamone and subsequently the output of the thyroid hormone is decreased.

Each section of thyroid was stained by the Mallory-Azan technic as described by DeRobertis (1941). The epithelial height was measured by means of an ocular micrometer. Twenty-five follicles in each of the seven thyroid sections were measured and the average values computed. The mean epithelial height in follicles of the three experimental dogs was 6.50 u. The mean epithelial height of the four control animals was 11.90 u. This constituted a 54.53% decrease in cell height as a result of the protamone given. (Plate V)

The data were submitted to a statistical analysis of variance. The analysis showed a very highly significant variance due to the treatment. The dosage of protamone produced prominent histological changes in the thyroid epithelium.

There was a significant difference between the animals, indicating a variation in epithelial height between the individual dogs irrespective of dosage. However, the difference in epithelial height between the control and the experimental groups was so large as to overshadow the differences between animals. (Plate V)

The accumulation of colloid in the follicles is usually associated with the response of thyroid to thyroxine. All the follicles were well filled with colloid; however, no marked accumulation was noted. (Plates VII and VIII)

Vacuoles were present in thyroids from both groups but were more numerous in the control group. Intracellular colloid was not identified for certain in any of the sections.

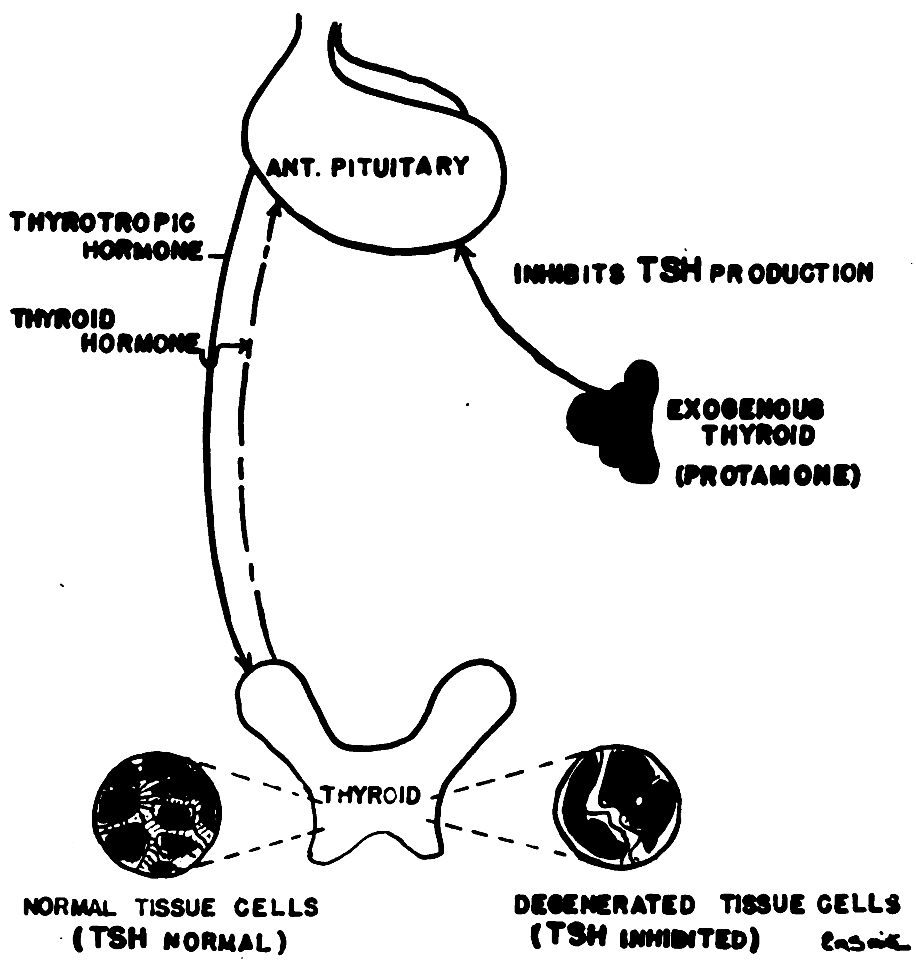
Parafollicular cells common in the thyroids of most dogs were not seen. This supports the opinion of Stockard (1941), that these cells are practically absent from English bulldog thyroids.

The physiology of the English bulldog disputes the idea of thyroid hyperactivity. The basal metabolic rate is low and the animal is usually inclined to be slow, inactive and fat, although a comparatively small eater (Stockard 1941).

Stockard (1941) reported that the thyroid of the English bulldog contained an excessive amount of extra-follicular material consisting of epithelial cells not properly incorporated into the follicles. The author did not find this to be true. The follicles were well formed with distinct connective tissue bands separating them. This difference might have been due to seasonal or climatic differences. These animals were destroyed during summer months; however, the temperature was not exceedingly high. It may be of some interest to mention that they were housed in a brick building and not submitted to the direct sun or allowed to run in open kennels.

The degree of thyroid suppression obtained in this experiment was somewhat limited. Radioactive iodine determinations revealed only a 24% reduction on the function of the thyroid of those on protamone. This indicated that the thyroid was not suppressed to such an extent that it would not be able to resume normal function when thyroid administration was stopped.

Plate IV



EFFECT OF THYROXINE ON THE CYTOLOGY OF THYROID

Plate V

MEASUREMENT OF FOLLICULAR EPITHELIUM

Figures Computed on 25 Measurements

ANALYSIS of VARIANCE

SOURCE	F
TREATMENT	158.66 *
ANIMALS	4.587*

**HIGHLY SIGNIFICANT, $p < 0.01$*

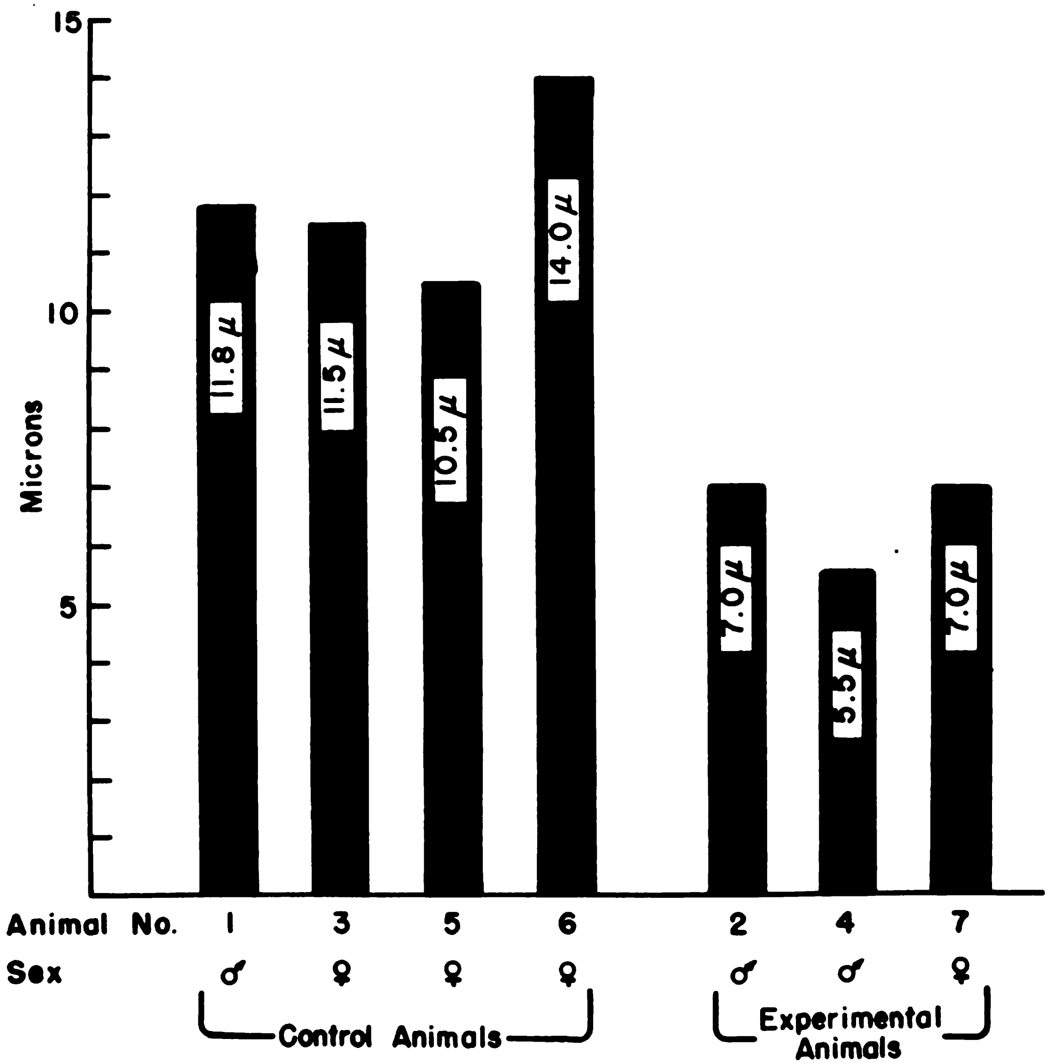


Plate VI Thyroid. Mallory-Azan stain. 165X.

Fig. 1. Experimental animal. Female.
Fig. 2. Control animal. Female.

Plate VI

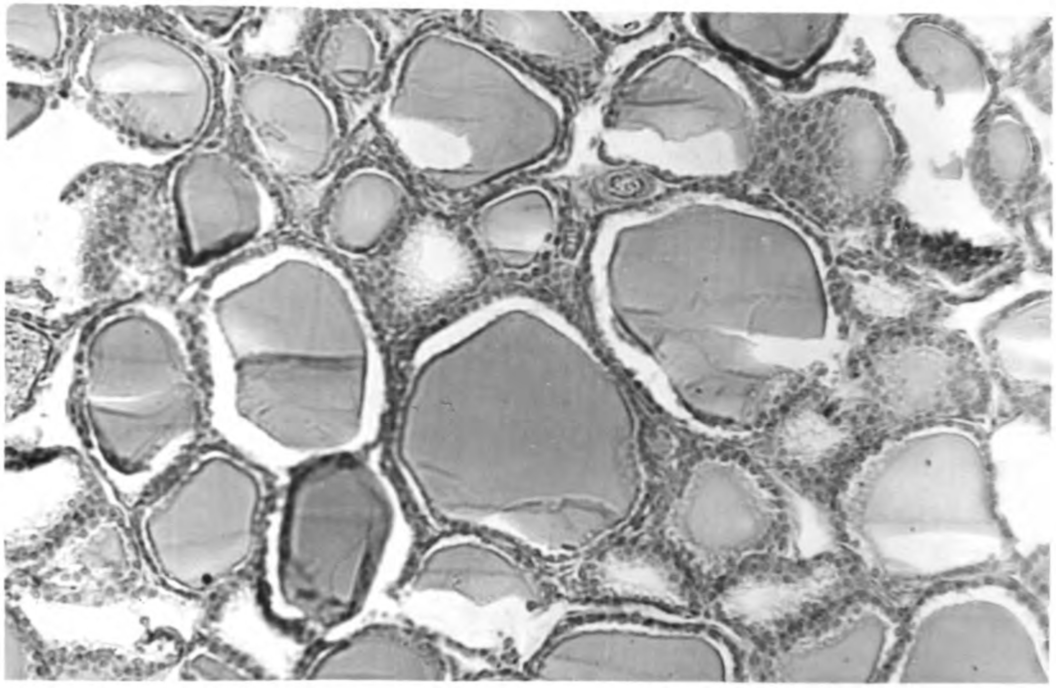


Fig. 1

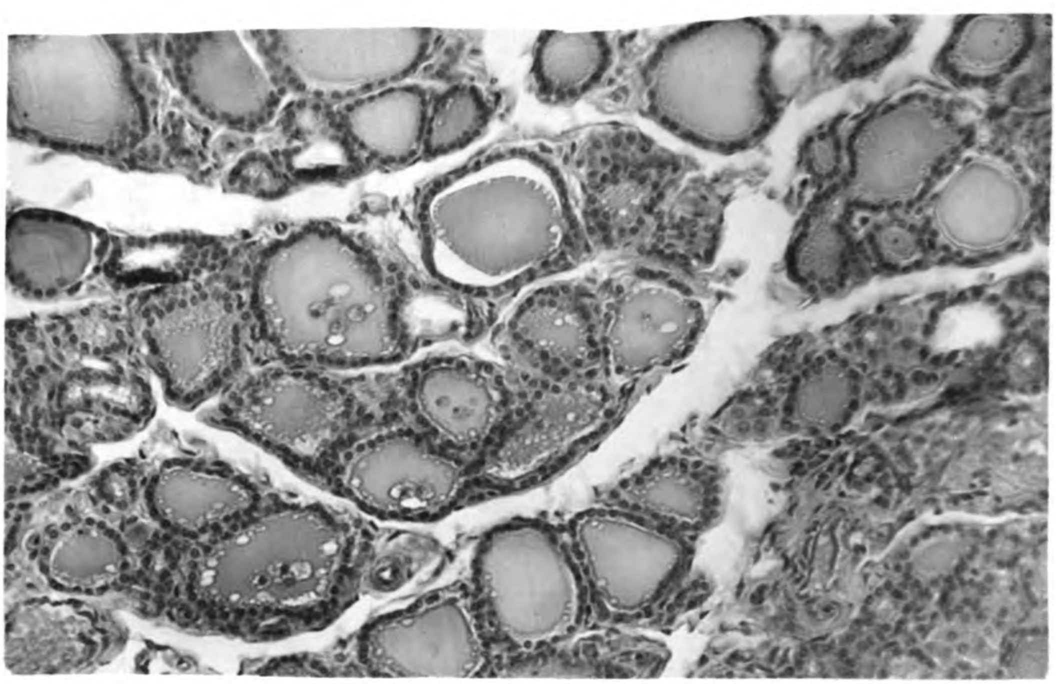


Fig. 2

Plate VII   Thyroid. Mallory-Azan stain. 530X.

Fig. 1. Experimental animal. Female.
Fig. 2. Control animal. Female.

These views illustrate the effect the protamone produced on the cytology of the thyroid. The control has many vacuoles in the colloid and taller follicular epithelium. The experimental animal shows a general accumulation of colloid, lowered follicular epithelium and fewer vacuoles.

Plate VII

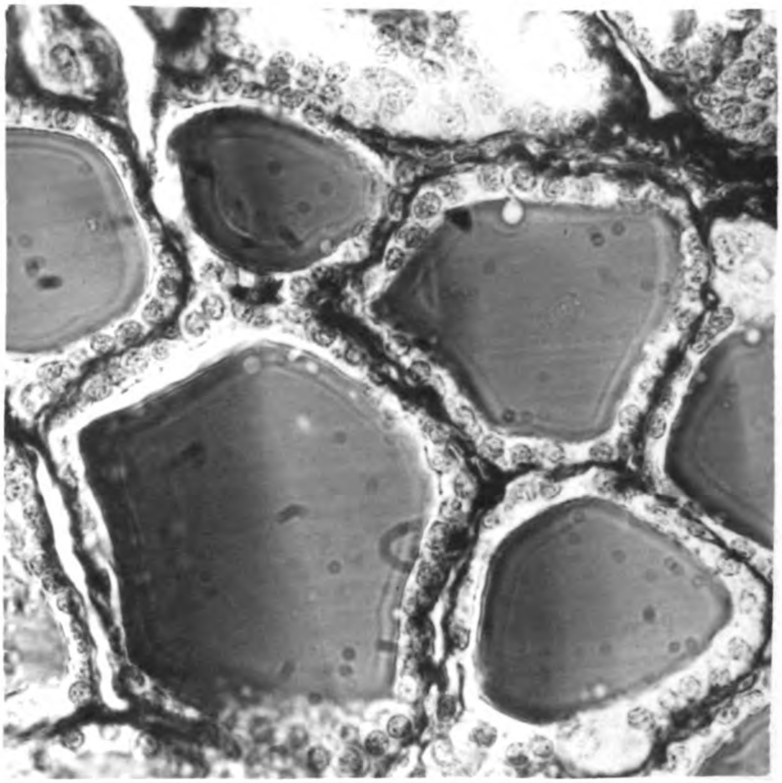


Fig. 1

Fig. 2

Plate VIII   Thyroid. Mallory-Azan stain. 1000X

Fig. 1. Experimental animal. Female.
Fig. 2. Control animal. Female

These are kodachrome prints of the same area as that shown on Plate VII.

Plate VIII

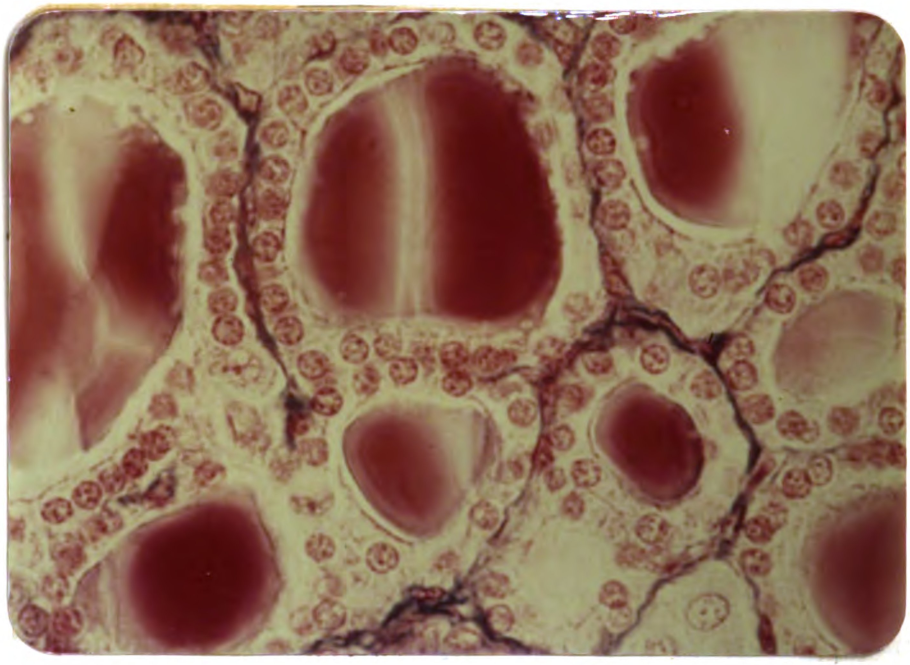


Fig.1.

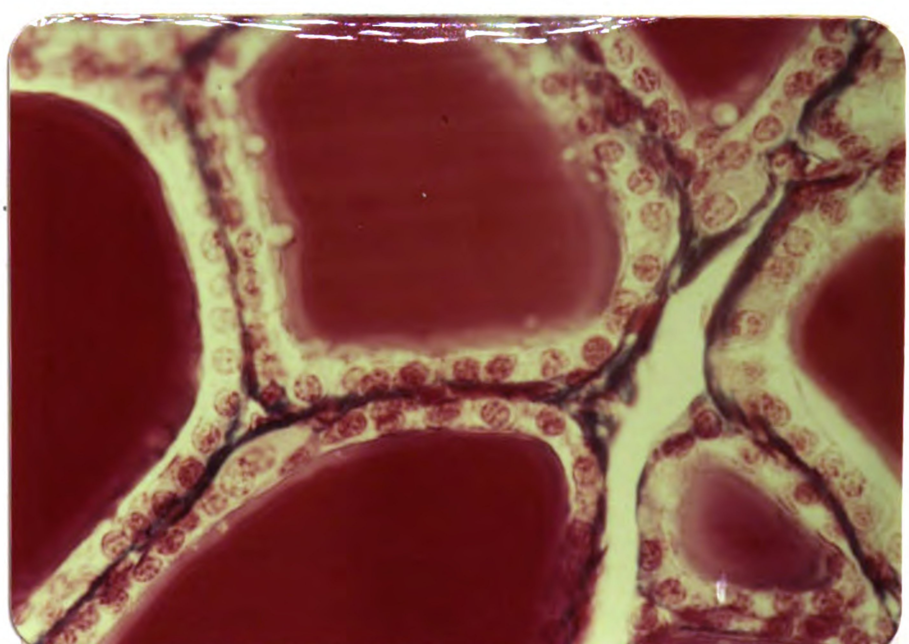


Fig. 2.

Anterior Pituitary

The relationship between the thyroid and the pituitary and the effect thyroxine produces is shown in plate IX. When excess thyroxine is administered the numbers of basophils are reduced.

In an effort to determine the effect of protamone upon the English bulldog pituitary, several different stains were employed in addition to the routine hematoxylin-eosin stain. MacCallums's modification of Cowdry's copper-hematoxylin stain for the pituitary gland, Mallory (1942), was used to differentiate the cells of the pars intermedia from the basophilic cells of the anterior lobe of the pituitary. (Plate X) The fourth cell type described by Wolfe et al. (1933) was brought out by the Erythrosin-Orange G-aniline blue method of Cleveland and Wolfe (1932). (Plate XI) An attempt was made to emphasize the basophils and acidophils with Masson's trichrome stain, Mallory (1942).

There were very few basophils present in the sections of the controls. The slides stained with the copper-hematoxylin stain revealed some cells considered to be of the basophilic type. However, counts made in numerous areas throughout the sections gave inconsistent results. The experimental sections were almost completely depleted of basophils. Because of this, any attempt to differentiate control from experimental animals by means of the

differential count was abandoned. This nearly complete absence of basophils in the pituitary of the English bulldog supports Stockard's (1941) findings of excessive acidophils and few basophils.

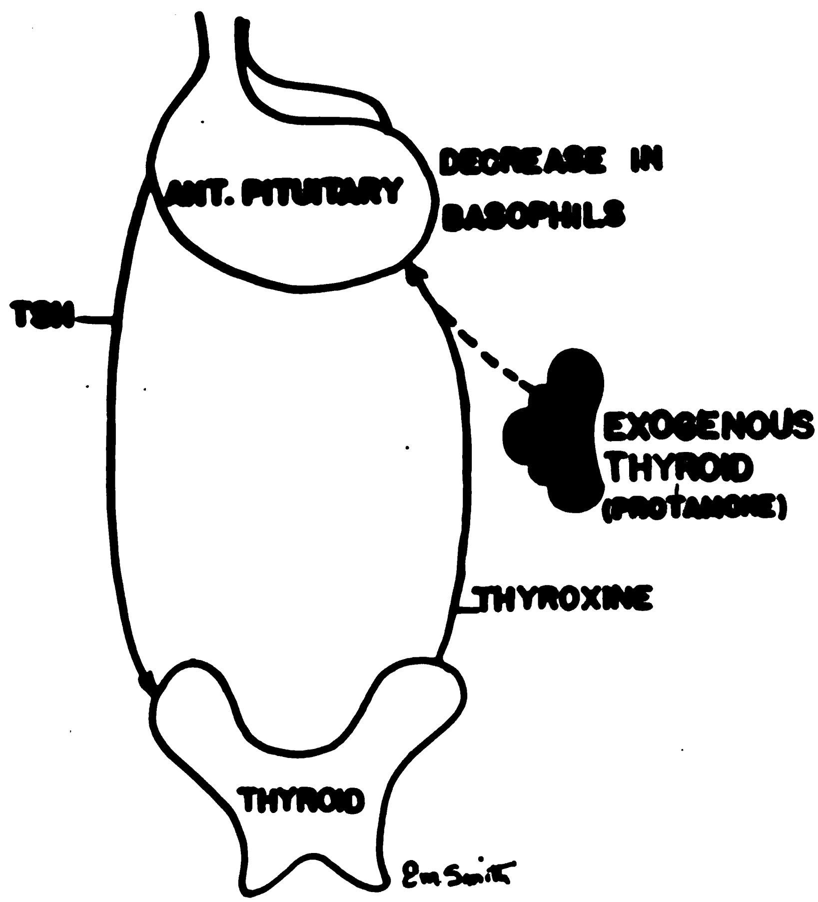
The sections stained with Masson's stain showed a large accumulation of acidophils in the experimental group (Plate XII, fig. 2) when compared to the control (Plate XII, fig. 1). These Masson-stained sections were compared with a section of canine anterior hypophysis (breed unknown) prepared at the University of Minnesota, Department of Veterinary Anatomy. (Plate XIII) Undoubtedly this section was not from an English bulldog since four cell types were very prominent. The basophils were sharp and distinct from the bright red stained acidophils. The chromophobes were colorless and another cell with a pale staining cytoplasm was presumed to be the fourth cell type reported for the dog.

It is reasonable to assume that the protamone reduced the basophil cells since the treated group contained larger numbers of acidophils when compared to the sections of the untreated group.

The histological sections showed some evidence that the fourth cell type, described by several workers, might exist in the English-bulldog pituitary. Different fixation methods than those employed here, should aid in clarifying this point.

Low basophil counts in the English bulldog indicate thyroid hypofunction and may account for the small litters produced by these dogs. Stockard (1941) classified the pituitaries of English bulldog as being of the male type, because of this abnormal acidophil-basophil ratio. Diets supplemented with protamone in growing English bulldogs and other brachycephalic breeds could possibly result in the production of larger litters.

Plate IX



EFFECT OF THYROXINE ON CYTOLOGY OF ANT. PITUITARY

Plate X   Anterior Pituitary. Copper-hematoxylin stain. 560X.

Fig. 1. Control animal. Male.
Fig. 2. Experimental animal. Male.

These views illustrate the presence of a few basophils in the anterior pituitary of the control compared to the complete absence of basophils in the anterior pituitary of the experimental animal. The large, spheroid, black-stained cells in the center of Fig. 1 are basophils.

Plate X

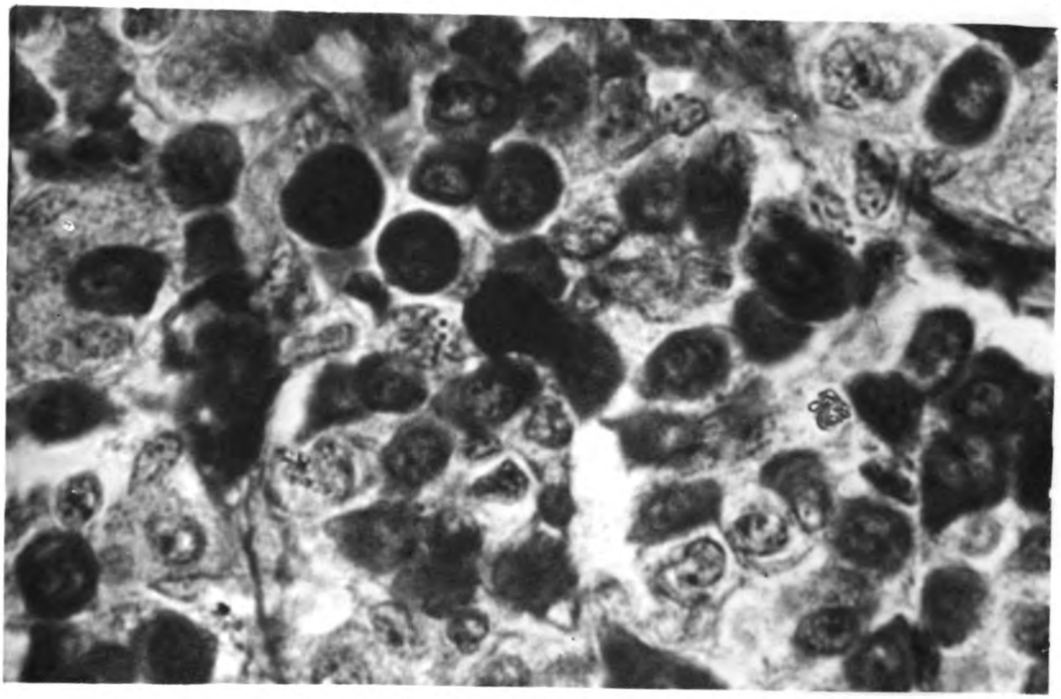


Fig. 1

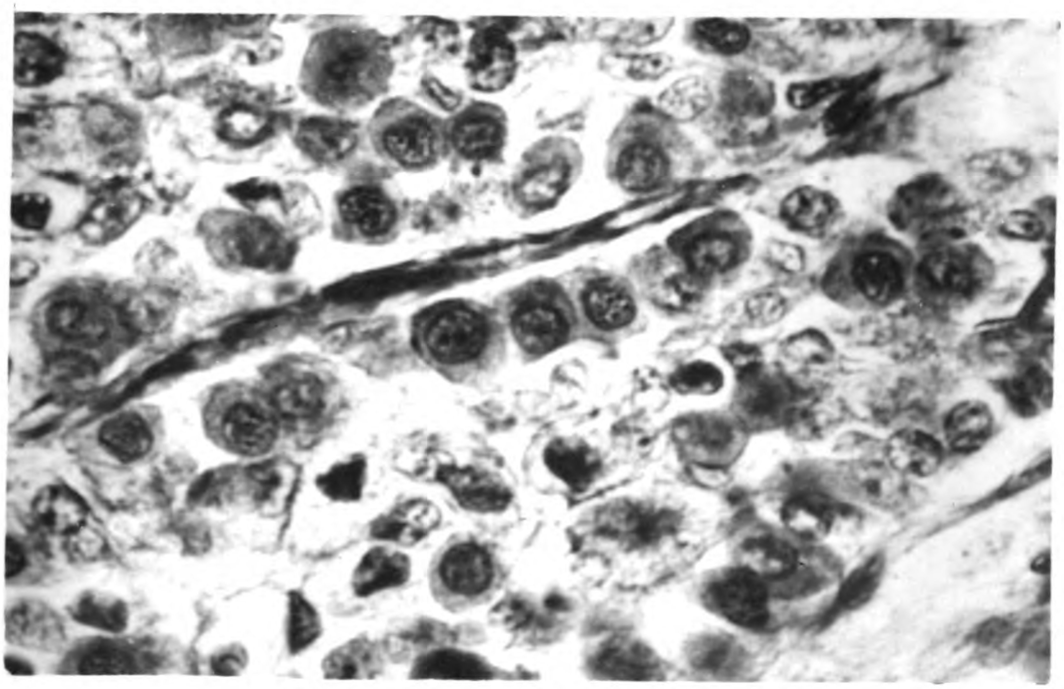


Fig. 2

Plate XI Anterior Pituitary. Erythrosin-orange-G-aniline blue stain. 760X

This is a kodachrome print of the anterior pituitary from a control animal which illustrates this special stain. Due to the printing process much of the color detail has been lost.

Plate XI

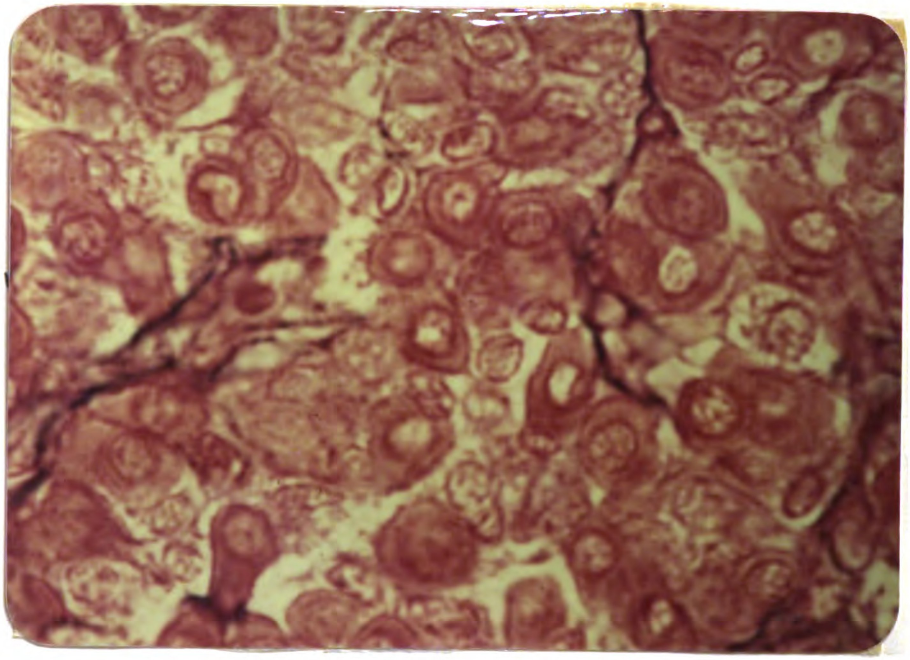


Plate XII Anterior Pituitary. Masson's trichrome stain. 760X.

Fig. 1. Control animal. Male.
Fig. 2. Experimental animal. Male.

These kodachrome prints illustrate the decrease in basophils produced by the protamone. Fig. 1. shows one basophil (a) and numerous chromophobes, while fig. 2. shows no basophils and numerous acidophils. The printing process has reduced the color differentiation.

Plate XII

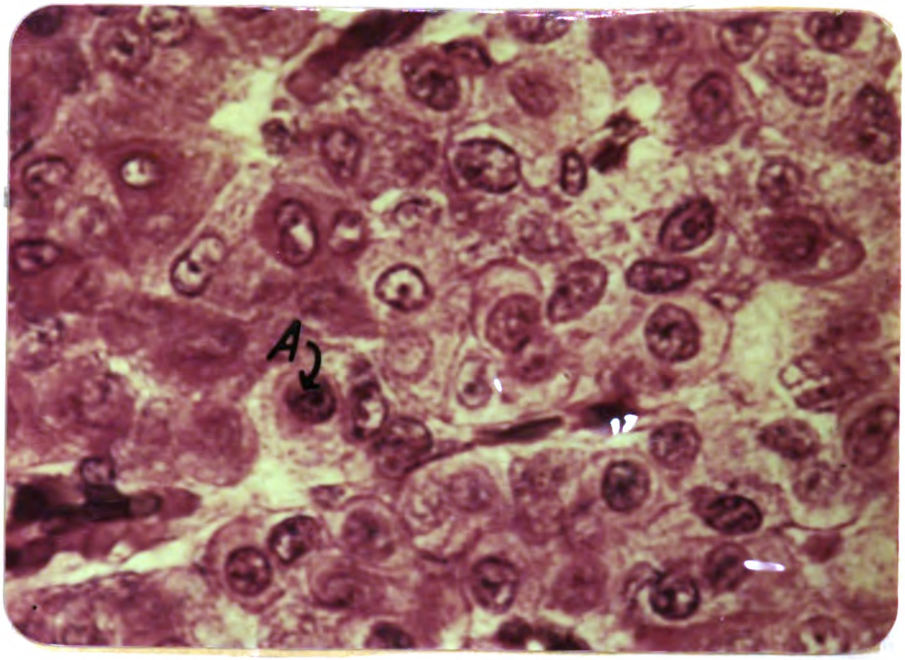


Fig. 1.

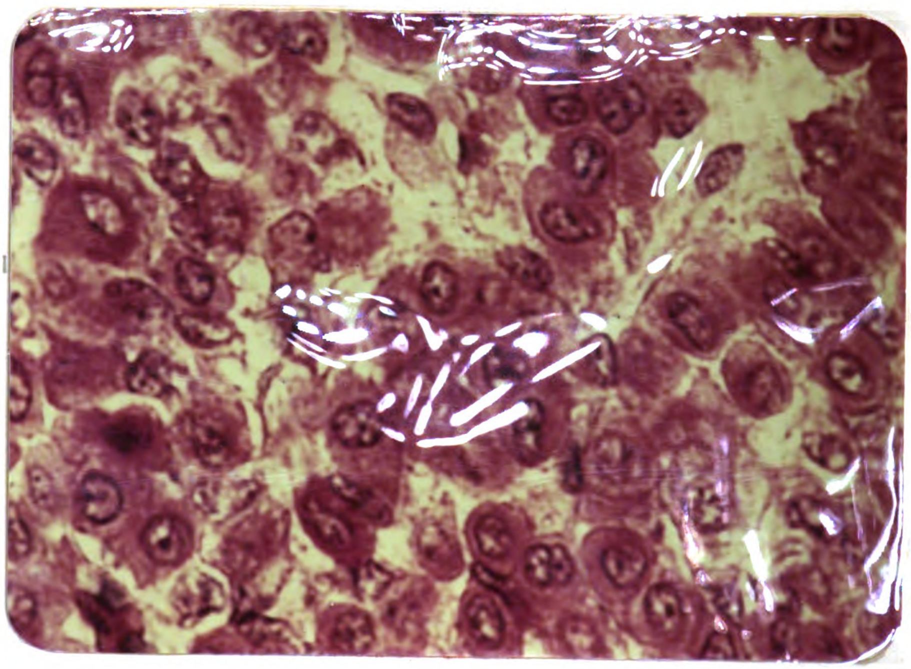


Fig.2.

CA

Plate XII

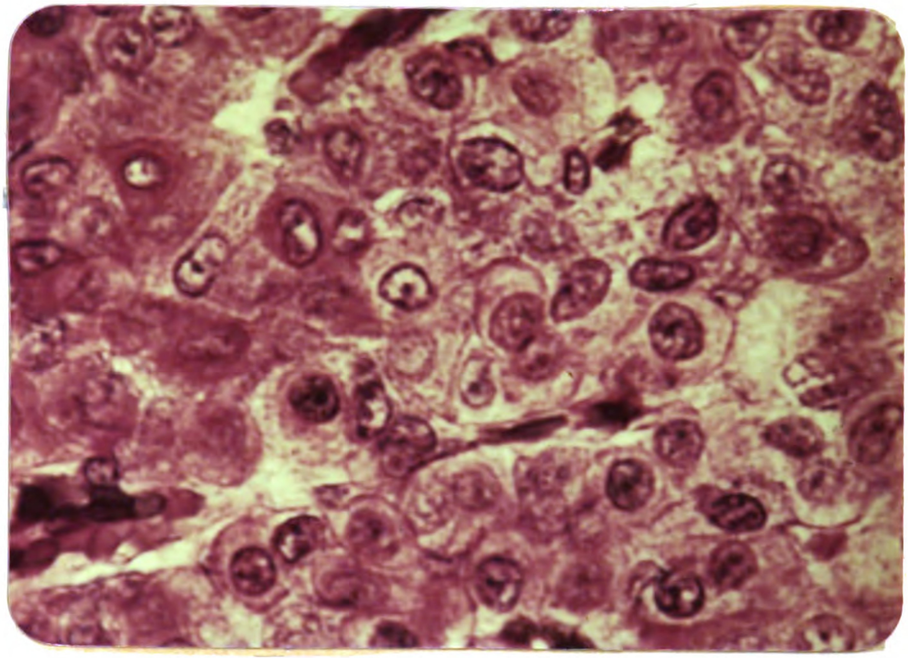


Fig. 1.

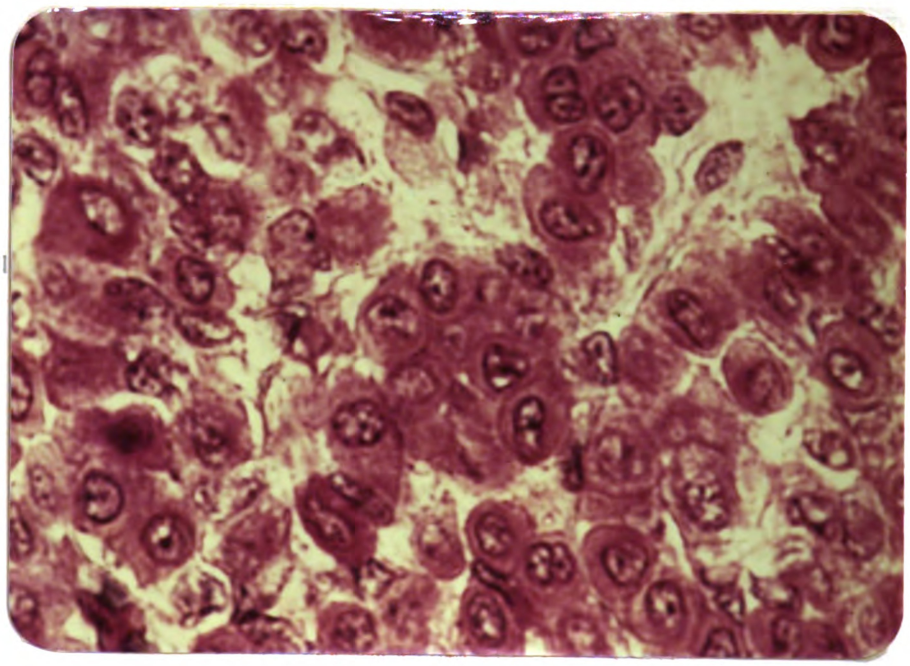


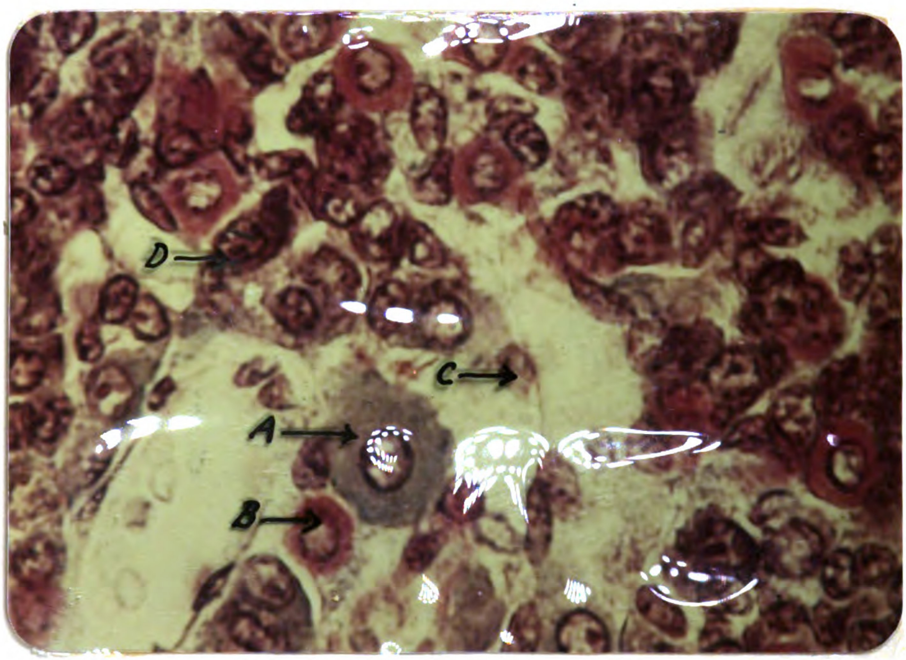
Fig.2.

Plate XIII Anterior Pituitary. Masson's trichrome stain. 760X.

This is a kodachrome print of a slide prepared in the Veterinary Anatomy Department of the University of Minnesota. The breed and sex of this dog was unknown.

A. Basophil.
B. Acidophil.
C. Chromophobe.
D. Fourth cell type described by Cleveland and Wolfe.

Plate XIII



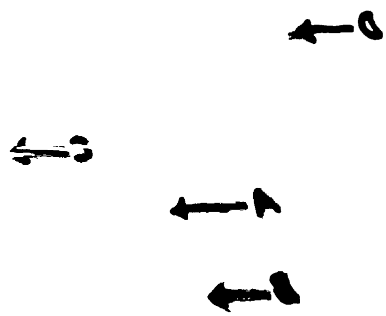
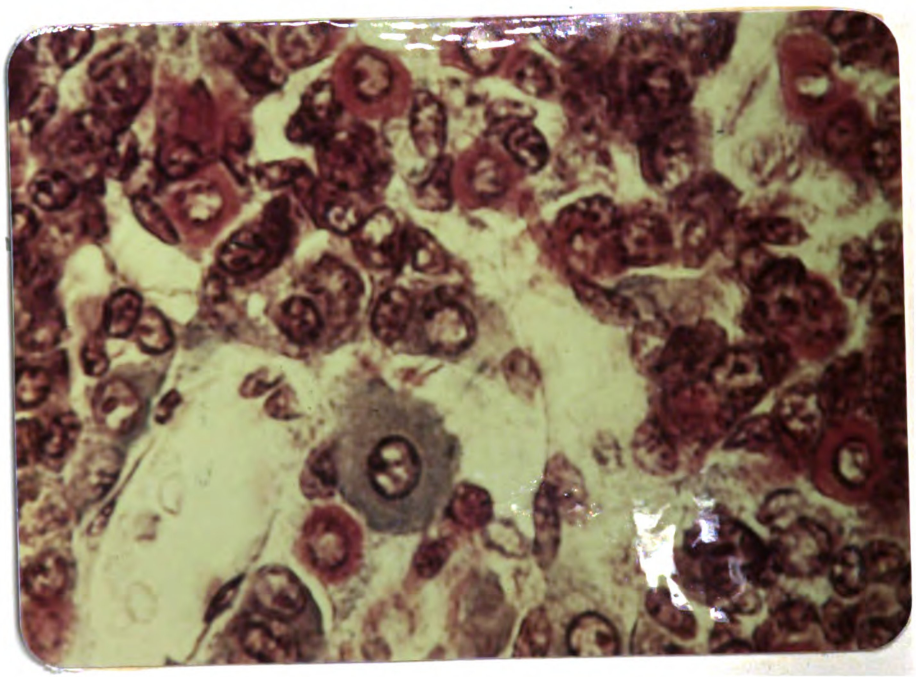


Plate XIII



Adrenal

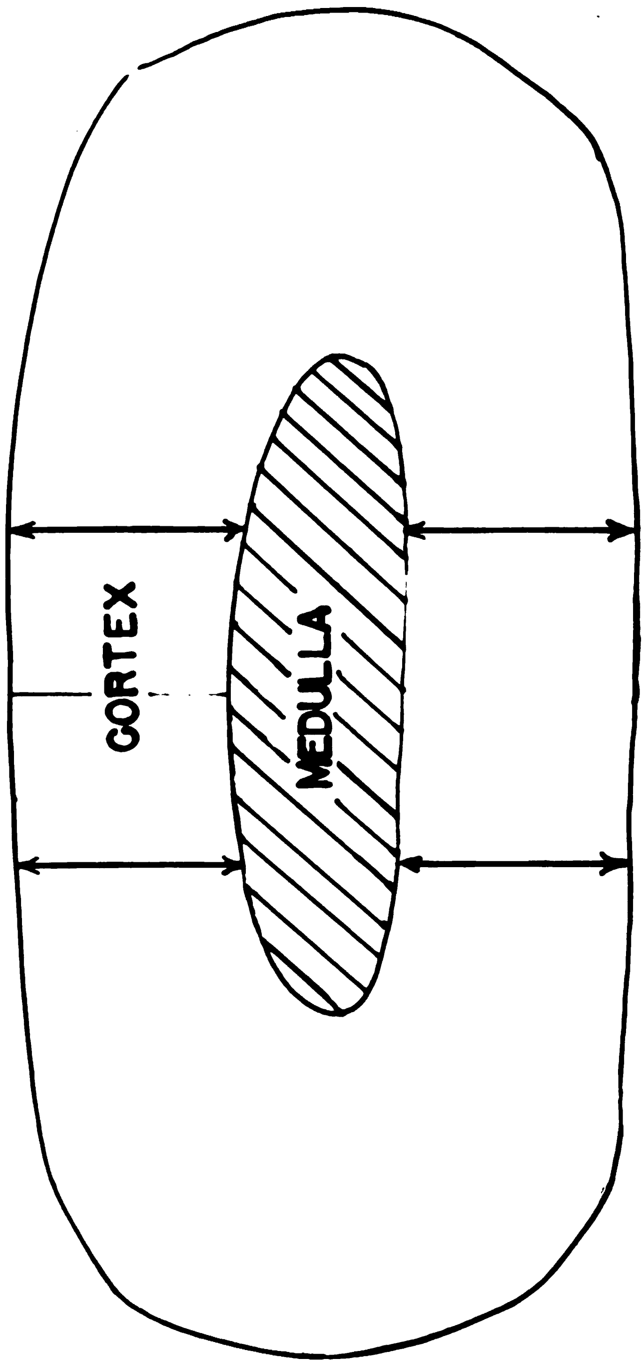
The adrenal glands were stained with Masson's triple stain. The were sectioned longitudinally and measurements were made in the areas shown on plate XIV. The entire cortex of each gland was measured as well as the component zones.

Analysis of variance of the measurements on the cortex indicated a highly significant difference due to the treatment. However, no difference was found between the individual animals within each group. When the analysis was applied to the individual zones, the same results were obtained. However, it should be noted that the glomerular zone was smaller in the treated animals than in the controls while the reticular and fascicular zones increased in size with the treatment. (Plate XV) This cortical hypertrophy produced as a result of the protamone, was probably due to hypersecretion of the adrenal. It has been said that the thyroid is essential for the full effect of adrenocorticotrophic hormone from the pituitary. It could be said that the thyroid acts as a sort of catalyst in this adrenal-pituitary relationship.

The thyroid and adrenal medulla appear to be mutually stimulating. Increased activity of one stimulates the other. Marked hyperemia of the medulla was seen in all adrenals of the experimental animals, while no hyperemia was present in any of the adrenals from the control animals. (Plate XVI) This same result was reported by Preston (1928). The exact mechanism of this hyperemia is not entirely understood.

Plate XIV

ADRENAL



AREAS IN WHICH MEASUREMENTS WERE TAKEN

Plate XV

MEASUREMENTS OF THE ADRENAL CORTEX WITH EACH COMPONENT PART

ANALYSIS of VARIANCE

SOURCE				
TREATMENT	524.81*	14.84*	50.33*	134.09*
ANIMALS	1.50	1.38	< 1	1.15

***HIGHLY SIGNIFICANT, $p < 0.01$**

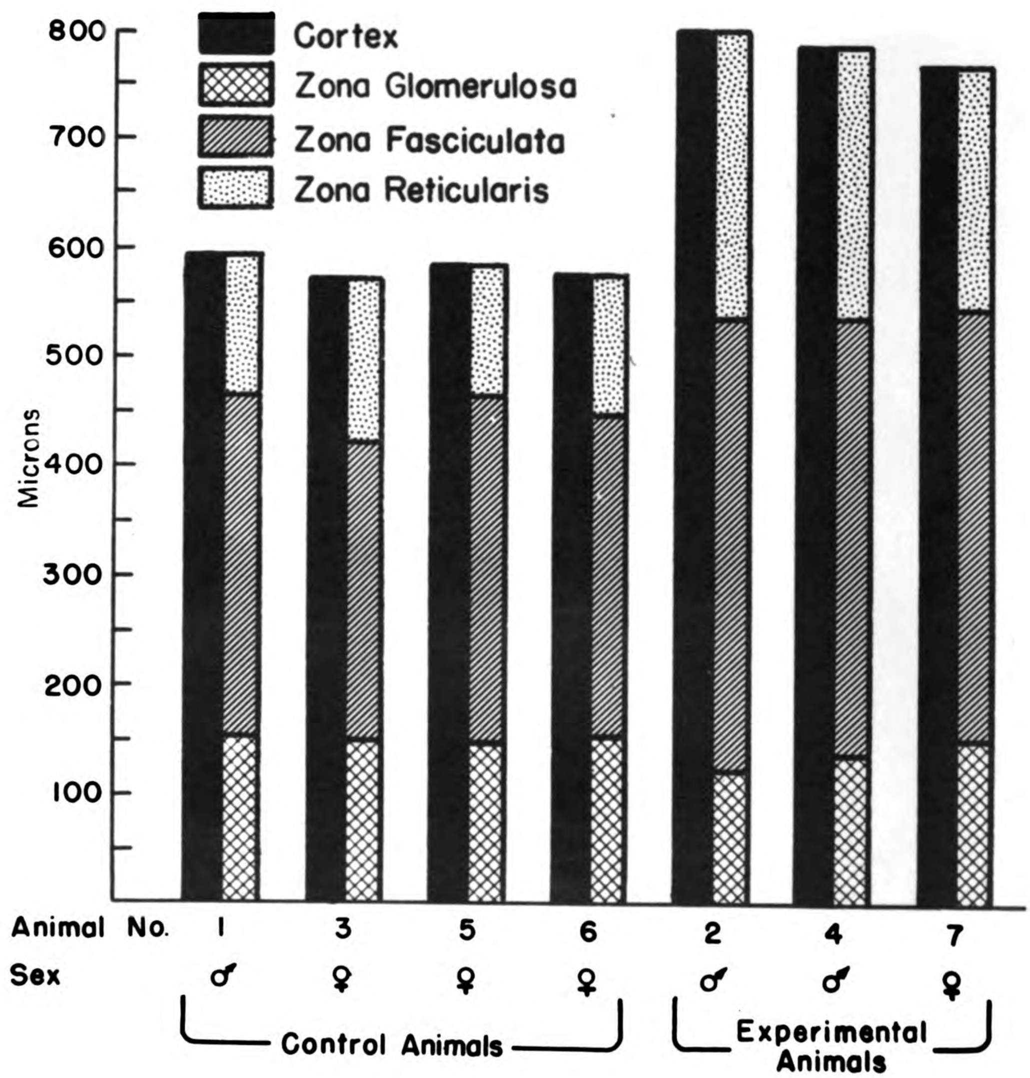


Plate XV Adrenal Cortex. Masson's trichrome stain. Male. 95X.

These illustrate the cortical hypertrophy produced by the protamone. Compare the width of the cortex in Fig. 1 with that in Fig. 2.

Fig. 1. Control animal. Male.
- A. Zona Glomerulosa.
- B. Zona Fasciculata.
- C. Zona Reticularis.
- D. Beginning of Medullary portion.

Fig. 2. Experimental animal. Male.
- A. Zona Glomerulosa.
- B. Zona Fasciculata.
- C. Zona Reticularis. The entire reticularis is not shown.

Plate XVI

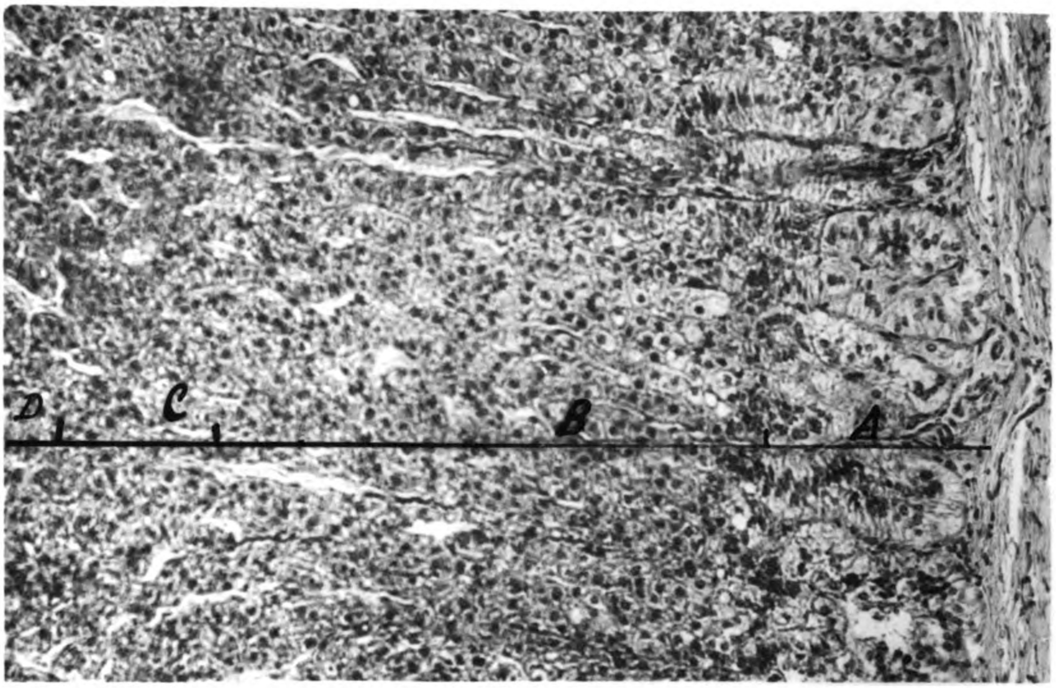


Fig. 1

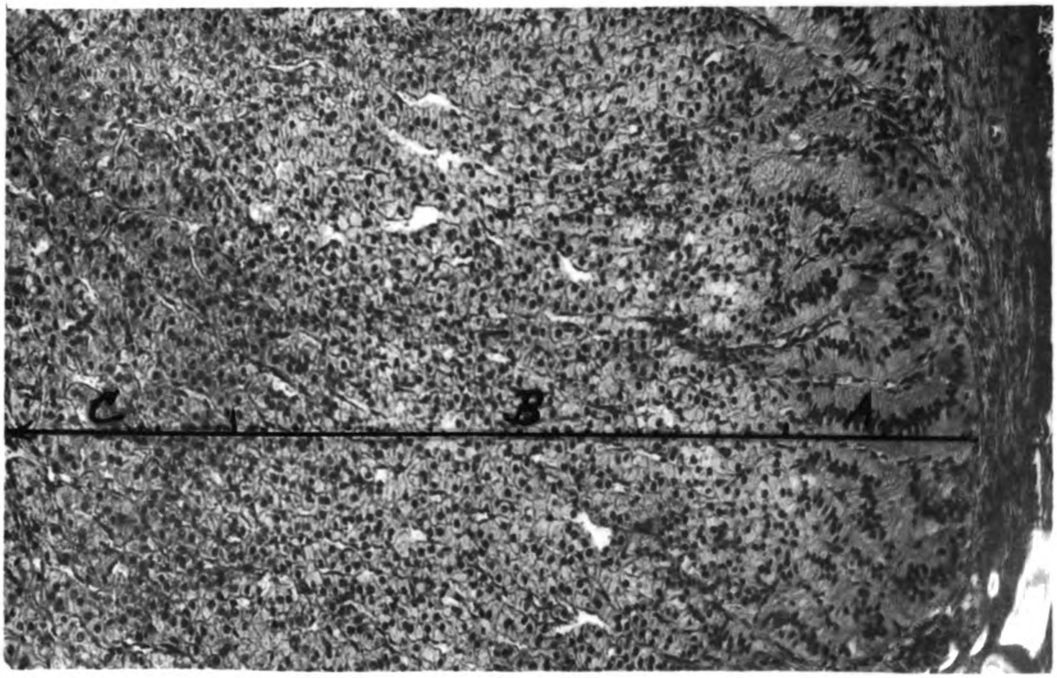
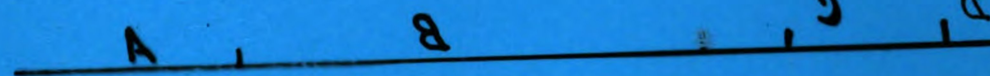


Fig. 2


A
B
C
D

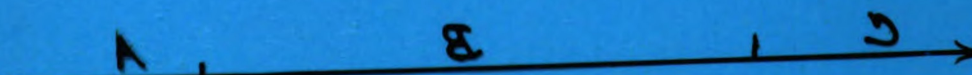

A
B
C

Plate XVI

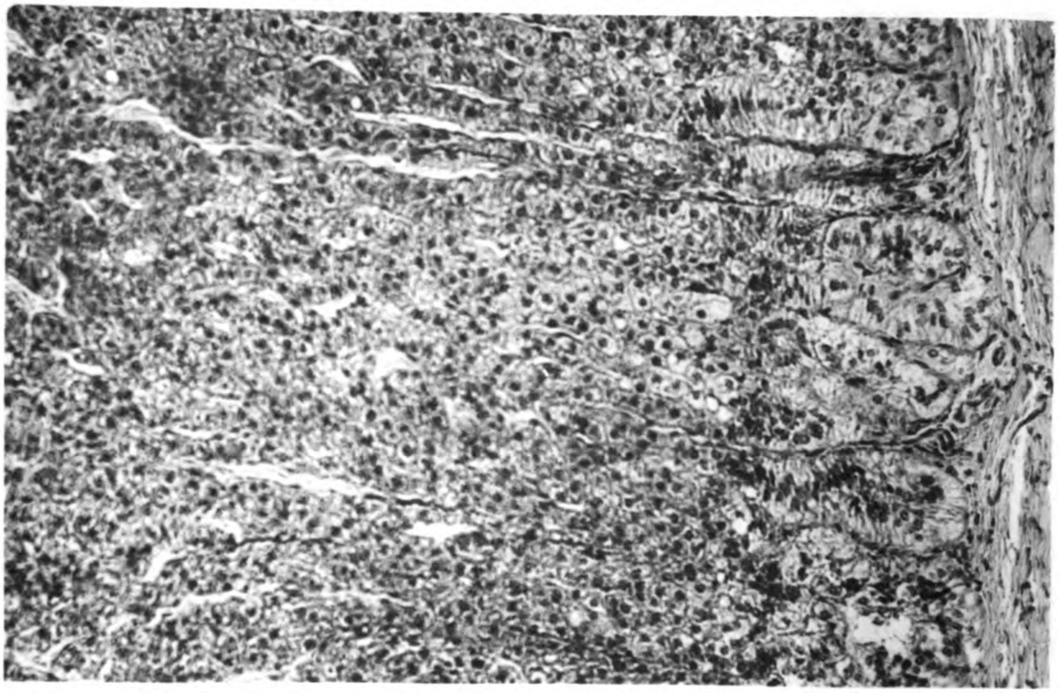


Fig. 1

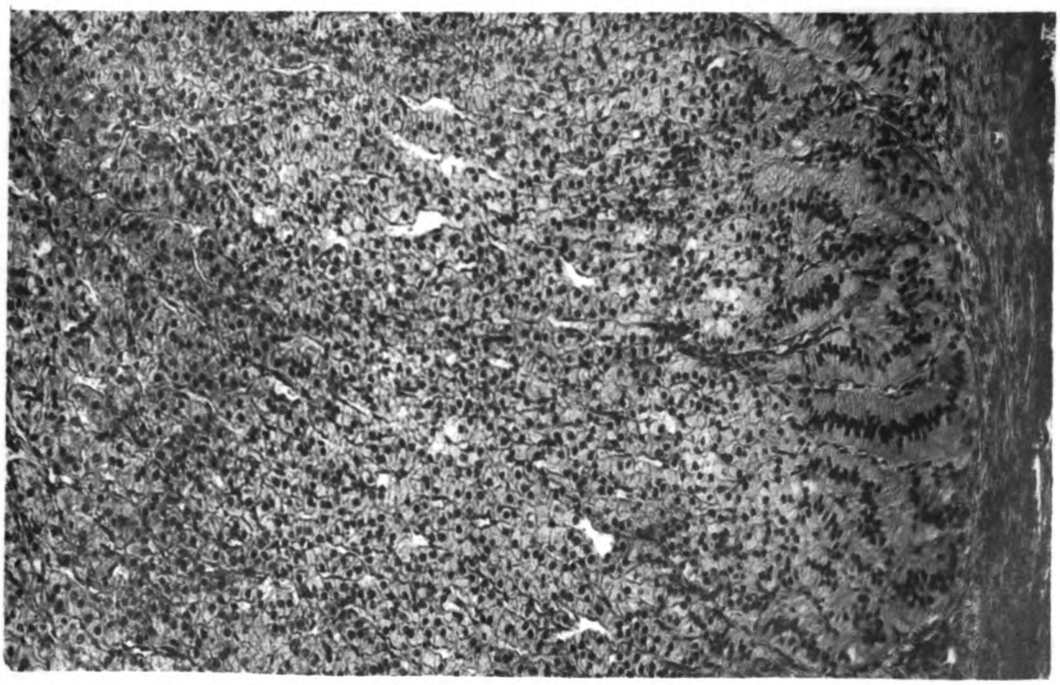


Fig. 2

Plate XVI Adrenal Medulla. Masson's trichrome stain. 190X.

Fig. 1. Control animal. Male.
Fig. 2. Experimental animal. Male.

These kodachrome prints illustrate the hyperemia present in the adrenal medulla of the experimental animal. There is no blood in the medullary sinuses of the control group.

Plate XVII

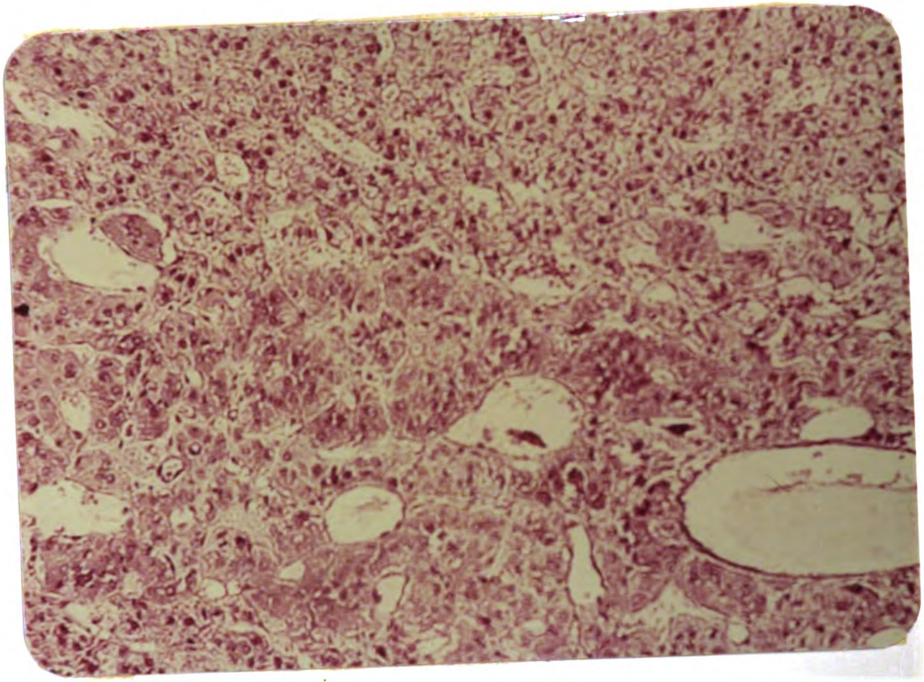


Fig. 1.

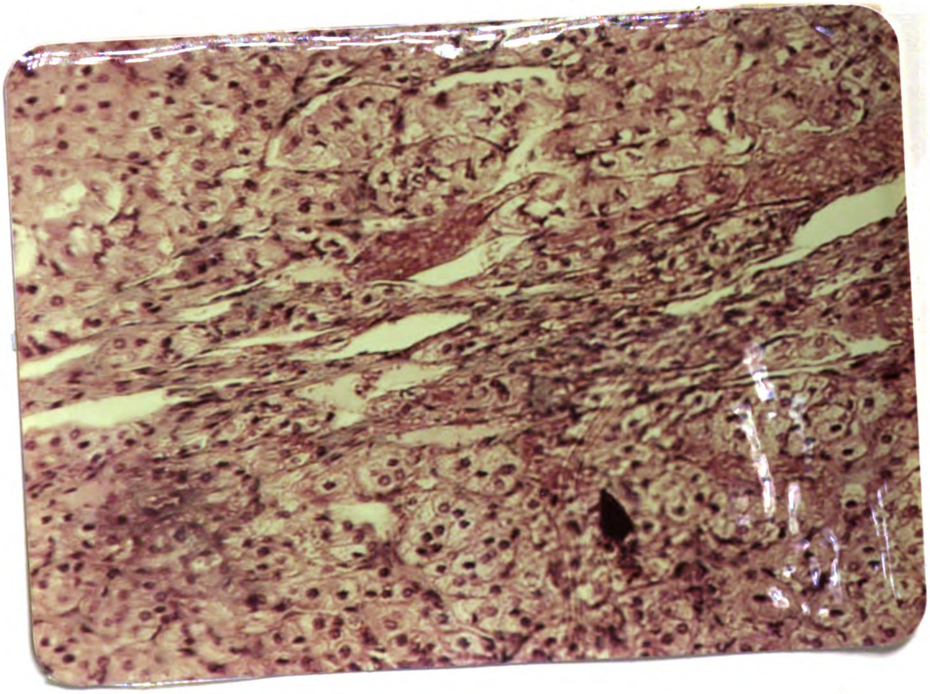


Fig. 2.

SUMMARY AND CONCLUSIONS

Seven purebred English bulldog puppies were used in this experiment. They were divided into two groups, separated according to sex and littermates. Three were experimental dogs and were fed a total of 21.745 gms of protamone over a five month period. Four were the control animals and they were fed exactly as the others, omitting the protamone.

They were autopsied at eight months of age and histological studies were done on the thyroid, anterior pituitary and the adrenal.

The thyroids of the experimental group revealed a general accumulation of colloid and a 54.57% decrease in follicular epithelial height, when compared to those of the control group. Statistical analysis of the data showed a highly significant variance due to the protamone.

The basophils in the anterior pituitary were decreased at the expense of an increase in acidophils. Normal anterior pituitaries of English bulldogs contained very low numbers of basophils. The anterior pituitaries of the experimental dogs were almost completely depleted of basophils. Because of this breed difference, differential counts were attempted, but finally abandoned.

The adrenal glands of the experimental animals showed a marked cortical hypertrophy. Measurements of the cortex were made on all adrenals. The data were analyzed statistically and proved to show a highly significant variance. Hemorrhage into the sinus spaces of the medulla was present in the adrenals of the experimental animals, but not present in the control dogs.

It is obvious that a low dose of protamone such as this, given to a normally hypothyroid animal will produce significant histological changes in these particular endocrine organs.

The thyroid activity of the English bulldog is low and histologically shows a picture of general hypofunction. After protamone administration this activity was further diminished and the gland was put in a resting state.

The anterior pituitary of the English bulldog contains very low numbers of basophils. After exogenous thyroid was given, these basophils decreased in numbers still further and the gland was nearly depleted of them. This absence of basophils was due to the presence of protamone in the body indicating that the basophil cell is a source of thyrotropic secretion.

Exogenous thyroid stimulated the cortex of the adrenal causing hypertrophy, with the reticular zone showing the greatest increase. This effect was apparently mediated through the anterior pituitary, since it must be present for the full effect.

Protamone could possibly be used as a supplement to the normal diet for dogs of those breeds with tendencies toward a hypothyroidism. This treatment might result in increased litter size.

LITERATURE CITED

Arey, L. B., 1947. Developmental Anatomy. W. B. Saunders Company, Philadelphia.

Astwood, E. B., 1949. Mechanisms of action of various antithyroid compounds. Annals of New York Acad. of Sciences, 50:419-443.

Bensley, R. R., 1916. The normal mode of secretion of the thyroid gland. Am. J. Anat., 19:37-42.

Blaxter, K. L., E. P. Reineke, E. W. Crampton and W. E. Peterson, 1949. The role of thyroidal materials and of synthetic goitrogens in animal production and an appraisal of their practical use. J. An. Science, 8:306-352.

Borgman, R. J. and E. P. Reineke, 1949. The response of English bulldog puppies to thyroidal stimulation. J. Am. Vet. Med. Assoc., 115:480-486.

Cleveland, R. and J. M. Wolf, 1932. A differential stain for the anterior lobe of the hypophysis. Anat. Rec., 51:409-413.

Cortell, R. and R. W. Rawson, 1944. The effect of thyroxine on response of the thyroid gland to thyrotrophic hormone. Endocrinology, 35:488-498.

Dawson, A. B. and H. B. Friedgood, 1938. Differentiation of two classes of acidophils in the anterior pituitary of the female rabbit and cat. Stain Tech., 13:17-21.

Deanesly, R. 1931. The histology of adrenal enlargement under experimental conditions. Am. J. Anat., 47:475-498.

Dempsey, Edward W. 1949. The chemical cytology of the thyroid gland. Annals of New York Acad. Sciences, 50:336-357.

DeRobertis, E., 1941. The intracellular colloid of the normal and activated thyroid of the rat studied by the freezing-drying method. Am. J. Anat., 68:317-337.

DeRobertis, E., 1949. Cytological and cytochemical basis of thyroid function. Annals of New York Acad. of Sciences, 50:317-335.

Dvoskin, Samuel, 1947a. Intracellular colloid droplets as a basis for thyrotropic hormone assay in the chick. Endocrinology, 41:220-229.

Dvoskin, Samuel, 1947b. The spontaneous formation of intracellular colloid droplets in surviving chick thyroid tissue. Endocrinology, 41:403-416.

Dvoskin, Samuel, 1948. The effects of pituitary and non-pituitary gland factors on the formation of intracellular colloid droplets in the thyroid epithelium of hypophysectomized rats. Endocrinology, 43:52-70.

Gilmore, James W., 1940. Growth changes in body organs. Part II Growth changes in the thyroid of the normal dog. Am. J. Vet. Res., 1:66-72.

Goldsmith, E. D., 1949. Phylogeny of the thyroid: descriptive and experimental. Annals of New York Acad. Sciences, 50:283-312.

Griesbach, W. E. and H. D. Purves, 1945. The significance of the basophil changes in the pituitary accompanying various forms of thyroxine deficiency. Brit. J. Exp. Path., 26:13-17.

Gudernatsch, Fred, 1949. See Discussion of Paper by E. D. Goldsmith, Phylogeny of the thyroid: descriptive and experimental. Annals of New York Acad. Sciences, 50:283-312.

Hartmann, Frank and K. A. Brownell, 1949. The Adrenal Gland. Lea and Febiger, Philadelphia.

Hartmann, J. F., W. R. Fain and J. M. Wolf, 1946. A cytological study of the anterior hypophysis of the dog with particular reference to the presence of a fourth cell type. Anat. Rec., 95:11-27.

Herring, P. T., 1917. The effect of thyroid-feeding on the weight of the suprarenals and on their adrenalin content. Quart. J. Exp. Physiol., 11:47-57.

Hoskins, R. G., 1941. Endocrinology: the Glands and their Function, W. W. Norton and Company Inc., New York, New York.

Johnson, Eunice, F. N. Andrews, and C. L. Shrewsbury, 1943. The preparation of muscular tissues for histological study. J. Animal Science, 2:244-250.

Leblond, C. P., 1949. See Discussion of Paper by De-Robertis, Cytological and cytochemical basis of thyroid function. Annals of New York Acad. Sciences, 50:317-335.

Mallory, F. B., 1942. Pathological Technique. E. B. Saunders Co., Philadelphia.

Means, J. H., 1948. Thyroid and Its Diseases. J. B. Lippincott Co., Philadelphia.

Nonidez, J. F., 1932. Origin of the parafollicular cell, a second epithelial component of the thyroid gland of the dog. Am. J. Anat., 49:479-505.

Preston, M. I., 1928. Effect of thyroxine injections on the suprarenal glands of the mouse. Endocrinology, 12:323-334.

Schmidt, I. G. and L. H. Schmidt, 1938. Variations in the structure of adrenals and thyroids produced by thyroxine and high environmental temperature. Endocrinology, 23:559-565.

Stockard, C. R., 1941. The Genetic and Endocrine Basis for Differences in Form and Behavior. Wistar Inst. of Anat. and Biol., Philadelphia.

Uhlenhuth, E., 1923. The elaboration and release of the colloid of the thyroid. Proc. Soc. Exp. Biol. and Med., 20:494-503.

Wallach, D. P. and E. P. Reineke, 1949. The effect of varying levels of thyroidal stimulation of the ascorbic acid content of the adrenal cortex. Endocrinology, 45:75-81.

Williams, R. G., 1944. Some properties of living thyroid cells and follicles. Am. J. Anat. 75:95-117.

Wolfe, J. M. and Rucker Cleveland, 1932. Cell types found in the anterior hypophysis of the dog. Anat. Rec. 52:43-44.

Wolfe, J. M., R. Cleveland, and M. Campbell, 1933. Cyclic histological variation in the anterior hypophysis of the dog. Zeit. Zellforsch., 17:420-452.