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THE EFFECT OF ESTRADIOL BENZOATE ON THE ANTERIOR PITUITARY OF ADULT FEMALE CASTRATE RATS

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THE EFFECT OF ESTRADIOL BENZOATE ON THE ANTERIOR PITUITARY

OF ADULT FEMALE CASTRATE RATS

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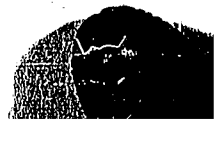


THE EFFECT OF ESTRADIOL BENZOATE ON THE ANTERIOR PITUITARY OF ADULT FEMALE CASTRATE RATS

CHAPTER I

INTRODUCTION

Harvey Cushing (1910), after successfully hypophysectomizing dogs, noted that these animals developed sexual impotency with atrophy of the gonads. In 1921, Herbert Evans found that alkaline extracts of beef pituitary tissue when injected intraperitoneally would produce enlargement of the rat ovary. Tremendous impetus to the experimental study of the pituitary gland followed the development of the parapharyngeal method of hypophysectomy by Phillip E. Smith (1926).

After uniting a normal and a castrate rat in parabiosis, Kallas (1930) found that precocious sexual development of the normal partner was prevented by injecting an ovarian extract into the castrated parabiont. Since the early maturation of normal rats under these circumstances was thought to be due to the increased secretion of the pituitary of the castrate partner, it became clear that the ovary exerted some control over the pituitary gland.

Many studies have been conducted to establish the importance of pituitary gland in gonadal function, and conversely, numerous studies have been made to determine the gonadal influences on the hypophysis.

Although many facts concerning the interrelationship between hypophysis and gonads have been established, the specific manner in which the hormones from these two glands affect the intracellular processes of one another has not been made clear.

Two factors have hindered the accumulation of specific information pertaining to the functional reciprocity of the pituitary gland and the gonads, namely: (1) Differences of opinion existed concerning the nature of the gonadotropic factors. Some investigators maintained that the pituitary gland contained a single gonadotropin and believed that its different effects were due to quantitative variations. Others maintained that the gland contained multiple gonadotropic factors with each exhibiting specific effects. (2) The lack of a standardized method of study which has resulted in the use of many different methods, including numerous variables, as enumerated in the following: a) Many strains of numerous species of animals have been used to study the relationship between the pituitary gland and gonads. The responses observed in these studies have been variable and are often difficult to correlate. b) The age range of the animals has included those from the newborn to the senile adult. This is an important consideration, since a reduced sensitivity to estrogens and androgens has been observed with increase in age. c) The estrogen and androgen dosages have varied widely. d) The experimental time intervals have ranged from three days to two years. e) Different types of animals -- normal, castrate and hypophysectomized -- have been used in these studies with each type giving a different response. f) Both normal and hypophysectomized male and female animals (usually rats) have been used to assay the pituitary glands for the gonadotropic

hormones. The results have varied depending upon age and weight of the animal, time of injection and time of autopsy. g) The pituitary glands have been prepared for assay in the following manner: 1) fresh tissue implanted immediately post-operative, 2) suspensions made from dried tissue, 3) extracts, 4) homogenates. h) The routes of administration have varied; some being made subcutaneously, others intraperitoneally and still others intravenously.

In spite of the apparent confusion and discrepancies, certain concepts have been established and are generally accepted. It is known that the pituitary gland and gonads are closely interrelated functionally. The pituitary gland contains three gonadotropic factors which are present in both sexes and are essential for normal gonadal function. Follicle stimulating hormone (FSH) and luteinizing hormone (LH) affect comparable tissues in the two sexes and the site of action of the luteotropic hormone (LTH) is established in the female but not in the male.

The gonads, under the influence of the pituitary gonadotropic hormones, produce several estrogens and androgens. These steroid hormones are transported throughout the body in the blood and affect many tissues, particularly muscle and bone. The two types of hormones have similar effects on the different gonadotropins.

The specific actions of estrogens and androgens on the pituitary gonadotropins are not completely elucidated. They are both known to have an influence upon the function of FSH and LH, since they both induce a release of LH and inhibit FSH. However, a smaller dose of estrogen is required to produce these influences upon the gonadotropins.

The specific purpose of this study is to endeavor to determine whether estrogen, in addition to causing a release of LH, influences its synthesis. A 1.0 µg. daily dose of estradiol benzoate for 30 days causes a release of LH with relatively little remaining at the termination of the injection period. If 1.0 µg. of estradiol benzoate is capable of producing this effect in 30 days and if estrogen influences LH synthesis, 25.0 µg. given over a longer injection period should accentuate the estrogenic activity upon this process.

Consequently, a comparison of the effects of small and large amounts of estrogen injected for relatively long and short periods has been evaluated.

CHAPTER II

HISTORY

Physiology of the Pituitary and Ovary

The functional significance of the pituitary gland was recognized in the description of acromegaly by Pierre Marie in 1886. Almost 25 years later, dogs were successfully hypophysectomized by Harvey Cushing (1910) and as a result of his work, attention was focused on the effect of the pituitary upon the gonads. New impetus was again added to this field of study when Phillip E. Smith (1926) introduced the parapharyngeal method of hypophysectomy. Since then our knowledge of pituitary function has accumulated rapidly.

The important discovery of two gonad stimulating substances in the urine of women was made by the noted German investigators, Ascheim and Zondek (1927). One hormone was found in the urine of pregnant women, the other in the urine of menopausal women. They assumed that both hormones were of pituitary origin. However, the hormone found in pregnancy urine has since been shown to be of placental origin and of a different nature from those found in the pituitary gland, while the hormone found in the menopausal urine has been shown to be of pituitary origin and is primarily follicle stimulating hormone.

The presence of the ovary in women has been known for centuries,

but it was not until as late as 1884, that Bischoff proved that the ova mature periodically and it was not until 1927 that Corner made the important observation that menstruation could occur without ovulation.

Biochemists were making intensive efforts in the late 1920's to isolate, crystallize and synthesize the sex hormones. Although the female sex hormones had been isolated earlier in the impure form, it remained for Doisy (1927) and Butenandt (1929), working independently, to isolate and crystallize estrone. Butenandt (1933) synthesized estrone from palm nuts. It has since been shown that estradiol is also a secretory product of the ovary.

As information accumulated concerning the function of the pituitary gland and the gonads, it became apparent that they were interrelated. Mocre and Price (1932) reported that most reproductive phenomena could be explained on the basis of a gonado-pituitary interrelationship.

Two pituitary gonadotropic hormones were postulated by Fevold, et al., (1931) and Leonard (1932). Fevold, Hisaw, Hellbaum and Hertz (1933) and Wallen-Lawrence (1934) along with others, fractionated chemically two pituitary gonadotropic factors.

Much of the criticism concerning the presence of two pituitary hormones was related to the fact that the separation of the two factors was accomplished chemically and many workers believed that they represented chemical artefacts. However, Hellbaum (1933, 1935) studied the pituitary glands of horses varying in age from unborn fetuses to senile adults and established the fact that FSH and LH were separate entities under varying physiological conditions. All of the pituitaries were treated in an identical manner chemically.

In the meantime it has been shown by many workers that the pituitary gland contains two gonadotropic factors which have separate and distinct activities. It has also been noted that these two hormones function together and complement each other (Fevold, Hisaw and Greep, 1937; Fevold, 1939).

More recently it has been established that the pituitary gland contains a third gonadotropic factor, namely, the luteotropic hormone (LTH), in addition to the follicle stimulating and luteinizing hormones.

When pure, crystalline estrogenic compounds became available for use in the early 1930's, their influence on the hypophyseal gonadotropic complex was studied. As knowledge accumulated regarding the influence of pure estrogens on the gonadotropins, it became evident that a difference of opinion had developed as to what the exact nature of those effects were. One concept of estrogen action emphasized an inhibition or suppression of pituitary gonadotropins while another concept stressed the idea that in addition to a suppression of FSH, it caused a release of LH.

Moore and Price (1930) introduced the concept of estrogen inhibition of the pituitary gonadotropic complex. By injecting two rat units of estrone into immature female rats for periods varying from 21 to 66 days Leonard, Meyer and Hisaw (1931) were able to prevent normal development if the injections were continued beyond the time when the animals became sexually mature. If the injections were stopped prior to the time of sexual maturity, normal development occurred. It was thought that estrogen had inhibited pituitary function since the response produced in the assay animals was reduced.

It was concluded by Meyer, Leonard, Hisaw and Martin (1932) that

estrogen had an inhibiting effect upon the anterior pituitary gland, since the pituitaries of animals which were castrated for 90 days and then treated with estrone had a reduced gonadotropic content as evidenced by an assay response which was 41 per cent less than the controls.

Lipschutz (1935) asserted that the gonadotropins were depressed in male rats by the injection of estrogen. Jungck, Heller and Nelson (1947) found that large "unphysiological" doses of estrogen were potent inhibitors of pituitary gonadotropic hormones while "physiological" amounts in the circulation exert very little inhibitory action on the gonadotropins.

Finerty and Meyer (1950) injected immature female ovariectomized rats with dienestrol in daily doses varying from 0.025 to 5.0 μg. for ten days. They found that estrogen in the smaller dosage stimulated while the higher doses suppressed all the gonadotropic functions.

It was stated by Greep and Jones (1950a) that the fundamental effect of estrogen on the pituitary is to reduce synthesis of LH. The reasons for their belief are as follows: (1) Immature male rats injected with estrogen show no growth of accessory sexual structures. (2) Estrogen administered to mature male rats is notably effective in producing marked atrophy of the testes and accessory sexual structures. (3) Estrogen is completely effective in abolishing the LH potency of the castrate rat pituitary glands and in direct correlation, the hypophysis of the castrate increases in LH potency. Studying 30 and 55 day old rats with varying doses of estradiol (0.015 to 0.175 μg. per day for 10 days) Byrnes and Meyer (1951b) thought that the secretion of FSH was decreased with very small amounts of estrogen and higher doses caused secretion of

LH while estrogen given in the highest dose, 0.175 µg., decreased the secretion of LH. They found a close correlation between the amount of gonadotropin in the pituitary and the amount in the blood as indicated by the weight of the ovaries and uterus. However, specific findings as to the histological structure of the ovaries of the donor or assay animals were not given. These authors point out that some confusion results from failure to distinguish between the gonadotropic content of the pituitary and the amount of hormone being secreted by the gland. These same authors (1951b) observed an inhibiting effect upon the gonadotropic "hormone" in 65 to 75 gm. normal and castrate parabiotic pairs with 0.009 µg. estradiol and 0.075 µg. estrone.

A marked reduction in total gonadotropins in the monkey with 100 µg. of estradiol benzoate per day are reported by Salhenick, Hisaw and Zarrow (1952).

A release phenomenon was postulated by Engle (1931) as the mechanism of action of estrogen on the pituitary gonadotropins. He referred to this action as one which stimulated the secretion or liberation of the gonad stimulating factors.

Using normal female rats varying in age from 15 to 66 days, Lane and Hisaw (1934) found that estrogen produced an increased output of LH in animals older than 22 days of age. Mechanical stimulation of the cervix of 22 day old rats produced ovarian changes in five days similar to those produced by injected LH. They believed that estrogen stimulated the formation of LH. Hohlweg (1934, 1937) reported that estrogen produced a liberation of LH and corpus luteum formation in the rat.

Biddulph, Meyer and Gumbreck (1940) injected estradiol, estrone

and progesterone and observed a reduction of the hypersecretion of gonadotropins in the pituitary gland of the castrated partner of the normal-castrate parabiotic pair. Hypersecretion was prevented in the castrate partner with 0.025 µg. of estradiol benzoate per day. The females required 1.5 µg. of estriol and the males 0.15 µg. estradiol or 10 µg. of estriol to prevent the hypersecretion of gonadotropins. (Meyer and Biddulph (1941) indicate the amount of secretion is probably very small in the rat at this age, therefore requiring the extremely small dose.)

It was concluded that for the first ten days after castration, the male secretes no more gonadotropic hormone than the female since little difference was noted in the ovarian weight in the normal female parabiont partner, whether the castrate was male or female. Follicle stimulating hormone is primarily secreted for the first ten days as indicated by the absence of corpora lutea, however, the ovaries of the intact partner contain corpora lutea after the ten day period. It was believed that the corpora lutea are the result of LH being secreted by the intact rat as a consequence of the estrogen being produced by the rat's own ovaries. The conclusion was reached that the quantities of estrogen and androgen necessary to prevent castration hypersecretion of gonadotropic hormone in male and female rats either prevent production or release of FSH and at the same time increase the production and release of LH.

The normal parabiont of an adult normal-castrate female parabiotic pair comes into continuous heat after a period of time averaging 39 days following castration (Meyer and Biddulph, 1941; Witschi and Levine, 1934;

and many others). Estradiol administered to the castrate partner stopped the continual estrus in the normal partner within ten days with reappearance of its cycles. If the castrate parabiont was hypophysectomized, the normal rat regained its normal cycles. It was concluded that estrogen prevents the hypersecretion of the gonadotropic hormone (FSH) when given to castrate animals. Meyer and Biddulph (1941) also demonstrated by injecting LH into the castrate partner that it can cross the parabiotic barrier if it is present in sufficient quantities.

Estrogen increased the production and release of LH when it was given over a three day period as evidenced by what Fevold, Hisaw and Greep (1936) refer to as "augmentation". Augmentation is defined as a greater increase in size and weight of the ovaries than can be accounted for by the additive effects of FSH and LH. When estrogen administration was continued over an eight day period, the "augmentation" did not occur. The results were explained on the basis that estrogen had resulted in a reduction in FSH, consequently, the ovary was not adequately prepared for the complete action of LH and subsequent "augmentation" reaction. This work was verified by Fevold and Fiske (1939).

Hellbaum and Greep (1943) working with normal and castrate adult male rats administered testosterone propionate for periods varying from 15 to 45 days and found that this steriod would bring about a release of LH while inducing an inhibition of FSH. The observation was also made that while the pituitaries of normal males contained primarily FSH, the blood serum contained LH with small amounts of FSH. Following castration, the LH as well as FSH were markedly increased in the pituitary, FSH was present in greater amounts in the blood serum while serum LH

appeared to have decreased. Treatment with testosterone propionate reduced the content of LH in the pituitary with little effect on the FSH. The blood serum FSH concentration was lowered with testosterone propionate whereas the LH appeared to have increased after 15 to 30 days of treatment, but not after 45 days of treatment.

According to the work of Hellbaum and Greep (1946), the pituitaries of normal adult female rats under the normal influence of estrogen, stimulated moderate luteinization when assayed in immature normal and hypophysectomized female rats. Pituitaries of adult females that had been oophorectomized produced extensive luteinization, indicating liberation of the pituitary luteinizing factor was minimal in the absence of estrogen. Release of LH by estrogen was suggested by the type of response (only follicular development) produced by the pituitary glands when assayed after being treated daily for 30 to 45 days with estradiol benzoate. Only a minimal amount of LH was present since there were no corpora lutea in the ovaries.

The fundamental principle of estrogen action on the hypophyseal gonadotropins, whether it influences the production of gonadotropins in addition to causing a release of LH and inhibition of FSH was investigated by Bradbury (1947). He found that the administration of one to two micrograms of estradiol to immature female rats increased the ovarian weights in 72 to 120 hours, but a decrease in gonadotropic content of the pituitary gland occurred in 72 to 96 hours. He thought the results indicated a release of LH had occurred under estrogen stimulation rather than an inhibition or depression of function. It was also noted that when oophorectomy was performed one day prior to estrogen administration,

the pituitary gonadotropins were less sensitive to estrogen. Greep and Jones (1950b) were not able to substantiate Bradbury's work. However, they did observe a small but significant increase (14 to 17 mg.) in the ovarian weight of 30 day old rats. The increase in weight was considered to be a release of a small store of both FSH and LH.

By placing ovarian transplants into the spleens of castrate guinea pigs, Lipschutz, et al., (1948) found only bloody and cystic follicles, but after the injection of estradiol, corpora lutea were formed, indicating the release of stored LH. The investigation of Inglesias, Lipschutz and Rojan (1950) with simultaneous intrasplenic ovarian grafts and implantation of estradiol pellets subcutaneously also indicated the release of LH with corpora lutea formation in the implanted ovaries.

Greep and Jones (1950b) using 30 day old normal and castrate male and female rats of the Hisaw strain, and employing a wide range of dosages of both estradiol benzoate and testosterone propionate found that a release of LH was produced by these steroids in both the normals and the castrates. A smaller dose of estradiol was required to produce this effect. Instead of inhibiting FSH, testosterone propionate was thought to produce a storage of FSH in addition to causing a release of LH in the intact females.

Byrnes (1951) studied 30 day old normal and castrate rats in parabiotic pairs and found that by administering estrogen to the castrate at the time the parabiosis and oophorectomy were performed, the hypersecretion of the gonadotropin and ovarian hypertrophy could be prevented in the normal rat. If the rats were castrated ten days prior

to parabiosis and estrogen administered at the time of parabiosis, 70 per cent more estrogen was required to prevent hypersecretion of the hypophyseal gonadotropin. This fact confirms Bradbury's work (1947) that if the animal is allowed to wait for a few days following castration, more estrogen is required to produce the desired effect, that is, it is less sensitive to estrogens and androgens.

Funnell, Keaty and Hellbaum (1951) in studying menopausal patients, found that those patients with severe symptoms had relatively high titers of FSH in the urine. With administration of estradiol benzoate in 1.66 to 3.32 mg. doses per day, LH could be demonstrated in the urine, indicating a release of LH from the pituitary gland by estrogen. Their work indicated that storage and liberation of the separate gonadotropic hormones (FSH and LH) are independently influenced by estrogens. This work also indicated that the intracellular synthesis of the factors is a separate process from that involving the liberation of the hormones from the gland.

Histology of the Pituitary

As early as 1892, Shönemann described three types of cells in the pituitary gland. To the cells lying mostly in the center of the cell cords, he gave the name, chromophobes, since their cytoplasm was devoid of granules. Occupying the periphery of the cords adjacent to the blood sinusoids were cells with distinct granular cytoplasm. The cells which stained with the plasma dyes were named acidophiles, and the cells whose cytoplasmic granules stained with the basic dyes were named cyanophiles (basophiles). These three types of cells have been described in all species studied (Severinghaus, 1939).

Severinghaus (1939) stated that the cells of the pituitary gland are not arranged in any fixed pattern, aside from the general tendency of chromophobes to occupy the center of cell cords in those species in which cord-like structures occur. In man the basophiles are most numerous in the peripheral portion of the gland, the center being highly acidophilic. In the rat the basophiles are most prominent at the anterior and posterior margins. Not only do species differences exist, but individual differences in distribution are the rule.

Early in the study of pituitary gland cytology, the distribution of cells and variation in cell types became of interest. Fischera (1905) first called attention to the castration cell. Comte (1898) had earlier described cellular changes which accompany pregnancy.

As the histology of the pituitary gland developed, it soon became apparent that distinct differences in the cells occurred in the two sexes (McQueen-Williams, 1934). Cellular differences were also noticed during the estrus cycle (Charipper and Haterius, 1930) as well as other functional changes.

It is generally known that estrogens affect the pituitary gland functionally and there is a great body of evidence indicating they also affect it structurally. Fischera (1905) reported that the normal structure of the hypophysis of the castrate hen could be restored by injecting ovarian extracts. Since the work of Fischera, numerous studies have been made of the effects of estrogen on the pituitary gland and it is generally agreed that it produces definite cytological changes.

In 1934, Severinghaus reported that estrogen administration produced a marked degranulation of the pituitary basophiles in addition to

stimulating the formation from the chromophobes of many new small basophiles with large Golgi apparatuses. Acidophiles were actively degranulated in the adult female but not in the immature female or in the adult male although prominent areas of cellular atrophy were present in the glands. There was similarity between the cells of these pituitary glands and those of pregnant animals. The cytological findings indicated that the previously reported loss of potency of hypophysis (Meyer et al., 1932; Leonard, 1933) was due to widespread degranulation of the secretory cells. Wolfe and Chadwick (1936), using graded doses, concluded that estrogens affect both the basophiles and acidophiles. Small doses caused degranulation of basophiles, but large doses given for a prolonged period degranulated the acidophiles and produced an increase in acidophiles through active mitoses.

Evidence that estrogens are not only able to prevent hypophyseal changes incident to castration, but will also repair the changes produced by castration was pointed out by Severinghaus (1939). Estrogens cause a marked depletion of the granular cells if given in larger than physiological doses. Also associated with the above mentioned effects of estrogen is the failure of pituitaries of castrates treated with estrogen to develop increased gonadotropic potency or to retain increased gonadotropic potency following castration. A marked subnormal potency also results from any continued estrogen treatment. The generally accepted interpretation of these observations has been that the estrogens inhibit the secretory activity of the anterior lobe cells.

Severinghaus believed that it was impossible to interpret the cytological data in this manner, if secretory activity was to be

interpreted as the production and release of the secretory substances. The association of potency with granule filled cells and its absence with the granule depleted chromophobes can hardly be questioned.

Estrogens will cause the pituitary gland to release its increased storage of IH and even deplete its store far below normal. There is, therefore, no inhibition of this secretory release but rather a stimulation to release its secretion. The marked degranulation of the basophiles and acidophiles in the hypophyses of estrogen treated animals is cytological confirmation of secretion release, as is also the hypertrophy of the mitochondria. Severinghaus goes on to say that the question of secretion production is more difficult.

Since the classical experiments of Nassonov (1923) and Bowen (1923), now repeatedly confirmed, cytologists have regarded the hypertrophy of the Golgi apparatus in secretory cells as evidence of active elaboration of secretion. Production of secretion is directly proportional to the degree of hypertrophy of the Golgi apparatus. Judged on this basis, the anterior lobe of estrogen treated animals must be regarded as highly active in the elaboration as well as release of its secretory products. With small doses and a brief injection period, estrogens cause hypertrophy of the Golgi apparatus in the basophiles. Mitochondria also become more prominent. Larger doses and more protracted treatment intensify these changes in the basophiles and extend them to the acidophiles. Eventually, hypertrophy of the Golgi regions in the acidophiles become so great that the entire cytoplasm of the cell is occupied.

Finerty and Meyer (1950) reported the physiological and cytological

effects of graded doses of dienestrol (0.025 to 5.0 µg. per day) for ten days on immature oophorectomized rats. They concluded that hypophyseal gonadotropic activity is influenced by the amount of estrogen circulating in the blood, in which low concentrations stimulate the acidophile cells and LH production and high concentrations reduce the number of acidophiles and suppress all gonadotropic activity as evidenced by the pituitary assays.

They noted that estrogen in any concentration reduced the number and activity of basophile cells in direct proportion to its concentration in the blood. Byrnes, Meyer and Finerty (1951) found that progesterone given alone or with estrogen prevented the post-castration basophilia when administered in amounts sufficient to inhibit gonadotropic secretion. The progesterone-estrogen combination produced this effect with a smaller dose than would have been required by either one alone.

Victor and Anderson (1937) have introduced evidence of an entirely different nature. By using the Warburg apparatus, they found a distinct rise in metabolic activity of the anterior lobe of the pituitary gland after the injection of estrogen as compared with control tissues (kidney, liver).

The opinion that both the acidophiles and the basophiles are involved in pituitary gonadotropic secretions has been maintained until recent times.

McManus (1946) described a new technique for staining tissues in which he used periodic acid followed by Schiff's reagent. This new stain provided a method by which glycoprotein could be demonstrated. The mechanism of action of this procedure is the change of the glycoprotein to a

polyaldehydeprotein under the influence of periodic acid. The polyaldehyde can then react with Schiff's reagent to give a red color. Hotchkiss (1948) also employed the periodic acid Schiff technique to study both plant and animal tissues which contained carbohydrate. Catchpole (1949), utilizing this stain to study frozen pituitary tissue, was able to demonstrate the accumulation of glycoprotein granules in the basophiles. He also correlated this study with the staining of the presumably purified FSH and LH preparations.

Purves and Griesbach have made an extensive study of the relationships between pituitary gland cytology and its function. These authors (1945) reported a direct correlation between increases in the number and size of "basophile" cells and an increasing thyrotropin secretion. A year later (1946) they reported that the acidophile cell degranulation which follows total thyroidectomy is due to a failure of hypophyseal function in the complete absence of thyroxine. They believed that the basophile could be considered the source of thyrotropin. In 1951 these investigators reported that two distinct types of basophiles existed in the pituitary, one type being an oval or rounded cell staining intensely with the PAS (Periodic Acid Schiff) stain and localized near the upper and lower surfaces of the pituitary and adjacent to the pars intermedia. This cell type appeared to be inhibited by estrogen and appeared to be the long recognized "castration" cell. It was concluded that it is the site of gonadotropin production. The other glycoprotein containing cell was inhibited by thyroxine administration and responded to thyroxine deficiency by producing the "Thyroidectomy" cells, which were located centrally in the gland, were more angular in shape and became enlarged

as a result of thyroid deficiency. The conclusion was that those cells are the source of thyrotropin. These workers thought it unlikely that either of these cell types, which together constitute the so called "basophiles", was responsible for the production of any hormone other than the specific glycoprotein hormones from which their suggested names are derived.

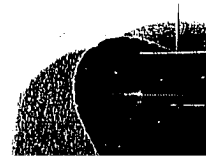
Li and Evans (1948) reported that there are three hypophyseal hormones which contain sugar in significant amounts, the follicle stimulating hormone, luteinizing hormone and thyrotropic hormone. However, McShan and Meyer (1939) reported that on the basis of digestive enzyme studies, FSH was dependent upon a carbohydrate fraction for maintenance of its action, while LH was dependent upon a protein fraction. These facts were determined by studying the activity of trypsin and ptyalin on three different extracts of sheep pituitary gland. Extract "A" was rich in both FSH and LH, extracts "B" and "C" had very small amounts of protein in them. It was found that trypsin completely destroyed the LH activity of extracts "A" and "B", but did not affect the FSH of the extracts. Ptyalin destroyed the FSH activity of extract "A", which had the high content of LH, but had very little effect upon extracts "B" and "C".

Since it was suggested by Purves and Griesbach (1951) that specific cells in the pituitary gland produced specific hormones, and since Li and Evans (1948) reported that the hypophysis possessed three sugar containing hormones, Siperstein, Nichols, Griesbach and Chaikoff (1954) thought that perhaps the basophiles could be responsible for all three hormones. The growth and development of the acidophiles and of the thyrotropin-producing and gonadotropin-producing cells were studied from birth to 11 weeks of age. In keeping with recent knowledge, three

types of glycoprotein containing cells (basophiles) were noted. Two distinct types of gonadotropes could be distinguished, those in the periphery of the gland and those in the central portion. The thyrotropes were strictly confined to the central and posterior regions of the anterior lobe. The gonadotropes preceded the thyrotropes in development as judged by the appearance of the granules. The cells were PAS positive on the first day after birth as evidenced by the diffuse pink color, although the granules did not appear until the seventh day.

Between the 35th and 42nd days, degranulation of the gonadotropes in the female took place, and this was almost certainly due to the release of gonadotropic hormones, coinciding with the first estrus. In seven out of twelve rats the degranulation occurred in the peripheral cells before it occurred in the central gonadotropes. From day 42 onward, no storage of gonadotropin glycoprotein was recognized.

Purves and Griesbach (1954, 1955) reported that in addition to the basophiles being concerned with thyrotropic hormone secretion, the anterior pituitary gland has two specific types of basophile cells which produce gonadotropic hormones. The influence of castration and estrogen administration on the immature female rat pituitary (Lockhart and Finerty, 1955) and the electron microscopic studies of Farquhar and Rinehart (1954) are in agreement with those of Purves and Griesbach. The peripherally situated gonadotropes are distinguished by a coarse cytoplasmic granulation due to glycoprotein with a high sugar content and are considered to be responsible for secretion of FSH. The centrally located gonadotropes have a finer granulation and the glycoprotein reaction is less intense. These are considered to be responsible for the secretion of LH.



Testosterone propionate has a differential effect upon the gonadotropic cells of the adult female rats, promoting granule storage in the peripheral (FSH producing) cells and depressing the granule content (LH producing) cells. These findings support the work of Greep and Jones (1950b) which demonstrated that testosterone propionate stimulated the storage of FSH in the intact female rat.

CHAPTER III

EXPERIMENTAL PROCEDURE

A total of 392 adult female castrate rats were injected with estradiol benzoate in daily doses of 1.0 or 25.0 µg. and each dose being administered for 30 and 180 days and the time determined when the luteinizing hormone (LH) again became demonstrable after cessation of the estrogen administration. The rats were of the Sprague-Dawley and Holtzman strains and were approximately four to seven months of age at the time of oophorectomy. Two months post-operative, subcutaneous administration of estradiol benzoate dissolved in cottonseed oil was begun. The injections were made in 0.05 cc. quantities every other day with the injection sites distributed over the dorsum of the animals. The pituitary glands from these rats were assayed in a total of 323 immature normal or hypophysectomized rats, also of the Holtzman and Sprague-Dawley strains.

In order to determine the length of time required for reappearance of LH in the four groups of rats, the donor animals were autopsied at times ranging from zero to 50 days after terminating the injections, zero indicating the day the injections stopped. At the time of autopsy, the pituitary glands were removed, some being placed in acetone and prepared for assay while others were placed in Zenker-Formol solution in preparation for cytological study.

Those glands placed in acetone were dried, subsequently ground into a fine powder and prepared for administration by being suspended in water (Hellbaum and Greep, 1940). In most cases individual pituitary glands were used for the assay. In other instances, two or more glands were necessary to obtain a response. The majority of the assay animals were hypophysectomized, being operated at 24 days of age and the first injection given 48 hours post-operative. The normal assay rats were 20 to 21 days of age with a weight range of 35 to 40 gms. at the time of the first injection.

The suspensions of dried pituitary tissue were administered in dosages of 0.5 cc. twice daily for three days and the autopsies performed 48 hours after the last injection. At autopsy the results of the injected pituitary tissue were noted by the response in the ovarian weights as well as the presence or absence of corpora lutea and the relative stimulation of the uterus.

The animals from which the pituitary glands were taken for assay were autopsied on the days indicated below:

(1) Estradiol benzoate 1.0 µg. - 30 days and autopsied 0, 2, 6, 9, 12, 15, 18 and 21 days after the injections were stopped.

(2) Estradiol benzoate 1.0 µg. - 180 days and autopsied 4,6, 9, 12, 17, 21, 24 and 28 days after the injections were stopped.

(3) Estradiol benzoate 25.0 µg. - 30 days and autopsied 12, 15, 18, 20, 25 and each five days thereafter to 50 days after injections were stopped.

(4) Estradiol benzoate 25.0 µg. - 180 days and autopsied 15, 18, 19, 20, 21, 24, 25, 26 and 33 days after the injections were stopped.

Observations made at the time of autopsy of the assay animals were as follows: (1) Body weight (2) Vaginal membrane open or intact

(3) Weight of ovaries (4) Gross examination of the ovaries by transillumination to determine the presence or absence of corpora lutea. If corpora lutea could not be discerned grossly, the ovaries were sectioned and studied microscopically. (5) Relative stimulation of the uterus. The stimulation of the uterus is indicated as 1 + to 4 +, one plus indicating that stimulation was present and 4 + representing maximum stimulation.

Two or three pituitary glands were taken for cytological study from each group of injected animals at autopsy at the times specified below:

(1) Estradiol benzoate 1.0 µg. - 30 days and autopsied 2, 12 and 18 days after the injections were stopped.

(2) Estradiol benzoate 1.0 µg. - 180 days and autopsied 4, 12 and 28 days after the injections were stopped.

(3) Estradiol benzoate 25.0 µg. - 30 days and autopsied 2, 12, 18 and 20 days after the injections were stopped.

(4) Estradiol benzoate 25.0 µg. - 180 days and autopsied 6, 25 and 26 days after the injections were stopped.

The cytological studies of the pituitaries were made by Dr. J. C. Finerty, Professor of Anatomy at the University of Texas School of Medicine, Galveston, Texas. The tissue sections were treated with the McManus-Hotchkiss periodic acid Schiff reagent (PAS) which stains glycoprotein and the results were compared with those of Purves and Griesbach (1951, 1954), Wolfe (1949) and Siperstein et al., (1954).

CHAPTER IV

EXPERIMENTAL RESULTS

The results of this study are summarized in Tables I, II, IV, VI and VIII. The tables are divided into two parts. One part pertains to the donor animals which were injected with estradiol benzoate and from which the pituitary glands were taken. The other part pertains to those immature female test rats which were recipients of the donor pituitary glands.

The results of the statistical analysis are summarized in Tables III, V, VII and IX. Since qualitative changes were measured in this experiment, the Chi Square (X^2) method of analysis was used.*

The donor division of the tables as exemplified in Table I summarizes: (1) The number of days intervening between cessation of the injections and the autopsy of the animals. "0" indicates the animals were autopsied the day the injections were stopped, "2" indicates two days after the injections were stopped, etc. (2) The number of pituitary glands tested from each group.

The recipient division of the tables consists of six parts, each part summarizing a specific group of finding as follows: (1) Number of

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*The analyses were made by Carl R. Doering, M.D., C.P.H., D.Sc., Professor of Biostatistics, Department of Preventive Medicine and Public Health, University of Oklahoma School of Medicine.

test animals for each test group. (2) Specific type of test animal, "APx" indicating hypophysectomized immature female rats and "Nor" indicating normal immature female rats. (3) Average body weight of the test animals at the beginning and end of the five day test period. The mean body weight is recorded to demonstrate the weight gain which occurred over the five day assay period. The normal recipients usually gained much more weight than the hypophysectomized animals due to the presence of the rat's own pituitary gland. (4) Vaginal membrane open or intact indicated as "O" and "NO" respectively. (5) Relative uterine stimulation designated in terms of 1 / to 4 / depending on the degree of response. One / indicated that uterine stimulation was present and 4 / indicated a maximum uterine stimulation. (6) Mean ovarian weight in mg. at the end of the five day injection period. (7) The number of animals whose ovaries contained no corpora lutea or contained corpora lutea in addition to follicles, being designated as "NoC" and "C.L." respectively.

The Effects of 1.0 µg. of Estradiol Benzoate Daily for 30 Days on the Pituitary Glands of Castrated Female Rats

Pituitary glands from 154 adult female castrated rats were assayed in 147 immature normal and hypophysectomized rats. Thirteen of the pituitaries were from uninjected animals and served as controls. These were assayed in 26 normal immature test rats. The ovaries of all 26 animals contained corpora lutea. This response occurred regardless of the quantity of pituitary tissue administered or whether the resultant ovaries were large or small. The ovarian weights averaged 53.8 mg. with a range of 21.0 to 143.0 mg. The castrate female rats from which the

pituitaries were obtained were approximately the same age as the animals treated with estradiol benzoate and they were castrated for the same length of time (Table I).

The remaining 141 pituitaries were from animals injected with 1.0 µg. estradiol benzoate daily for 30 days and were assayed in 121 immature normal and hypophysectomized recipients. Twenty donor animals were autopsied on the day the injections were stopped and the type of response induced by the pituitary glands from these rats was determined. Four hypophysectomized recipients received one pituitary gland each while four other hypophysectomized and four normal test rats each were injected with two donor glands.

Of the four recipients receiving only one pituitary gland, three had open vaginas and the uteri of all four stimulated to the extent of 2 +. The mean ovarian weight averaged 22 mg. The ovaries of three of the four recipients contained no corpora lutea; whereas one had corpora lutea in addition to the follicles. All four of the hypophysectomized recipients which received two pituitary glands had open vaginas and 2 + uterine stimulation. The mean ovarian weight was 50 mg. and three of the four possessed ovaries containing no corpora lutea.

Four normal recipient animals each received two donor glands from animals killed on the last day of injections. All four rats had open vaginas and 2 + uterine stimulation. The mean ovarian weight was 46 mg. The ovaries of three of the four recipients contained corpora lutea in addition to follicles.

Six of the eight hypophysectomized recipients injected with pituitary glands from adult rats treated daily for 30 days with 1.0 µg.

Table I

Pituitary Activity of Adult Female Castrate Rats Receiving 1.0 µg. Estradiol Benzoate per Day for 30 days

Donor		Recipients									
Days After Inj.	No.Pits. Stop Adm.	No.	Type	Av.Body Wt. Begin	Av.Body Wt. End	Vagina O	Vagina NO	Ut. Stim.	Av.Wt. Ovary	St. of Ov. NoC.	St. of Ov. C.L.
Uninjected	13	26	Nor	---	---	26	0	/	53.8	0	26
0	4	4	APx	48.5	54.5	3	1	//	22.0	3	1
0	8	4	APx	48.0	54.5	4	0	//	50.0	3	1
0	8	4	Nor	37.0	54.0	4	0	//	46.0	1	3
2	9	9	APx	45.6	51.6	5	4	/	18.0	8	1
6	10	10	APx	49.4	56.6	8	2	//	32.6	5	5
6	6	3	APx	48.0	51.3	3	0	/	101.0	0	3
9	12	12	APx	46.8	49.8	12	0	/	67.6	1	11
9	4	2	APx	45.0	45.5	2	0	/	69.0	0	2
9	1	1	Nor	35.0	50.0	1	0	/	30.0	1	0
9	10	5	Nor	33.2	53.0	5	0	/	94.4	0	5
12	22	22	APx	46.7	49.7	22	0	/	67.2	3	19
12	4	2	APx	49.5	53.5	2	0	/	89.5	0	2
15	6	6	APx	48.2	48.3	6	0	/	61.5	1	5
15	7	7	Nor	36.1	54.4	7	0	/	58.9	2	5
18	15	15	APx	46.0	49.5	15	0	/	56.3	1	14
18	8	8	Nor	37.1	57.6	8	0	/	66.5	2	6
21	6	6	APx	47.0	53.5	6	0	/	73.7	0	6
-	-	21	APx	----	48.0	0	21	0	6.6	-	-
-	-	21	Nor	----	54.0	0	21	0	11.6	-	-

APx: Hypophysectomized animal. Nor: Normal animal. No.: Number Av. Body Wt.: Average body weight in gm. Vagina: O-open; NO-not open Ut. Stim.: Relative stimulation of uterus. Av.Wt.Ovary: Average weight of ovaries in mg. St.of Ov: State of Ovaries. NoC-No corpora lutea; C.L.-- corpora lutea.

of estradiol benzoate possessed ovaries containing no corpora lutea which indicated that relatively little LH was present in the glands. It is to be noted that the ovaries of hypophysectomized animals receiving two pituitary glands were stimulated approximately 600 per cent as compared to 200 per cent in those receiving one gland. The four normal recipients each received two glands, but three of the animals had corpora lutea.

Two days following cessation of the estrogen injections, nine donor animals were autopsied, and the pituitary glands were administered to nine hypophysectomized recipients. The mean ovarian weight was 18 mg. and the ovaries of eight of the nine recipients possessed no corpora lutea. The ovarian weight was increased approximately 200 per cent when compared with that of the controls.

Little or no difference was found in the ovarian weights of the hypophysectomized recipients receiving a single pituitary gland from the donors autopsied on the day estrogen administration stopped as compared with those killed two days later. Eleven of the 13 recipients in these two groups responded with ovaries containing only follicles.

Cytological study of the donor pituitary glands two days after cessation of estrogen administration, Table II, revealed an increased number of basophiles in the angles adjacent to the pars intermedia (Fig. 1).

Sixteen donor animals were autopsied six days after the estrogen injections were stopped, Table I. Ten of the pituitary glands were tested in ten hypophysectomized recipients, five of which had ovaries with no corpora lutea while the remaining five responded with corpora

Table II

Pituitary Cytology of Adult Female Castrate Rats on Different Days Following Cessation of Administration of Estradiol Benzoate

Dose	Duration of Adm.	Days Intervening After Adm.	No. Pits	No. Basophiles	Basophile Granulation	Basophile Degranula.	No. Chromophobe	Prediction of Gonadotropin
1.0 µg.	30 days	2	2	increase in angle, vac.	---	---	---	FSH
1.0 µg.	30 days	18	2	marked excess, vac.	heavy, dark	---	---	high LH
1.0 µg.	180 days	4	3	slight increase	light, near P.Intermed.	---	---	FSH
1.0 µg.	180 days	12	3	extreme basophilia conc, angles	light and dark. more dark, heavy	---	---	high LH
25.0 µg.	30 days	2	2	few	light	some	many hyperactive	low FSH and LH
25.0 µg.	30 days	18	2	marked increase	light and dark, in angle P.Intermed.	---	high conc., adenoma	moderate increase in FSH and LH
25.0 µg.	180 days	6	2	decrease	---	---	many hyperactive	reduced
25.0 µg.	180 days	25	2	moderate increase	light and dark, in angles P.Intermed.	---	possible increase	slight to moderate increase
25.0 µg.	180 days	26	3	1) few 2) increase 3) many	slight light, moderate sized light and dark castration cells	--- --- ---	--- --- ---	variable content

Adm: Administration. Pits: Pituitaries. Angle: Angle adjacent to Pars Intermedia (Fig.1). Vac: Vacuolation. Conc: Concentration. P.Intermed: Pars intermedia.

PITUITARY GLAND, CROSS SECTION
SHOWING GONADOTROPE ZONES
(SCHEMATIC)

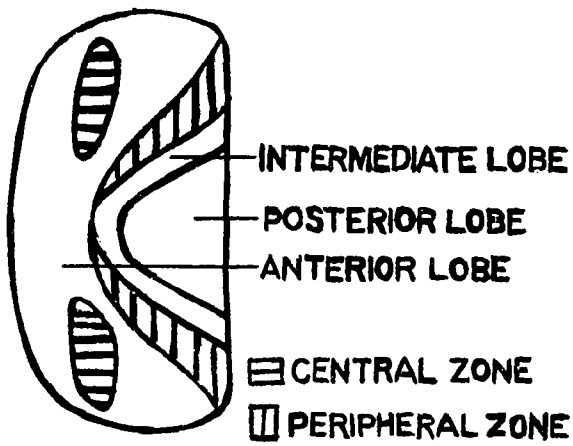

FIG. I

lutea in addition to follicles. The mean ovarian weight was 32.6 mg. Eight recipients showed open vaginas. The uteri responded with a 2 + stimulation. Three hypophysectomized recipients each received two glands. All three rats responded with ovarian corpora lutea, and the mean ovarian weight was 101.0 mg.

All of the responses to injected pituitary glands from rats autopsied six days subsequent to cessation of the injections, indicated that the amount of LH was increasing in the glands. Over 60 per cent of the recipients had ovarian corpora lutea. The tremendous increase in ovarian weight when two glands were administered was quite noteworthy. Recipients receiving one pituitary gland had a 400 per cent increase in

ovarian weight, while those receiving two glands had a 1500 per cent increase in weight. The increase in weight is probably due to the increased amount of both FSH and LH when two glands were administered. Slight increases in the amount of LH in the presence of an adequate amount of FSH have been shown to stimulate a marked increase in the weight of the ovary.

The statistical analysis of the responses from the rat pituitary glands which had been treated with 1.0 μg. of estradiol benzoate for 30 days and autopsied at various times subsequent to cessation of the injections, indicated a highly significant difference in the content of LH in the pituitaries depending on the time of autopsy. The number of recipient animals responding with ovaries containing no corpora lutea was significantly greater when the autopsies were made on or before the sixth day after estrogen treatment was stopped. The ovaries of 20 of the 34 recipients contained no corpora lutea. The probability that this response would have occurred by chance was one in a thousand; or the P value was $<.001$, Table III.

Between the ninth and twenty-first days following cessation of estrogen administration, 96 donor animals were autopsied and the pituitary glands were tested in 87 normal and hypophysectomized recipients. The mean ovarian weights ranged from 56.3 mg. to 94.4 mg., except for one normal recipient in which the ovaries weighed 30.0 mg. Eleven of the 87 recipients possessed ovaries in which no corpora lutea were present, while the ovaries of the remaining 76 contained corpora lutea. One pituitary gland was used per test animal in most of the assays.

The increase in ovarian weight ranged from 500 to 1500 per cent

Table III

Ovarian Response of the Assay Animals to the Pituitary Glands of Castrate Female Rats Treated with 1.0 μg. Estradiol Benzoate for 30 Days

	Days After Cessation of Estrogen Administration				
St.Ov.	0 - 6	9	12 - 15	18 - 21	Total
C.L.	14	18	31	26	89
No C.L.	20	2	6	3	31
Total	34	20	37	29	120
% No C.L.	59	10	16	10	26

$X^2 = 26.9$ $P =< .001$

St.Ov.: Status of the ovaries.
C.L. : Corpora lutea.
No C.L. : No corpora lutea, or follicles only.

over that of the controls. It has been established that in addition to FSH, LH must be present in order for the ovary to develop such an increase in size and weight in a period of 120 hours. The presence of both FSH and LH are also required for the secretion of estrogen by the ovary (Hisaw, 1947). That LH and FSH were present in significant quantities after the ninth day following estrogen administration was further evidenced by the fact that sufficient estrogen was being produced by the ovaries of the recipients to stimulate growth of the uterus and secretions in the uterus to the extent that the vaginas were opened.

A definite change occurred in pituitary cytology by the ninth day after stopping the estrogen injection, evidenced chiefly as a marked increase in vacuolated basophiles, Table II. The most apparent increase was in the central gonadotrope zone (Fig. 1).

Pituitary Gland Assays after Administering 1.0 µg. of Estradiol Benzoate for 180 Days

The recipient responses to the pituitaries of this group, totaling 64 adult female castrate donor rats, are tabulated in Table IV. The glands were tested in 51 normal and hypophysectomized recipients.

Twelve donor animals were autopsied four days after the estrogen injections were terminated. Two glands were tested in two hypophysectomized recipients and neither of them had open vaginas nor uterine stimulation. The mean ovarian weight was 12 mg., an increase of less than 100 per cent, and the ovaries contained no corpora lutea. This response suggested that the pituitaries had a low content of both FSH and LH, since only follicles were present in the ovaries and no estrogen was being

Table IV

Pituitary Activity of Adult Female Castrate Rats Receiving 1.0 µg. Estradiol Benzoate per Day for 180 Days

Donor		Recipients									
Days After Inj.	No.Pits. Stop Adm.	No.	Type	Av. Body Wt. Begin	Av. Body Wt. End	Vagina O	Vagina NO	Ut. Stim.	Av.Wt. Ovary	St. of Ov. NoC.	St. of Ov. C.L.
4	2	2	APx	45.0	51.5	0	2	0	12.0	2	0
4	10	5	APx	45.0	44.2	5	0	+	57.8	1	4
6	1	1	APx	50.0	51.0	0	1	0	8.0	1	0
6	12	6	APx	46.2	51.5	5	1	+	70.7	1	5
9	12	12	APx	47.4	53.1	8	4	+	28.7	6	6
12	11	11	APx	49.2	54.5	11	0	+	57.4	3	8
12	1	1	Nor	35.0	53.0	1	0	+	89.0	0	1
17	2	1	APx	47.0	49.0	1	0	+	63.0	0	1
21	7	7	APx	51.3	55.9	6	1	+	50.4	2	5
21	1	1	Nor	35.0	55.0	1	0	+	107.0	0	1
21	2	1	Nor	35.0	55.0	1	0	+	122.0	0	1
24	1	1	APx	51.0	53.0	1	0	+	106.0	0	1
28	2	2	APx	51.0	53.5	2	0	+	69.0	0	2
-	-	21	APx	----	48.0	0	21	0	6.6	-	-
-	-	21	Nor	----	54.0	0	21	0	11.6	-	-

APx: Hypophysectomized animal. Nor: Normal animal. No.: Number Av. Body Wt.: Average body weight in gm. Vagina: O-open; NO-not open Ut. Stim.: Relative stimulation of uterus. Av.Wt.Ovary: Average weight of ovaries in mg. St.of Ov.: State of Ovaries. NoC-No corpora lutea; C.L.--corpora lutea.

produced by the ovaries as evidenced by the lack of uterine stimulation and intact vaginal membranes.

The remaining ten donor pituitary glands from rats autopsied four days after termination of the injections were tested in five hypophysectomized recipients. The response was somewhat different from that obtained when one gland was administered. The ovaries of four of the five animals responded with the formation of corpora lutea. All five animals had open vaginas and 1 + uteri. The mean ovarian weight was 57.8 mg. which was approximately an 800 per cent increase of that of the controls.

The cytological study, summarized in Table II, of the pituitary glands of animals treated with 1.0 µg. of estradiol benzoate for 180 days and autopsied four days after cessation of the injections showed a slight increase in the number of basophiles. The cytoplasm of the basophiles stained lightly and were concentrated in an area adjacent to the pars intermedia (Fig. 1).

On the sixth day following the termination of estrogen administration, 13 donor rats were autopsied. One pituitary gland was tested in one hypophysectomized recipient with no apparent response as evidenced by an intact vaginal membrane, no uterine stimulation and ovaries weighing 8 mg. with no corpora lutea.

Twelve donor pituitary glands were tested in six hypophysectomized recipients. The mean ovarian weight was 70.7 mg. with five of the six recipients possessing ovaries with corpora lutea. Five of the six recipients also had open vaginas and the mean uterine stimulation was 1 +.

Twelve donor animals were autopsied on the ninth day after estrogen administration was stopped, and their pituitary glands were

tested in 12 hypophysectomized rats. Eight of the 12 animals responded with open vaginas. The mean uterine stimulation was 1 +. The mean ovarian weight was 28.7 mg., a 300 per cent increase over that of the controls. Six of the 12 animals possessed ovaries containing no corpora lutea. The response was suggestive of the fact that LH had returned to the pituitaries in relatively large quantities. Only one gland was necessary to produce a response in each test rat.

Of the 26 recipients receiving pituitary glands from animals autopsied on the fourth to ninth days, the ovaries of 11, or 42 per cent showed no corpora lutea, Table V. On the other hand, of the 25 test animals receiving glands from animals autopsied on the 12th to 28th days after the estrogen injections were stopped, the ovaries of only five, or 20 per cent, contained no corpora lutea.

From the cytological studies, see Table II, of the pituitary glands of the donors autopsied on the 12th day after terminating the estradiol benzoate injections which had been given in a 1.0 µg. daily dose for 180 days, it was apparent that an extensive basophilia had occurred. The cells were concentrated in the angle adjacent to the pars intermedia. Some of the cells contained light staining granules and some contained dark staining granules. However, those with dark, coarse granules were present in larger numbers.

An analysis, using the "t" test, indicated that no significant difference existedin the time required for the return of LH in the two groups which received 1.0 µg. estradiol benzoate per day for 30 and 180 days.

Table V

Ovarian Response of the Assay Animals to the Pituitary Glands of Castrate Female Rats Treated with 1.0 µg. Estradiol Benzoate for 180 Days

	Days After Cessation of Estrogen Administration		
St.Ov.	4 - 9	12 - 28	Total
C.L.	15	20	35
No C.L.	11	5	16
Total	26	25	51
% No C.L.	42	20	31
	$X^2 = 2.13$	$P =< .10$	

St.Ov.: Status of the ovaries.
C.L.: Corpora lutea.
No C.L.: No corpora lutea, or follicles only.

Changes in the Pituitary Glands of Donor Rats Receiving 25.0 µg. of Estradiol Benzoate for 30 Days

This injection group included 131 donor animals and 97 recipients, Table VI. The recipients were normal and hypophysectomized immature female rats.

Ten donor animals were autopsied on the day the estrogen injections were terminated and their pituitary glands were tested in three hypophysectomized recipients. Three glands were administered to each of two recipients and four glands were given to one animal. There was no stimulation in these animals as indicated by the lack of corpora lutea, significant increase in ovarian weight, or stimulation of the uterus. This response suggests that no significant amounts of either LH or FSH were present.

The pituitary cytology, Table II, of the two glands subjected to 25.0 µg. of estradiol benzoate per day for 30 days and autopsied two days later, showed a remarkable change in the cellular structure due to the effect of estrogen. Few basophiles were present and those possessed only a slight granulation. Some degranulation was also apparent and many hyperactive chromophobes were noted.

On the twelfth day after termination of estrogen administration 22 donor animals were autopsied and their pituitary glands were tested in ten hypophysectomized receipients. Sixteen glands were administered to eight recipients. The mean ovarian weight was 18.8 mg., a 200 per cent increase. Seven of the eight recipients had ovaries containing no corpora lutea. The mean uterine stimulation was 1 +.

Table VI

Pituitary Activity of Adult Female Castrate Rats Receiving 25.0 µg. Estradiol Benzoate per Day for 30 Days

Donor		Recipients									
Days After Inj.	No.Pits. Stop Adm.	No.	Type	Av. Body Wt. Begin	Av. Body Wt. End	Vagina O	Vagina NO	Ut. Stim.	Av.Wt. Ovary	St. of Ov. NoC.	St. of Ov. C.L.
0	6	2	APx	50.0	58.5	0	2	0	8.0	2	0
0	4	1	APx	41.0	41.0	0	1	0	8.0	1	0
12	16	8	APx	47.1	52.3	5	3	/	18.8	7	1
12	6	2	APx	46.0	51.0	2	0	/	90.5	0	2
15	1	1	APx	53.0	52.0	0	1	0	8.0	1	0
15	8	4	APx	48.3	56.3	4	0	/	34.0	3	1
18	20	20	APx	49.0	55.1	13	7	/	21.6	13	7
20	3	3	APx	45.7	52.0	3	0	/	42.0	1	2
25	12	12	APx	45.1	47.4	11	1	/	61.4	1	11
25	8	4	APx	46.0	48.3	4	0	//	66.5	2	2
25	2	2	Nor	35.0	49.0	2	0	//	59.0	0	2
30	6	6	APx	46.2	50.2	6	0	/	63.7	1	5
30	4	2	APx	44.0	54.5	2	0	/	78.0	0	2
35	8	8	APx	46.5	46.9	7	1	//	53.0	0	8
40	7	7	APx	46.6	48.9	7	0	/	60.1	0	7
45	9	9	APx	47.7	48.3	6	3	//	42.8	2	7
50	3	3	APx	46.7	48.7	3	0	/	62.7	0	3
-	-	21	APx	----	48.0	0	21	0	6.6	-	-
-	-	21	Nor	----	54.0	0	21	0	11.6	-	-

APx: Hypophysectomized animal. Nor: Normal animal. No.: Number Av. Body Wt.: Average body weight in gm. Vagina: O-open; NO-not open Ut. Stim.: Relative stimulation of uterus. Av.Wt.Ovary: Average weight of ovaries in mg. St.of Ov: State of Ovaries. NoC-No corpora lutea; C.L.--corpora lutea.

Six of the donor glands from animals autopsied 12 days after stopping the injections were tested in two hypophysectomized recipients. The response to three glands appeared to be quite different from those which received two glands. Both recipients had ovaries containing corpora lutea. The mean ovarian weight was 90.5 mg., approximately a 1400 per cent increase over that of the controls as compared to a 200 per cent increase in those receiving two glands. The vaginas were open and the uteri showed a 1 + stimulation.

Nine donor animals were autopsied 15 days after the estrogen injections were terminated. The pituitary glands were all tested in hypophysectomized recipients. One gland was tested in one recipient and the response was no stimulation. Eight donor glands were tested in four recipients, three of which possessed ovaries containing no corpora lutea. The mean ovarian weight was 34.0 mg., a 400 per cent increase. All of the recipients responded with open vaginas and 1 +.

On the 18th day after the injections were terminated, 22 donor animals were autopsied. Twenty glands were tested in hypophysectomized recipients and two were fixed for cytological study. Thirteen of the 20 recipients had ovaries containing no corpora lutea. The mean ovarian weight was 21.6 mg., a 200 per cent increase. The vaginas were open in all of the 13 recipients.

The cytological appearance of the pituitary glands taken 18 days after termination of the estrogen injections was quite different from that of those glands removed two days after stopping the injections. The response in these pituitaries was one of marked basophilia. Both gonadotrope cell types were increased, i.e., those with the light staining

granules. The cells were especially numerous in the angles adjacent to the pars intermedia. A high concentration of chromophobes was also present.

The statistical summary of the responses of the pituitary glands taken on or before the 18th day after termination of the estrogen administration is given in Table VII. Thirty of the 41 recipients, or 73 per cent, responded with ovaries which contained no corpora lutea. Testing this response with the Chi Square method indicated a significant difference in the number of responses with no corpora lutea in those animals autopsied on or before the 18th day and those autopsied after the 18th day. The P value was <.001. After the 18th day, quite a different response was apparent. The ovaries of 49 of the 56 recipients contained corpora lutea. Between the 20th and 30th days after cessation of the injections, 17 per cent of the recipients had no ovarian corpora lutea, between the 35th and 50th days only 7 per cent responded with no ovarian corpora lutea.

The Alterations in Pituitary Gland Activity After Administering 25.0 μg. of Estradiol Benzoate per Day for 180 Days

A summary of responses produced in 44 normal and hypophysectomized recipients when given pituitary glands from 56 donor animals injected with 25.0 μg. of estradiol benzoate for 180 days is found in Table VIII.

Cytology of the donor pituitary glands treated with 25.0 μg. of estradiol benzoate for 180 days and removed from the animals six days after the cessation of the estrogen injections showed a reduction in the number of basophiles, Table II. The number of chromophobes was much increased.

Table VII

Ovarian Response of the Assay Animals to the Pituitary Glands of Castrate Female Rats Treated with 25.0 µg. Estradiol Benzoate for 30 Days

	Days After Cessation of Estrogen Administration			
St.Ov.	0 - 18	20 - 30	35 - 50	Total
C.L.	11	24	25	60
No C.L.	27	5	2	34
Total	38	29	27	94
% No C.L.	71	17	7	36
	$X^2 = 34.0$		$P =< .001$	

St.Ov.: Status of the ovaries.
C.L.: Corpora lutea.
No C.L.: No corpora lutea, or follicles only.

Table VIII

Pituitary Activity of Adult Female Castrate Rats Receiving 25.0 µg. Estradiol Benzoate per Day for 180 Days

Donor		Recipients									
Days After Inj.	No.Pits. Stop Adm.	No.	Type	Av. Body Wt. Begin	Av. Body Wt. End	Vagina O	Vagina NO	Ut. Stim.	Av.Wt. Ovary	St. of Ov. NoC.	St. of Ov. C.L.
15	3	3	APx	57.3	62.0	1	2	/	14.7	3	0
15	12	6	APx	54.3	58.8	5	1	/	28.0	5	1
15	1	1	Nor	35.0	50.0	1	0	/	12.0	1	0
18	2	2	APx	46.5	54.5	2	0	//	15.0	1	1
18	4	2	APx	44.5	45.0	2	0	/	54.5	0	2
19	3	3	APx	47.0	50.0	3	0	/	48.3	0	3
19	2	1	Nor	34.0	52.0	1	0	/	102.0	0	1
20	10	10	APx	47.7	51.0	5	5	/	11.4	9	1
20	2	1	APx	47.0	47.0	1	0	/	93.0	0	1
21	2	1	APx	46.0	52.0	1	0	/	77.0	0	1
24	2	1	APx	53.0	65.0	1	0	/	27.0	1	0
25	10	10	APx	45.6	46.6	10	0	/	47.6	1	9
26	2	2	APx	48.0	56.0	2	0	/	59.0	0	2
33	1	1	APx	48.0	56.0	1	0	/	54.0	0	1
-	-	21	APx	----	48.0	0	21	0	6.6	-	-
-	-	21	Nor	----	54.0	0	21	0	11.6	-	-

APx: Hypophysectomized animal. Nor: Normal animal. No.: Number
Av. Body Wt.: Average body weight in gm. Vagina: O-open; NO-not open. Ut. Stim.: Relative stimulation of uterus. Av.Wt.Ovary: Average weight of ovaries in mg. St. of Ov: State of Ovaries. NoC-No corpora lutea; C.L.-corpora lutea.

Sixteen donor animals were autopsied on the 15th day after terminating 180 days of estrogen administration. Three donor pituitaries were tested in three hypophysectomized recipients. One animal had an open vagina. The mean uterine stimulation was 1 /. The mean ovarian weight was 14.7 mg., an increase of approximately 100 per cent and all three recipients possessed ovaries which contained corpora lutea.

Twelve donor pituitaries from animals autopsied on the 15th day were tested in six hypophysectomized recipients. Five of the recipients had open vaginas. The mean uterine stimulation was 1 / and the mean ovarian weight was 28.0 mg., a 300 per cent increase. Ovaries of five of the six animals responded with no corpora lutea.

One donor gland from an animal autopsied on the 15th day was tested in a normal recipient. The response was an open vagina, 1 / uterus, an ovarian weight of 12.0 mg. and ovaries containing no corpora lutea.

On the 18th day after termination of the estrogen injections, six donor animals were autopsied, and the pituitaries were assayed in hypophysectomized recipients. The mean ovarian weight of the test animals was 15.0 mg. The ovaries of one animal contained no corpora lutea. Both animals had open vaginas and 2 / uteri. The remaining four donor pituitaries were tested in two hypophysectomized recipients. The ovaries of both animals contained corpora lutea, and they both had open vaginas and 1 / uteri. The mean ovarian weight was 54.5 mg., an 800 per cent increase.

On the 19th day after termination of estrogen administration, five donor animals were autopsied. Pituitaries from three of them were tested in three hypophysectomized recipients. The ovaries all contained corpora lutea and the uteri were all stimulated. The mean ovarian weight

was 48.3 mg., an 800 per cent increase. Two pituitaries were tested in one normal recipient. The response was an open vagina, 1 / uterus, 102.0 mg. ovaries and the ovaries contained corpora lutea.

Twelve donor animals were autopsied on the 20th day after termination of the estrogen injections. Ten pituitaries were tested in ten hypophysectomized recipients. The mean ovarian weight was 11.4 mg., less than a 100 per cent increase. Five of the recipients responded with open vaginas and 1 / uteri. None of the ten recipients possessed ovaries with corpora lutea. Two donor pituitaries were tested in one hypophysectomized test animal. The response was an open vagina, 1 / uterus and 93.0 mg. ovaries which contained corpora lutea.

By referring to Table IX, one sees a significant difference between the recipients killed before the 20th day and those killed after the 20th day. The ovaries from 19 of 29, or 66 per cent, of the recipients killed before the 20th day contained no corpora lutea as compared to two of 15, or 13 per cent, of those killed after the 20th day. The P value was $< .001$.

Cytological studies were made of the pituitary glands of animals treated with 25.0 µg. of estradiol benzoate per day for 180 days and autopsied 25 and 26 days after termination of the injections. The pituitaries from those animals killed on the 25th day appeared to have a moderate increase in basophiles. Both light and dark granulated cells were present, especially in the angles adjacent to the pars intermedia. A possible increase in the number of chromophobes was also present. In those killed the 26th day after stopping the injections, a variable response was apparent. The three pituitaries of this group were

Table IX

Ovarian Response of the Assay Animals to the Pituitary Glands of Castrate Female Rats Treated with 25.0 µg. Estradiol Benzoate for 180 Days

	Days After Cessation of Estrogen Administration		
St. Ov.	15 - 20	21 - 33	Total
C.L.	10	13	23
No C.L.	19	2	21
Total	29	15	44
% No C.L.	66	13	48
	$X^2 = 11.4$	$P = < .001$	

St.Ov.: Status of the ovaries.
C.L.: Corpora lutea.
No C.L.: No corpora lutea, or follicles only.

characterized as follows: (1) A few basophiles, with slight granulation. (2) Increased number of basophiles, light granulation and moderate sized cells. (3) Many basophiles with both dark and light granulated cells. Castration cells were also present.

A statistical analysis, using the "t" test, indicated that no significant difference existed between the responses elicited from animals receiving pituitary glands from donors being treated with 25.0 µg. daily doses of estradiol benzoate for 30 and for 180 days.

However, a definite significant difference did exist between the responses induced in the recipients from pituitaries of donors receiving 1.0 µg. and those receiving 25.0 µg. of estradiol benzoate per day. The period of time required before the pituitaries from estradiol benzoate treated animals would again produce ovarian corpora lutea, was two to three times longer in those receiving 25.0 µg.

Ovaries from the assay animals were sectioned and stained with hematoxylin and eosin. Photomicrographs (16x) were made of the ovarian sections which were representative of the responses to pituitary suspensions for each of the four injection groups (Fig. 2).

Figure 2. Ovarian sections from assay animals which have been injected with pituitary tissue suspensions from four groups of rats treated with estradiol benzoate in 1.0 and 25.0 µg. daily doses for 30 and 180 days. (See next page.)

1. Ovarian section from an immature test rat which received pituitary tissue from an animal injected with 1.0 µg. estradiol benzoate daily for 30 days. Autopsy was performed shortly after terminating the injections and before LH had reappeared in the pituitary gland. The ovary shows only follicles with no lutein tissue present.

2. Ovarian section from an immature test animal which received pituitary tissue from an animal injected with 1.0 µg. estradiol benzoate daily for 30 days, but autopsied at a time after LH had reappeared in the pituitary gland. The ovary is much denser, with more interstitial tissue, with corpora lutea and a few follicles.

3. Ovarian section from assay animal which was given pituitary tissue from a donor animal injected with 1.0 µg. estradiol benzoate daily for 180 days. Autopsy was performed shortly after stopping the injections and before LH had reappeared. Only follicles are noted.

4. Ovarian section from a test animal which was given pituitary tissue from an adult female castrate rat injected with 1.0 µg. estradiol benzoate daily for 180 days. Autopsy was after LH had reappeared. A number of corpora lutea are present in addition to follicles.

5. Ovarian section from an asaay animal treated with pituitary tissue from a donor rat which was injected with 25.0 µg. estradiol benzoate daily for 30 days. Autopsy was prior to the time of LH return. No lutein tissue is present, however, numerous follicles are.

6. Ovarian section from an assay animal given pituitary tissue from a donor animal which had been treated with 25.0 µg. estradiol benzoate daily for 30 days and autopsied after LH had reappeared. An enlarged ovary, thickened interstitial tissue, lutein tissue (corpora lutea) and numerous follicles are noted.

7. Ovarian section from test animal treated with pituitary tissue from a donor injected daily for 180 days with 25.0 µg. estradiol benzoate. Autopsy was performed prior to return of LH. No lutein tissue is present.

8. Ovarian section from an assay animal which was injected with pituitary tissue from an animal which was treated with 25.0 µg. estradiol benzoate daily for 180 days. Autopsy was performed after the return of LH to the pituitary gland as evidenced by the presence of corpora lutea, thickened interstitial tissue and relatively few cystic follicles.


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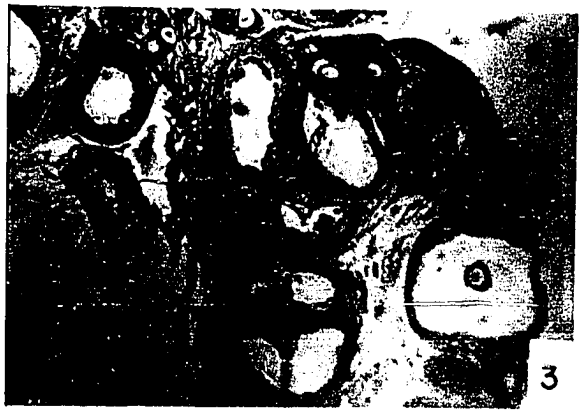
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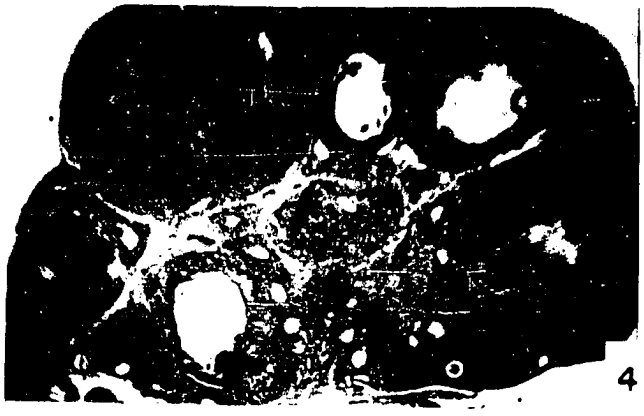
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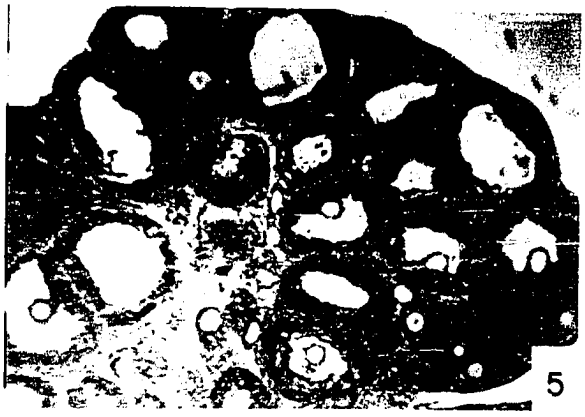
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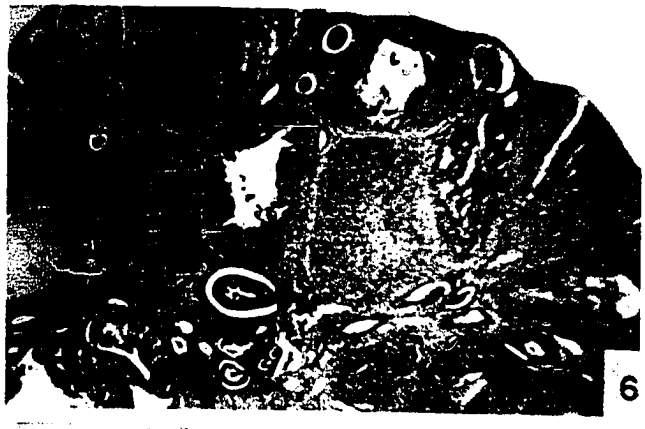
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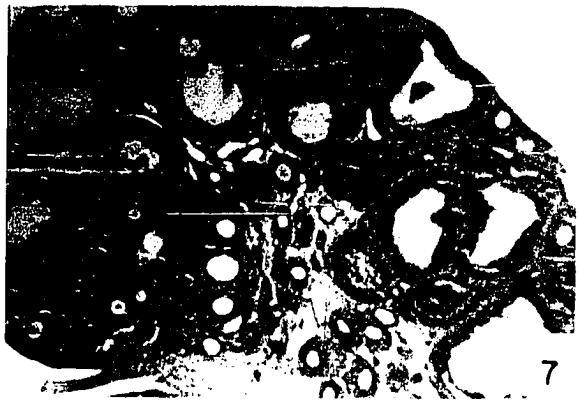
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CHAPTER V

DISCUSSION

For two decades it has been evident that hormones are produced by the pituitary gland as well as by the gonads, and that the hormones from each influenced the function of the other gland reciprocally. The specific phases of the synchronized interrelationships and interactivity of the anterior pituitary gland and the gonads have not been clarified. In such a relationship it is important to recognize that the gonadotropic influence of the pituitary does not stem from a single hormone but consists of several factors, each capable of separate and independent action. Likewise in considering a secretory product of an endocrine gland the term "secretion" implies two phases, namely: 1) That which is concerned with the production, synthesis or elaboration of a substance, as distinquished from 2) Its release, liberation or expulsion from the gland.

Severinghaus (1939), from the histological picture of the pituitary gland, believed that the secretion occurred in three phases: synthesis (elaboration), storage and release (elimination) of secretory products. Dawson (1946) concurred with Severinghaus. Wolfe (1949) reported cytological evidence of stimulation of both elaboration and release of pituitary secretions after estrogen administration.

The changes in the pituitary gland cytology following castration have been known since Fischera's observation (1905) who also described

the castration cell. The increased potency of the pituitary gland following castration in stimulating the gonads has been demonstrated repeatedly by many investigators. Engle (1929) noted that the ovarian response to pituitary implants was much increased when the pituitary glands were obtained from castrate animals. Evans and Simpson (1929) reported that the accumulation of gonadotropic activity occurred most rapidly within the first two weeks after castration. Hellbaum and Greep (1940) observed that a demonstrable increase in the concentration within the pituitary gland of LH and FSH, as separate entities, likewise occurred within two weeks post-castration. The work of Purves and Griesbach (1955) is in agreement with observation of Hellbaum and Greep and is further substantiated by the morphological findings.

With the knowledge that LH and FSH are found in the pituitary gland in increased quantities following castration, the question arises concerning the mechanism of action of the gonadal hormones as they influence the different pituitary gonadotropic factors. There is quite general agreement that the effect of estrogen upon FSH is one of inhibition or suppression. The concept of estrogen inhibition was introduced by Moore and Price (1930). Since that time numerous workers have concurred with the inhibition concept (Leonard, Meyer, Hisaw, 1931; Meyer, Leonard, Hisaw and Martin, 1932; Lipschutz, 1935; Byrnes and Meyer, 1951a, b). Post-castration hypersecretion of FSH was prevented in parabiotic immature female rats with small doses of estrogen (Byrnes, Meyer and Finerty, 1954; Byrnes, 1951; Finerty and Meyer, 1950; Miller and Pfeiffer, 1950) and with ovarian intrasplenic grafts (Barahona, et al., 1950; Lipschutz, et al., 1948; Jungck, et al., 1947).

The specific nature of the influence of estrogen on FSH secretion as demonstrated in these investigations can not be stated with any certainty. It is believed, however, that a definite suppression of FSH secretion does occur since direct assays demonstrated a definite dimunition in the amount of FSH present in the pituitary glands. The cylological studies also gave evidence of a decreased amount of FSH, since the dark granulated basophiles, considered to be the site of FSH secretion (Purves and Griesbach, 1954, 1955), were decreased in number and were degranulated.

The effect of estrogen on the luteinizing hormone is somewhat different from the effect upon FSH. It appears to stimulate a release of LH from the pituitary gland (Fevold, et al., 1936; Fevold and Fiske, 1939; Hellbaum and Greep, 1943; Hellbaum and Greep, 1946; Hisaw, 1947; Greep and Jones, 1950b; Funnell, Keaty and Hellbaum, 1951).

The general consensus of opinion among investigators is quite consistent regarding the release effect of estrogen upon the LH. However, there is no general agreement as to how estrogen affects the synthesis of LH. Greep and Jones (1950a) believe that the fundamental effect of estrogen is one of reducing the synthesis of LH. Three reasons are given for this concept: 1) Immature male rats injected with estrogen show no growth of accessory sexual structures. 2) Estrogen given to mature male rats is notably effective in producing marked atrophy of the testes and accessory sexual structures. 3) Estrogen is completely effective in abolishing the LH potency of the castrate rat's pituitary and contrariwise results in enriched pituitary LH potency.

When the work of Victor and Anderson (1937) is examined, the

concept of Greep and Jones (1950a) is less tenable. They found by using the Warburg apparatus that anterior pituitary tissue which had been subjected to estrogen treatment showed a distinct rise in metabolic activity. It appears somewhat contradictory for a tissue to be inhibited while at the same time possessing an increased metabolic activity.

The present study is an attempt to determine the effect of estrogen upon the interglandular synthesis of LH. The study was designed to determine the relative periods of time required for LH to return in significant amounts after it had been removed from the pituitary glands of adult female castrate rats treated with estradiol benzoate in doses of 1.0 and 25.0 µg. daily for 30 and 180 days. Animals were autopsied on different days after terminating the estrogen treatments and the pituitary glands tested for the presence of LH.

The response of the recipient assay animals to the pituitary glands from the donor castrate female rats was not significantly different whether a given dose of estradiol benzoate was administered for 30 or for 180 days.

The amount of LH in the pituitary glands of the donor animals treated with a 1.0 µg. daily dose of estradiol benzoate was much reduced at the time the injections were terminated as well as two to four days afterward. Corpora lutea were found in but a small number of the recipient's ovaries during this time. It was also apparent that these donor pituitaries were not completely devoid of LH, since with the injection of larger amounts of pituitary tissue or the use of a normal assay animal with its own pituitary contributing LH, corpora lutea were present in a greater number of the recipient's ovaries.

The cytological appearance of the pituitary glands treated with a 1.0 µg. daily dose of estradiol benzoate and autopsied two to four days after stopping the injections also gave the impression that both FSH and LH were present in small quantities, with LH being present in the least amount. It was also apparent that FSH was increasing in amount even at two to four days after stopping the injections. This was evidenced by the fact that the FSH producing basophiles were concentrated in numbers greater than normal in the area adjacent to the pars intermedia (Fig. 1). It is a known fact that changes in the amounts of estrogen in the body are reflected in the pituitary cytology within a very few days or hours (Lockhart and Finerty, 1955; Purves and Griesbach, 1955).

The pituitary gland assays indicate a gradual but progressive increase in the amount of LH as the period of time lengthens after the estrogen injections were stopped. A precipitous and significant increase occurred between the sixth and twelfth days after the 1.0 µg. dose of estradiol benzoate was terminated. This break was not as clear cut in those animals treated for 180 days as it was in those treated for 30 days, but it was thought that this may have been due to the fact that most of the recipients received two pituitaries which increased the amount of FSH and LH to the extent that the ovaries of a greater number of the test animals contained corpora lutea.

Both the cytological appearance and the recipient responses to the pituitary glands from rats treated with a daily dose of 25.0 µg. indicated that the concentration of LH as well as FSH was markedly reduced in comparison with those treated with 1.0 µg. Up to four pituitaries were required to produce a response in the ovaries of the recipients.

Cytologically, the number of basophiles was greatly reduced and many hyperactive chromophobes were present.

A longer period was required for LH to return to the pituitaries from animals treated with 25.0 µg. than in those treated with 1.0 µg. It was again apparent in the pituitaries subjected to the high doses of estrogen that LH returned gradually and progressively, but the sharp increase in LH did not occur until between the 18th and 21st days. After the 21st day the assays as well as the cytological appearance indicated that LH and FSH had returned.

The cytological study of the pituitaries of all animals which received 1.0 µg. estradiol and were autopsied at different times after terminating the estrogen treatment showed some evidence of the reappearance of the basophiles, although this was least apparent in the glands of those autopsied two and four days after injections were stopped. In these the greatest concentration of basophiles was located at the angles of the junction between the edge of the gland and the pars intermedia.

In those animals receiving the low dose of estradiol and autopsied at 12, 18 and 24 days following cessation of injections, cytology revealed typical long-term castration effects in all cases. The 1.0 µg. dose was insufficient to produce complete inhibition.

On the other hand, those rats which received the 25.0 µg. dose and were autopsied two and six days after stopping the injections, showed evidence of degranulation and suppression of basophiles, typical of high estrogen treatment. Many hyperactive chromophobes were present and contained enlarged, prominent negative Golgi images. These glands were suspected to have little or no gonadotropin content and assays confirmed

the suspicion. In those animals which were autopsied 18, 25 and 26 days after cessation of the injections, the pituitary glands were returning toward a castrate condition, but not to the same extent as those which received the lower dose. The pituitaries of all the high-estrogen treated rats showed areas of chromophobe hyperplasia which indicated incomplete recovery from the shock of the high dose of estradiol benzoate.

The early reappearance of the dark granular cells, tends to indicate that FSH had returned to the basophiles prior to the return of LH. This is verified by the assay studies, since the first stimulation occurring in the assay animal after stopping the injections was primarily follicles followed later by corpora lutea.

The physiological level of estrogen appears to be the equivalent of approximately 0.050 to 0.075 µg. of estradiol benzoate (Hellbaum, McArthur and Pritschow, 1954; Byrnes and Meyer, 1951b). Estrogen at this level may have little or no effect upon LH synthesis. However, estradiol benzoate administered to castrate female rats in doses ten to 250 times that of the physiological level (1.0 to 25.0 µg.) does have a demonstrable effect upon the synthesis of LH. This is indicated by the fact that several days are required for LH to return to the pituitary gland after terminating the estrogen injections.

The results obtained in the present investigation do not present a sharp demarcation to indicate when LH was present. There was no time when the immature test ovaries exhibited only follicles without corpora lutea in 100 per cent of the animals which would indicate an absence of the luteinizing factor. Neither was there a period when all of the

ovaries contained corpora lutea. The differences were relative, requiring statistical analysis. The lack of a sharp dividing line might be attributable to a number of factors.

The donor animals were all approximately the same age, but as in any biological population variation in weight and size was considerable. Also, individual variation from animal to animal in their responsiveness or sensitivity to a given dose of estrogen might be a factor. This fact was particularly evident in one group of pituitaries treated with 25.0 µg. of estradiol benzoate for 180 days and killed 26 days afterward (Table II).

A daily dose of 1.0 µg. of estradiol benzoate might be sufficient to liberate the LH from the pituitaries in most animals, but in a certain few considerably more may have been required. A difference among the animals as to the degree of adaptability and recovery following the injection periods must be considered. Also, the health status of the individual animals could be expected to show considerable differences, and this might in turn influence their responsiveness to the administered estrogens.

An important consideration in this study is post-injected absorption of estradiol benzoate, since it was injected in oil and part of the injections were continued over a long period of time. The injections were scattered over the dorsum of the animals in an attempt to prevent pooling of the oily injection material. Since there was no significant difference between the time required for LH to reappear in the pituitary gland in those injected for 30 days as compared to 180 days, although the animals receiving the estrogen for 180 days received six times the

total dose of those receiving injections for 30 days, it is thought that post-injection absorption was not a factor which markedly altered the total results. However, it could have entered into the individual responses.

Hisaw (1947) stated that a balance between FSH and LH was necessary for pre-ovulatory swelling, ovulation and corpus luteum formation. This fact is important since a bioassay method which uses these phenomena was employed.

Hypophysectomized immature Holtzman rats were used for most of the pituitary assays. It is well established that not all such test animals, even though from a well standardized and genetically homogeneous group, respond equally to a given dose of gonadotropins. Also, it cannot be stated with certainty that all of the test animals were completely hypophysectomized. The sella turcica of each animal was examined grossly at autopsy and in those where complete removal of the pituitary gland was questioned, the results were not included. However, since the sellas were not examined microscopically, it is possible that microscopic amounts of pituitary tissue were present in some, consequently influencing the results.

CHAPTER VI

CONCLUSIONS

Estradiol benzoate stimulates the release of luteinizing hormone.

Estradiol benzoate inhibits follicle stimulating hormone.

Estradiol benzoate at physiological levels (0.050 to 0.075 µg.) may have little or no effect on the synthesis of LH, whereas demonstrable effects were attained when estradiol benzoate was administered in doses which were 10 to 250 times (1.0 to 25.0 µg.) that of the normal level.

No greater effect on LH synthesis was found when estradiol benzoate was given for 180 days than when given for 30 days.

The time required for LH to return to the pituitary gland after terminating the injections is 18 to 21 days in those animals receiving a daily dose of 25.0 µg. of estradiol benzoate as compared to six to nine days in those receiving a daily dose of 1.0 µg.

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