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FACTORS INFLUENCING RELEASE OF LUTEINIZING HORMONE FROM THE PITUITARY GLAND

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FACTORS INFLUENCING RELEASE OF LUTEINIZING HORMONE FROM THE PITUITARY GLAND

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FACTORS INFLUENCING RELEASE OF LUTEINIZING HORMONE FROM THE PITUITARY GLAND

CHAPTER I

INTRODUCTION

Observations of infertility following castration were recorded in the Bible and other early written history. This phenomenon was readily detected in the male, and a simple cause effect relationship was derived. When impotency occurred without surgical castration, the condition was not understood. In the female infertility due to the loss of ovarian function was not recognized because of the internal location of the ovaries.

With the advent of regular autopsies, the etiology of infertility was ascribed to abnormalities in several sites in the body. Chief among these were dysfunction of the testes or the ovaries, but abnormalities of these structures were not always observed after death. After some years and many observations it was realized that disturbed function of the pituitary gland was often related to infertility and testicular or ovarian atrophy.

These findings aroused considerable interest among many investigators and initiated a new field of biological

investigation. It was necessary to know whether or not a pituitary-gonadal interrelationship existed and if so, whether it was neurogenic or hormonal in nature. It was desirable to establish what anatomical portions of these structures were concerned in the physiology of reproduction, and a knowledge of the sequence of events which occur during normal physiological estrus was essential.

The purpose of the present report is to present further evidence for the existence of separate pituitary gonadotropins and to clarify the discrete and independent mechanisms which control their production and release by the pituitary gland. Although they work in close relationship with each other, their respective effects on the gonads are different. Particular emphasis will be given to the action of various factors influencing release of the luteinizing hormone (LH) from the pituitary gland.

Experiments were performed to evaluate the action and conditions of the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from pituitary implants as indicated by ovarian response in hypophysectomized immature female test rats. Some test animals receiving implants were subjected to estrogen treatment in an attempt to stimulate release of LH from the implants. The ability of certain androgens and other substances to liberate (LH) from the pituitaries of adult, castrate rats was tested by injecting aqueous suspensions of the acetone-dried, powdered glands into assay animals.

CHAPTER II

HISTORY

Experimentation in the field of pituitary-gonadal relationships prior to the twentieth century produced few results, and these were inconclusive. Increased interest in the field was evidenced after the turn of this century. Clairmont and Ehrlich (1909) made one of the early attempts to maintain a pituitary transplant. Harvey Cushing (1910) showed that the pituitary has a direct effect on the gonads. He hypophysectomized dogs and observed that atrophy of the gonads resulted.

The possibility of using replacement therapy to study the various activities of the pituitary was recognized early and many workers utilized this approach. Exner (1910) and Schafer (1911) attempted to maintain functional pituitary transplants but found that the implanted tissue was rapidly reabsorbed.

Klinger (1919), using a different approach for replacement therapy, injected saline homogenates of the glands. He obtained poor ovarian response because his injections were too small and too infrequent. Due to the protein nature of

the gonadotropins it is conceivable that the salt solution had some adverse effects. Smith (1927) in an extensive experiment showed conclusively that ovarian growth and function could be maintained by replacement therapy in hypophysectomized rats. He accomplished this by using repeated homogenate injections and transplants of pituitaries in different groups of animals.

Smith (1930) later outlined his method of the parapharyngeal approach for hypophysectomy of rats, which is one of the most important contributions to the field of endocrinology. This relatively simple surgical procedure allows the use of a small, inexpensive laboratory animal for the study of the pituitary gland and its many functions. Work previous to that time required the use of immature normal animals or those which were hypophysectomized by other methods. The latter usually exhibited undesirable effects such as obesity and gave erratic experimental results. Zondek and Ascheim (1927) by the repeated administration of pituitaries via transplantation obtained marked gonadal stimulation and precocious sexual maturity of immature rats. They also reported this procedure could produce estrus in old female rats. Following Smith's studies it was relatively easy to determine the effects of ablation of the pituitary gland and to evaluate replacement therapy without interference from the test animal's own hypophysis. However, progress in determining the actions of the secretions

elaborated by the pituitary gland did not proceed rapidly. The chief obstacle was the inability to isolate the pituitary gonadotropins in pure form. Such pure substances could have been utilized to determine their specific effects on various organs and target endocrine glands. The study of the action of gonadotropins on the ovary was also hindered by their synergistic effect as found by Fevold, Hisaw and Greep (1936). Since one augments the action of the other, the presence of a small concentration of one makes it very difficult to determine the true action of the other.

Many attempts have been made to isolate the pituitary hormones in pure or crystalline form. Since the gonadotropins are protein in nature it is difficult to obtain them in reasonably pure form and all purified preparations of either FSH or LH to date have been shown to be contaminated with the undesired component. Fevold, Hisaw, Hellbaum and Hertz (1933) were the first to outline a method for the fractionation and partial purification of FSH and LH. Fraenkel-Conrat, Simpson and Evans (1940) also published a chemical procedure for the isolation and purification of these hormones. At the same time Shedlovsky et al. (1940). reported their method for the isolation and purification of the luteinizing hormone. Since that time the efforts of all these investigators have been proven to be less successful than originally thought. The latter workers interpreted the presence of a single peak in the electrophoretic pattern of

their material to indicate the presence of a single substance. However, when the same material was assayed biologically, it was evident that more than one gonadotropic substance was present.

Since FSH and LH were not available in pure form, other means had to be devised for the assay of these organic compounds. One of the these was the use of fresh pituitary implants in hypophysectomized animals. Hypophysectomy removes the possibility of the recipient animal's own pituitary interfering with the assay procedure. Several routes of administration of implants have been utilized, and reports of these have given varied results. Hohlweg and Junkman (1932) used the kidney as the site for pituitary transplantation. Viability of the gland was maintained, but physiological function was not observed. Haterius, Schweizer and Charipper (1935) successfully transplated fresh pituitary tissue into the anterior chamber of the eye. They reported that the tissue retained its histological identity for four months. A short time later Hill and Gardner (1936) transplanted similar material into the testes of mice. They claimed that some grafts remained viable and exhibited their normal physiological functions.

An interesting experiment was conducted by Evans, Simpson and Pencharz (1935). They found that when the pituitary was removed from an adult male rat forty days after castration and implanted into a hypophysectomized

immature female recipient rat, the ovaries of the latter exhibited only follicle stimulation. If four glands were implanted, corpora lutea formation was also observed. Greep (1936) hypophysectomized immature female rats and used the evacuated sella turcica to receive transplanted pituitary glands. His results appear to be much more positive than similar preceeding work. He reported functional viability of the implants in an unusually high percentage (73 per cent) of animals as shown by growth and continued sexual potency. More recent work by Shilberberg and Shilberberg (1950) utilized the chest walls as a site for grafting of pituitary glands. Although their interest was not directed toward gonadotropic activity, their results indicated that the implants caused body growth and stimulation of the ovaries.

Martinovitch (1950) obtained maintenance of body growth by transplantation of hypophyseal glands into the anterior chamber of the eye. Histological study showed that the material remained viable, but his report did not mention stimulation of the ovaries. At the same time he cultured pituitary tissue in vitro and observed some acidophilic cells to be present after ninety days. No cells resembling basophiles were to be seen in these cultures. According to Lockhart and Finerty (1955) the delta basophile cells of the anterior pituitary gland located centrally and peripherally are thought to elaborate LH and FSH respectively.

They believe the production of the growth hormone to be a function of the acidophilic cells. Thus it is reasonable to expect continued body growth, but ovarian stimulation for only a limited time in animals receiving pituitary implants.

Concurrent with the experiments utilizing pituitary transplants, a great deal of interest in the study of the release and action of pituitary gonadotropins was exhibited by other investigators. Experimental procedures involved the use of pituitaries in several forms: homogenates, aqueous suspensions of acetone-dried glands, and extracts.

Engle (1929) found that the content of pituitary gonadotropins was increased after castration. Hellbaum and Greep (1940) established the time of maximal storage of the gonadotropins in the rat pituitary to be approximately two to six months after castration. That the total gonadotropic content of the pituitary gland could be decreased by giving sex hormones was reported by Allen (1939). Hellbaum and Greep (1943, 1946) demonstrated the release of the luteinizing hormone from the pituitary gland under the influence of androgens and estrogens. Hellbaum (1935) had previously determined that there was a decrease in LH activity in the pituitaries of old castrate horses in comparison to those of young horses.

Funnell, Keaty and Hellbaum (1951) reported a stabilizing action of the luteinizing hormone on the vasomotor

system. It is well established that androgens have a metabolic function related to protein metabolism and that estrogens influence calcium metabolism. It is possible that this activity is dependent on the presence of the luteinizing hormone. Various investigators have devised experiments to measure these metabolic functions. Papanicolaou and Falk (1938) utilized the increase in size of the small temporal muscle of castrated guinea pigs. McCullagh and Rossmiller (1941) measured gain in body weight of their experimental animals. Reifenstein (1942) used nitrogen retention as an indicator of protein anabolic effects. One of the most recently accepted methods for determining androgenic metabolic activity was presented by Eisenberg and Gordon (1950) who used an increase in weight of the levator ani muscle as an index of myotropic activity of androgens. Utilizing a modification of the latter method Hershberger, Shipley and Meyer (1953) determined the protein anabolic effects of several steroids. These were compared with the effects on ventral prostate and seminal vesicle weights, which served as a measure of androgenic reproductive activity as reported by Moore and Gallagher (1930).

CHAPTER III

METHODS

Immature female rats of the Holtzman strain were utilized as test animals in all experiments (Fig. 1). Some of these were hypophysectomized twenty-three to twenty-five days after birth, and others were used as unhypophysectomized controls. Hypophysectomy was performed according to a modification of the technique of Smith (1930). Donor animals, from which the pituitary glands were obtained for testing, were treated or untreated rats of the Holtzman strain which had been castrated two to six months prior to removal of the glands. Aqueous suspensions of acetone-dried, powdered, pituitary glands were prepared and injected according to the method of Hellbaum and Greep (1940). Fresh pituitary tissue was implanted at various sites in different groups of the test animals. These included the anterior chamber of the eye, the sella turcica, the chest wall, the rectus abdominus muscle, and in most instances the biceps femoris muscle. An implant was considered to be viable if under gross observation it appeared red and well vascularized. Histological examination of some implants was made to verify the gross findings.

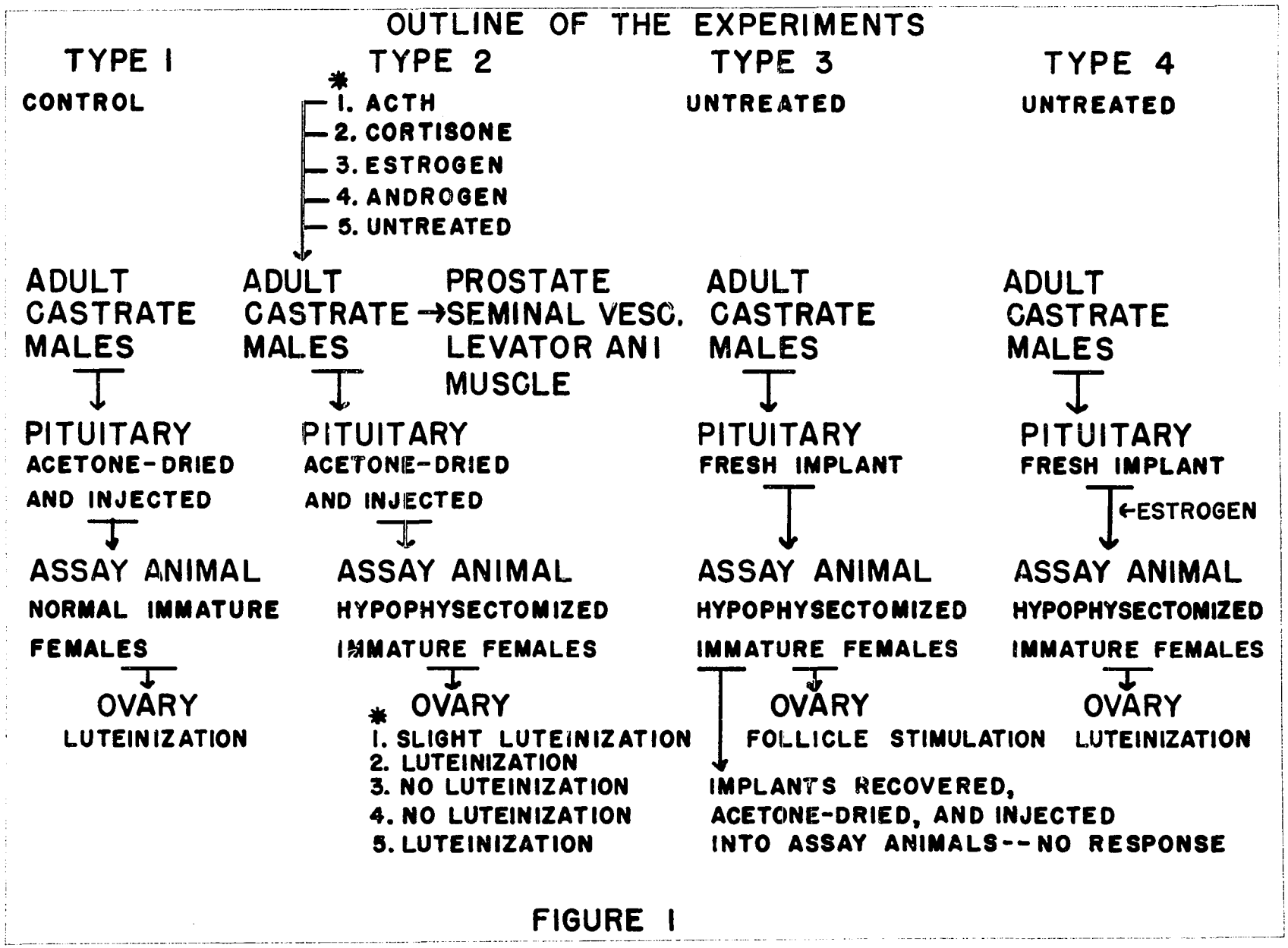

OUTLINE OF THE EXPERIMENTS
TYPE 1
CONTROL
TYPE 2
* 1. ACTH
2. CORTISONE
3. ESTROGEN
4. ANDROGEN
5. UNTREATED
TYPE 3
UNTREATED
TYPE 4
UNTREATED
ADULT CASTRATE MALES
ADULT CASTRATE MALES
PROSTATE SEMINAL VESC. LEVATOR ANI MUSCLE
ADULT CASTRATE MALES
ADULT CASTRATE MALES
PITUITARY ACETONE-DRIED AND INJECTED
PITUITARY ACETONE-DRIED AND INJECTED
PITUITARY FRESH IMPLANT
PITUITARY FRESH IMPLANT
ESTROGEN
ASSAY ANIMAL NORMAL IMMATURE FEMALES
ASSAY ANIMAL HYPOPHYSECTOMIZED IMMATURE FEMALES
ASSAY ANIMAL HYPOPHYSECTOMIZED IMMATURE FEMALES
ASSAY ANIMAL HYPOPHYSECTOMIZED IMMATURE FEMALES
OVARY LUTEINIZATION
* OVARY
1. SLIGHT LUTEINIZATION
2. LUTEINIZATION
3. NO LUTEINIZATION
4. NO LUTEINIZATION
5. LUTEINIZATION
OVARY FOLLICLE STIMULATION
OVARY LUTEINIZATION
IMPLANTS RECOVERED, ACETONE-DRIED, AND INJECTED INTO ASSAY ANIMALS--NO RESPONSE

FIGURE 1

Some of the animals receiving pituitary implants were treated with estrogen for various periods of time in an attempt to cause release of LH from the implant. Implants recovered after periods of five, ten, and fifteen days from assay animals not receiving estrogen, were reinjected as acetone-dried, powdered, suspensions into other assay animals. Ovaries in which follicle stimulation or corpora lutea formation were questionable by gross observation, were subjected histological examination. Completeness of hypophysectomy was considered to be indicated by weight gain of less than ten grams during the five day period following removal of the gland. Since implants are thought to release growth hormone causing an increased weight gain, only gross examination of the sella was made in some animals to determine completeness of hypophysectomy. Opening of the vagina, increase in uterine size, and ovarian weight (both ovaries) of sixteen milligrams or more were considered to be indicative of stimulation in the test animals.

Substances studied for their effect on LH release included adrenocorticotropic hormone (ACTH), cortisone, androgens and estrogens. ACTH and cortisone were injected daily as suspensions in isotonic saline, and the estrogens and androgens were administered every other day in five-hundredths milliliter of corn oil. The material was administered subcutaneously in different areas of the body. Only one type of injection material was used for a single

experimental group. The animals were killed by decapitation forty-five days after the start of the injections. The pituitary glands were removed from the donor animals and acetone-dried. The ventral prostate, seminal vesicles and the levator ani muscle of some of the rats which received androgen treatment were weighed and these values were compared with those of untreated groups.

LH release from the intact pituitary of the treated donor animals and from the implants in the assay animals receiving estrogen was determined by examination of the ovaries of the assay animals. If no corpora lutea were observed after five days, the material administered was considered to have been void of luteinizing hormone. Increased adrenal and body weights of the pituitary donor rats were used as indications of ACTH activity. The estrogen treated pituitaries should contain only FSH. Therefore, recovered implants were combined with pituitaries from donor animals in which the luteinizing hormone had been depleted by previous estrogen treatment. This material was acetone-dried, powdered, and injected into assay animals. If implants recovered from assay animals retain any LH activity, luteinization should occur in the ovaries of test animals receiving the combined material.

CHAPTER IV

RESULTS AND DISCUSSION

Part I

In order to establish the gonadotropin content of the pituitaries of the type of donor animal used in these experiments and to determine whether the surgical procedure of hypophysectomy interfered with ovarian responses in the assay animal, a control study was performed. As shown in Table I aqueous suspensions of acetone-dried, powdered pituitary glands of non-treated adult castrate male rats were injected into normal, immature, twenty-one day old, female rats. The effects on vaginal opening, uterine size, ovarian weight, follicle stimulation and degree of luteinization were used as criteria of gonadotropic stimulation.

These results may be compared with similar injections into hypophysectomized control animals as presented in Table II. The only difference in the two groups appears to be in body weight. Thus the surgical trauma of hypophysectomy is not considered to alter any physiological function of the test animals that would affect the results of these experiments. Hypophysectomy removes the complicating influence of the test animal's own pituitary.

Table I

Genital Response of Normal, Immature Twenty-One Day Old Rats Five Days After Being Injected With a Suspension of One-half Acetone-Dried, Powdered Pituitary Gland From Adult Castrate Male Rats.

Animal Number	Weight* Before & After	Vagina**	Uterine*** Response	Ovarian Weight	Ovarian Response
1	38 - 62 gm.	O	++	47.5 mg.	Many C.L.
2	38 - 62	O	++	31	Few C.L.
3	38 - 60	O	++	68	Many C.L.
4	38 - 62	O	++	16	No C.L.
5	38 - 60	O	++	27	One C.L.

* Weight of animal at start of injections and at autopsy

** O-open No-not open

*** + Slightly stimulated to ++++ Marked stimulation and distended with fluid

C.L. - Corpora lutea

Table II

Genital Response of Hypophysectomized, Immature Female Rats Five Days After Being Injected With a Suspension of One half Acetone-Dried, Powdered Pituitary Gland From Adult Castrate Male Rats.

Animal Number	Weight Before & After	Vagina	Uterine Response	Ovarian Weight	Ovarian Response
1	42 - 49 gm.	0	+	76 mg.	Many C.L.
2	41 - 43	0	+++	37	Several C.L.
3	41 - 46	0	++	59	Few C.L.
4	41 - 43	0	++	31	Few C.L.

C.L. - Corpora lutea

Table III

Genital Response of Hypophysectomized, Immature Female Rats Five Days After Receiving Sellae Implants of One-half Pituitary Gland From Adult, Castrate Male Rats.

Animal Number	Weight Before & After	Vagina	Uterine Response	Ovarian Weight	Ovarian Response
1	45 - 32 gm.	0	±	19 mg.	Foll. Only
2	45 - 57	0	±	32	Foll. Only
3	45 - 48	0	±	11	No C.L.
4	45 - 55	0	±±±	60	Foll. Only
5	45 - 47	0	±	18	Foll. Only

Foll. - Follicles
C.L. - Corpora lutea

Table IV

Genital Response of Hypophysectomized, Immature Female Rats Five Days After Receiving Chest-Wall Implants of One-half Pituitary Gland From Adult, Castrate Male Rats.

Animal Number	Weight Before & After	Vagina	Uterine Response	Ovarian Weight	Ovarian Response
1	48 - 48gm.	0	++	47 mg.	Foll. Only
2	48 - 52	0	+++	25.5 mg.	Foll. Only

Foll. - Follicles

The results of pituitary implants made in the evacuated sella turcica and the chest wall are recorded in Tables III and IV, respectively. It is seen that stimulation of the ovaries occurred without the formation of corpora lutea. These results are evidence for the release of FSH but retention of LH by the implant. These data suggest that FSH diffuses freely from the implant but that release of LH requires some physiological mechanism.

Single implants performed in this study usually maintained stimulation of the ovaries for periods of five days but not for periods of ten or fifteen days. In the few animals in which stimulation of the ovaries was maintained, follicle stimulation, as opposed to corpora lutea formation, predominated. The rare occurrence of luteinization was attributed to release of LH as a result of degeneration of the implant.

Preliminary observations indicated the leg to be the best site for implantation to cause maximal stimulation of the ovaries. Table V presents results obtained by utilizing the leg for implantation. Luteinizing hormone was not liberated by leg implants in a significant number of animals as evidenced by the small number of ovaries exhibiting corpora lutea formation. An attempt was made to cause the release of LH from similar implants by the use of estrogen. The results of that experiment appear in Table VI.

It is well established that estrogen causes the release

Table V

Genital Response of Hypophysectomized, Immature Female Rats Five Days After Receiving Leg Implants of One-half Pituitary Gland From Adult, Castrate Male Rats

Animal Number	Weight Before & After	Vagina	Uterine Response	Ovarian Weight	Ovarian Response	Implant
1	45 - 46 g	0	++	30 mg.	Foll.only	V
2	45 - 47	0	++	62	Foll.only	V
3	45 - 42	0	+++	20	Foll.only	N.V.
4	45 - 45	0	+	30	Foll.only	V
5	45 - 43	0	+	25	Foll.only	V
6	45 - 47	0	++	112	Foll.only	N.V.
7	45 - 47	0	+	42	Foll.only	N.V.
8	45 - 42	0	+	20	Foll.only	N.V.

Animal Number	Weight Before & After	Vagina	Uterine Response	Ovarian Weight	Ovarian Response	Implant
9	45 - 46 gm.	0	ǂ	48 mg.	1 C. L.	V
10	45 - 45	0	ǂǂ	38	Foll.only	N.V.
11	45 - 47	0	ǂ	182	Foll.only	V
12	45 - 46	0	ǂ	48	2 C. L.	V
13	45 - 43	0	ǂǂ	26	Foll.only	V
14	45 - 45	0	ǂ	8	No C. L.	Not Rec.
15	45 - 42	0	ǂ	14	No. C. L.	V
16	45 - 47	0	ǂ	110	Sev.C. L.	N.V.
17	45 - 46	0	ǂ	70	Few C. L.	V
18	45 - 44	0	ǂ	31	Foll.only	V
19	45 - 46	0	ǂ	66	Foll.only	V
20	45 - 42	0	ǂ	11	No. C. L.	N.V.
21	55 - 74	0	ǂ	38	Foll.only	Not Rec.
22	55 - 57	0	ǂ	38	Foll.only	V
23	55 - 70	0	ǂǂ	34	Foll.only	V
24	55 - 62	0	ǂ	70	Foll.only	V

Animal Number	Weight Before & After	Vagina	Uterine Response	Ovarian Weight	Ovarian Response	Implant
25	55 - 52	0	++	111	Foll.only	Abscess
26	55 - 52	0	+	59	Foll.only	V

V-Viable, N.V. - Non Viable, Foll. - Follicle, C.L. - Corpus luteum, C.L. - Corpora Lutea, Sev. C.L. - Several Corpora Lutea, Rec. - Recovered

Table VI

Genital Response of Hypophysectomized, Immature Animals Five Days After Receiving Leg Implants of One-half Pituitary Gland From Castrate, Male Rats and Five Micrograms Per Day of Estradiol Benzoate For Two Days Preceeding and Four Days After Implantation.

Animal Number	Weight Before & After	Vagina	Uterine Response	Ovarian Weight	Ovarian Response	Implant
1	45 - 40 gm.	0	++	25 mg.	foll.only	Not Rec.
2	49 - 57	0	++++	33	foll.only	N.V.
3	50 - 60	0	+++	36	foll.only	V
4	47 - 55	0	+++	18	foll.only	N.V.
5	50 - 60	0	++	93	foll.only	V
6	46 - 52	0	++	33	foll.only	N.V.
7	45 - 49	0	++	24	foll.only	N.V.
8	50 - 59	0	++	30	foll.only	N.V.
9	50 - 60	0	++	69	Few C.L.	Abscess
10	49 - 58	0	++++	14	No C.L.	V

Animal Number	Weight Before & After	Vagina	Uterine Response	Ovarian Weight	Ovarian Response	Implant
11	47 - 55 gm.	0	++++	30	foll.only	V
12	50 - 60	0	+	89	foll.only	Possible Abscess

Animal number one died and was autopsied four days after implantation. Animals numbers six and seven were given an injection of one milligram of cyclopental-testosterone thirty-six hours before autopsy. foll. - follicle, Rec. - Recovered, N.V. - Non viable, V. - Viable.

of LH from the intact pituitary gland of mature rats (Hellbaum and Greep 1946). Testosterone cyclopental propionate was administered to animals numbers six and seven (Table VI) to determine if the presence of androgen was necessary for LH to produce corpora lutea formation. This theory was investigated because of the possibility that the immature test animals lacked sufficient androgen, which in the case of adult animals is supplied by the adrenal glands. Since these ovaries did not exhibit corpora lutea formation, it was believed that by the time follicle stimulation occurred the implant had degenerated to such an extent that estrogen was unable to cause the release of LH. That LH was not released with degeneration of the implant may be due to the fact that it is a glycoprotein and is denatured in this process.

Another experiment was designed which utilized two implants, one-half of a fresh pituitary gland in each hind leg. The second was implanted three days after the first. The results of this experiment are recorded in Table VII.

The first six rats were used as implanted controls and were not treated with estrogen. As evidenced by previous experiments, the ovaries of these animals should have been stimulated by the first implant and sensitive to the action of LH had it been released from the second implant. Since only follicle stimulation occurred in four of these six test animals, LH was not believed to have been released by

Table VII

Genital Response of Hypophysectomized, Immature Female Rats Five Days After Receiving Right Leg Implants and Two Days After Receiving Left Leg Implants of One-half Pituitary Gland Each From Adult, Castrate Female Rats.

Animal Number	Weight Before & After	Vagina	Uterine Response	Ovarian Weight	Ovarian Response	Implant
1	47 - 64 gm.	0	++	165 mg.	Foll.only	L N.V.
					-	R N.V.
2	47 - 57	0	+++	52	Foll.only	L V
					-	R V
3	42 - 50	0	++	46	Foll.only	R Not Rec.
					-	L V
4	43 - 40	0	+	175	C.L.	L V
						R Abscess
5	47 - 60	0	++	64	Foll.only	L V
					-	R N.V.
6	43 - 48	0	+	106	C. L.	L Abscess
						R N.V.
7	47 - 57	0	++	119	C. L.	L N.V.
						R V
8	47 - 64	0	+	133	C. L.	L V
						R V

Animal Number	Weight Before & After	Vagina	Uterine Response	Ovarian Weight	ovarian Response	Implant
9	47 - 60	0	++	220	C. L.	L V R Abscess
10	46 - 58	0	+++	23	Foll.only	L V R N.V.
11	45 - 45	0	+	20	C. L.	L Not Rec. R Not Rec.

Five micrograms per day of estradiol benzoate was administered to animals numbers seven through twelve, two days preceeding and four days following implantation. Animal number twelve died and was autopsied four days after implantation. L - Left hind leg, R - Right hind leg, N.V. - non viable, V - viable, Rec. - recovered

these implants. That luteinization occurred in the ovaries of animals numbers four and six might have been expected since in preliminary studies it was observed that when an implant caused an abscess, it usually resulted in the formation of corpora lutea or no genital response whatever.

The ovaries of the last five rats in Table VII showed a different type of response, mainly one of corpora lutea formation in all but one animal. This was attributed primarily to the action of estrogen on the second implant since it was probably viable and functional during the forty-eight hour period before autopsy. As mentioned above the ovaries should have been stimulated by the first implant and sensitive to the effects of any LH liberated by the action of estrogen on the second implant. This mechanism is believed to have occurred because luteinization was observed in four out of five of these ovaries.

Part II

The ability of a substance to cause the release of LH is thought to be one measure of its metabolic activity. LH has been shown to be related to vasomotor activity and may well be concerned with bone calcium deposition and protein matrix anabolism. It is a well established fact that androgens and estrogens cause the liberation of LH from the pituitary, as is the fact that androgens have a role in protein metabolism. The androgenic steroid testosterone propionate has been shown to be effective in causing the

release of LH from the pituitary gland.

Several methods have been devised to measure the metabolic as well as the sex effects of androgens. In the following experiments LH release was utilized as a measurement of metabolic activity. Table VIII shows that one-fourth of the pituitary gland from an untreated castrate adult rat, when injected as an acetone-dried, powdered suspension into an immature, hypophysectomized female rat, resulted in ovarian luteinization.

In one experiment cortisone was used to see if this non-androgenic steroid would have effects on pituitary LH release similar to those of testosterone propionate. Table IX indicates this substance does not induce liberation of LH, as evidenced by luteinization of the test animal's ovaries. These results compare favorably with those in Table VIII in which luteinization by pituitaries from untreated, castrate donors is shown.

An attempt was made to bring about the release of LH with ACTH. It could have a direct effect on the pituitary gland or an indirect effect through its action on the adrenal glands. The latter possibility was postulated since ACTH may increase the production of the 17-ketosteroid androgens of the adrenal glands. It was thought these steroids might in turn cause the liberation of LH from the pituitary gland. Table X shows the ovaries of the test animals to have been luteinized, but the corpora lutea were fewer in number than

Table VIII

Genital Response of Hypophysectomized, Immature Female Rats Five Days After Being Injected With a Suspension of One-fourth Acetone-Dried Pituitary Gland From Adult Castrate Male Rats.

Animal Number	Weight Before & After	Vagina	Uterine Response	Ovarian Weight	Ovarian Response
1	55 - 60 g.	0	+	66 mg.	Many C.
2	50 - 60	0	+	51	Many C.
3	52 - 54	0	+	30	Few C.
4	54 - 48	0	+	28	Cloudy F.
5	50 - 45	0	+	58	Several C.
6	55 - 60	0	+	76	Many C.

C. - Corpora, F. - Follicles

Table IX

Genital Response of Hypophysectomized, Immature Female Rats Five Days After Being Injected With a Suspension of One-fourth Acetone-Dried Pituitary Gland From Adult, Castrate Male Rats.

Animal Number	Weight Before & After	Vagina	Uterine Response	Ovarian Weight	Ovarian Response
1	42 - 48 g.	0	+	48 mg.	Many C.L.
2	48 - 60	0	+	40	Many C.L.
3	43 - 53	0	+	70	Many C.L.
4	41 - 46	0	+	66	Many C.L.

Pituitary Donor Animals Were Injected with five-hundredths milligram of Cortisone a day for forty-four days. C. L. - Corpora Lutea

Table X

Genital Response of Hypophysectomized, Immature Female Rats Five Days After Being Injected With a Suspension of One-fourth Acetone-Dried Pituitary Gland From Adult, Castrate Male Rats

Animal Number	Weight Before & After	Vagina	Uterine Response	Ovarian Weight	Ovarian Response
1	44 - 36 g.	0	++	37 mg.	Few C.L.
2	46 - 45	0	+	27	Several C.L.
3	47 - 40	0	+	15	3 C. L.
4	45 - 45	0	+	36	3 C. L.
5	55 - 48	0	+	22	1 C. L.
6	50 - 52	0	++	62	Few C.L.
7	50 - 49	0	++	58	Few C.L.

Pituitary donor animals were injected with three units of ACTH a day for forty-five days. C. L. - Corpora lutea

in the ovaries of the control animals in Table VIII. This suggests that part of the LH content of the ACTH-treated pituitaries had been released.

Further studies are planned using an increased dose of ACTH to induce more complete release of the LH. The mechanism of action of this substance might be clarified by similar studies utilizing adrenalectomized donor animals.

Two androgenic steroids were also assayed, adrenosterone and 4-androstene-3,17-dione. The results of the experiment in which one milligram of adrenosterone was administered daily for forty-five days are recorded in Table XI. The ovarian response indicated LH had been liberated from the pituitaries assayed.

The genital response of these animals is in contrast with that produced by the action of pituitaries from castrate, untreated, donor animals as illustrated in Table VIII. However, when one-half of a pituitary of an adrenosterone treated animal was injected, the ovaries of the recipient animals were consistently luteinized. This would indicate that the dose of adrenosterone used removed only part of the LH content from the pituitaries assayed.

An experiment using 4-androstene-3,17-dione, two milligrams a day for forty-five days, produced results similar to those recorded in Table XI. These are presented in Table XII.

Preliminary studies are now being conducted with

Table XI

Genital Response of Hypophysectomized, Immature Female Rats Five Days After Being Injected With a Suspension of One-fourth Acetone-Dried Pituitary Gland From Adult, Castrate Male Rats

Animal Number	Weight Before & After	Vagina	Uterine Response	Ovarian Weight	Ovarian Response
1	50 - 54 g.	0	++	28 mg.	Foll.only
2	52 - 54	0	+	98	Many C.L.
3	50 - 56	0	+	26	Foll.only
4	62 - 65	0	+++	34	Foll.only
5	55 - 65	0	+	40	Cl. Foll.
6	55 - 65	0	+	25	Cl. Foll.
7	44 - 50	0	+	26	Foll.only
8	49 - 50	0	++	34	4 C. L.
9	45 - 45	0	++	42	Foll.only
10	40 - 35	0	++	28	Foll.only
11	50 - 55	0	+++	32	Foll.only
12	45 - 40	0	++	32	Foll.only

Pituitary donor animals were injected with 1 mg. of adrenosterone a day for forty-five days. Foll. - Follicle, C. L. - Corpora Lutea, Cl. - Cloudy.

Table XII

Genital Response of Hypophysectomized, Immature, Female Rats Five Days After Being Injected With a Suspension of One-fourth Acetone-Dried Pituitary Gland From Adult, Castrate, Male Rats

Animal Number	Weight Before & After	Vagina	Uterine Response	Ovarian Weight	Ovarian Response
1	53 - 65 g.	0	±	19 mg.	Foll.only
2	53 - 60	0	±	25	Foll.only
3	48 - 57	0	±	26	Foll.only

Pituitary donor animals were injected with two milligrams of 4-Androstene-3,17 dione a day for forty-five days. Foll. - Follicles.

adrenosterone and 4-androstene-3,17-dione concerning their effects on the levator ani muscle and the ventral prostate. Other experiments indicate that when administered at a level of two milligram a day for forty-five days, both substances cause almost complete liberation of LH from the pituitary gland. This is evidenced by the fact that one-half pituitary from an animal so treated produces no luteinization in the ovaries of test rats.

Part III

The vagina of immature female rats does not open within five days after hypophysectomy. Since opening of the vagina occurred in every animal that received an implant, it is another indication of genital stimulation. Increased uterine stimulation was observed in most of the assay animals receiving implants and estrogen treatment.

Several other observations were made during the progress of this investigation. It was noted that when luteinization occurred in the ovaries of a test animal which received a one-half pituitary implant, the number of corpora lutea was very small. The same amount of pituitary tissue injected as an acetone-dried, powdered suspension resulted in the formation of a large number of corpora lutea. As previously mentioned, the implants may have released a small amount of LH in these cases, but the same results might have been expected if a few secreting gonadotrophic cells remained

in the sella due to incomplete hypophysectomy.

The rare occurrence of corpora lutea formation in the ovaries of animals receiving implants might also have been due to biological variation; some assay animals may have been hypersensitive to a minute amount of LH released by the implant. An extremely small amount of LH would induce a genital response because of the augmentation phenomenon of FSH and LH.

The pituitary gland of the castrate male rat has been shown to be several times more potent in gonadotropic activity than that of the castrate female. Experiments were performed utilizing pituitaries from castrate female rats for implantation and injections. One such gland was required to obtain genital stimulation comparable to the stimulation produced by one-fourth of a pituitary gland from a castrate male.

When implants were made using one pituitary gland from a castrate male donor animal which had been treated with three micrograms of estrogen for thirty-eight days, no genital response occurred. Pituitary glands so treated were shown to retain FSH activity by injecting two glands as an acetone-dried, powdered suspension and observing that the only genital response was follicle stimulation in the ovaries of the assay animals.

The animals represented in Table X received three

units of ACTH daily for forty-five days and exhibited a two hundred and twenty-five per cent increase in adrenal weight at autopsy in comparison to untreated controls. There was no significant change in body weight during the forty-five day period. The adrenal glands are thought to supply almost all of the total body androgens in the castrate male and in the normal female animal. Following the menopause in the female and the climacteric in the male a relative condition of physiological castration exists. Since androgens possess important metabolic functions, any decrease in adrenal activity in the aged presents a serious geriatric problem. It is thus desirable to find therapeutic agents to treat the condition of adrenal androgen insufficiency. These agents should possess androgenic metabolic activity without undesirable side effects on the primary and secondary sex characteristics.

The judicious use of ACTH may be of value in this situation by stimulating the production and release of the androgenic 17-ketosteroids of the adrenal cortex. Adrenosterone and 4-androstene-3,17-dione appear to possess desirable metabolic properties, but further work is necessary to determine their activity relative to undesirable side effects.

There is general agreement among investigators in this field that not all of the metabolic functions of LH are known. An important step forward will be made when a

method is devised to study the independent actions of FSH and LH. One such method is suggested by the results of experiments presented in this study. FSH can be isolated from LH in vivo by estrogen treatment of castrate adult rats. The isolation of LH has not been accomplished to date.

An attempt was made in this investigation to isolate LH by physiological means using pituitary implants. FSH appears to diffuse out freely from the implants (Table V) and LH appears to be retained by the implant (Table VI). The presence of LH in such implants is substantiated by the fact that estrogen causes its release (Table VII).

Experiments have already been performed using as many as ten recovered implants and injecting them as acetone-dried suspensions into test rats. There was no genital response in any of the assay animals, which indicates that no FSH was present in the recovered implants. Further experiments are planned using recovered implants to supply physiologically pure LH, and pituitaries from estrogen treated donor animals to supply physiologically pure FSH. Procedures of this type may produce a more complete fractionation of these substances than can be accomplished by chemical means. When a method for the complete separation of FSH and LH is devised, the separate and independent actions of these substances can be better understood.

CHAPTER V

SUMMARY

A method is outlined for the release of LH from pituitary implants by estrogen treatment. FSH diffuses freely from the implants, and the isolation of LH may be accomplished by its retention in the implant. Information concerning the production and increased storage of FSH and LH was obtained by castrating adult rats two to six months previous to removing their pituitaries for assay. Release of these pituitary factors was determined from observations involving the degree of follicle stimulation and luteinization in ovaries of immature hypophysectomized rats which received fresh implants of pituitary glands. The results so obtained were compared with the effects of injecting aqueous suspensions of acetone-dried pituitaries into immature hypophysectomized test rats.

FSH and LH are not available in pure form. In order to observe the specific action of each, two methods were used in this study. Pituitaries containing only the FSH activity were obtained by injections of estrogen or androgen into castrate adult rats. On the other hand, recovered

pituitary implants, originally obtained from non-treated castrate rats, appear to exhibit only LH activity when injected into immature hypophysectomized assay rats. Since LH is considered to have metabolic functions independent of its sex regulatory mechanisms, it has become increasingly important to understand more about this hormone.

Several other substances were studied to determine their ability to release LH from the pituitary gland. Cortisone appeared to be ineffective. ACTH may be effective through its ability to release the 17-ketosteroids from the adrenal glands and these substances may then induce the liberation of LH. Adrenosterone and 4-androstene-3,17-dione appeared to be effective in causing the release of LH from the intact pituitary gland.

CHAPTER VI

CONCLUSIONS

Fresh pituitary implants release FSH within a five day period as evidenced by ovarian follicle stimulation in assay animals.

Fresh pituitary implants do not release LH within a five day period as shown by their failure to produce ovarian luteinization.

Estrogen causes the liberation of LH from viable pituitary implants as indicated by corpora lutea formation in the ovaries of the test rats.

Cortisone is ineffective in causing the release of LH from the intact pituitary gland of adult male rats.

17-Ketosteroids elaborated by the intact adrenal glands under the influence of adrenocorticotropic hormone may be effective in partially depleting the pituitary gland of its LH content.

Injections of the 17-ketosteroids, adrenosterone, and 4-androstene-3,17-dione in adequate doses, results in the depletion of LH from the pituitaries of adult castrate male rats.

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