THE INHIBITORY HORMONE OF THE TESTICLE

by

BEN VIDOFF

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[redacted]

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INTRODUCTION

It has been known for some time that the interstitial cells of the testicle elaborate a hormone which is necessary in the development of the secondary sex organs and characters of the male. This was isolated, later made synthetically and is now known as testosterone. Certain evidence, which will be developed further on in this thesis, has brought to light instances in which the presence of this lipoid soluble hormone alone, will not explain all the known facts in the relationships of the testicles to other endocrine organs.

Probably the first reference to the presence of a second hormone principle resident in the testes was made by Hoskins (14) in 1911, who stated that an endocrine principle resident in the male gonads has an inhibitory effect on the anterior pituitary; the removal of this principle or secretion should cause hypertrophy of the pituitary providing the rest of the gonadal secretion remains intact.

The purpose of this thesis is to follow the chronological development of the experimental work which makes the existence of this testicular secretion tenable, to review the attempts to isolate and assay this hormone, and, specifically, to review the published work from the Laboratory of Pharmacology of the University of Oregon Medical School, and including recent work not yet published. Brief tribute herein is paid to Brown-Sequard for his fundamental explorations in this field. For completeness, mention is made of another testicular substance, hyaluronidase, which is an aqueous derivative of the generative epithelium of the testis but is not of endocrine nature.

HISTORICAL DEVELOPMENT OF KNOWLEDGE OF HORMONAL FUNCTION OF THE TESTES

(A TRIBUTE TO BROWN-SEQUARD'S PIONEER STUDIES)

Brown-Sequard's announcement in 1889, reporting the effects of an aqueous extract of the testicle on himself, represents the actual birthday of Endocrinology as we know it today. He proclaimed the effects as a marked rejuvenation on himdelf, then 72 years of age, with such intimacy and enthusiasm that the medical world began to think along the line of internal secretion. In other words, Endocrinology began with an error in subjective interpretation. We now know that his extracts could not have contained the male hormone (lipoid soluble), the injection of which would cause some of the effects described by him.

Brown-Sequard's report provoked an interesting scientific uproar. There were many scientific and quasi-medical men who took advantage of the opportunity of Brown-Sequard's claims for their own material advancement. Either they accepted this new concept that such extracts would cause or produce the desired results or, with tongue in cheek, they began to use them. Thus treatment with these testicular extracts soon became widespread. However, there were those who scoffed and berated the author of a new concept in medicine, forgetting, in the heat of the moment, the scientific integrity of the worker, and his past scientific accomplishments. A few maintained a more mature and cautious aspect. Some made an honest scientific attempt to correlate Brown-Sequard's results with the potentiality of the extract. For instance, Dixon (6) used the extract as a basis for a very scientific pharmacological treatise based on laboratory experiments. He found that the aqueous extract produced certain effects on the blood pressure, myocardial construction, and respiration of the dog, cat and goat. He also found that boiling the extract nullified some of

these results. From the standpoint of this thesis, this work merely represents an attempt to place Brown-Sequard's work on a scientific level. It is of historic interest only.

However, Dixon missed the endocrinological concept of Brown-Sequard's results entirely. In fact, he did not even mention it. Later, Stockwell (57) and Loomis (21), who used the extracts on senile men, gave some support of clinical nature to the theory as pronounced by Brown-Sequard but were very cautious in their claims.

Endocrinologists owe a great deal to Brown-Sequard. At the time he made his report, while the work of others such as that of Claude Bernard was known, Thomas Addison had described the disease bearing his name (1855), Milton-Fagge had described sporadic cretinism (1871), the Reverdin Brothers described postoperative myxedema, and Gull had described myxedema (1873), no single one of these workers gave the same impetus to endocrinology. It was Brown-Sequard's erroneous conception of the efficacy of his aqueous testicular extract, reported and described in his very picturesque way, which opened up the way for the start of Endocrinology on a scientific basis.

HYALURONIDASE

Recently a factor which increases the permeability of the skin to injected fluids has been obtained from mammalian testes by aqueous extraction. This factor came to be known at first as the "spreading factor". It resides in the germinal epithelium of the testis and also has been extracted from spermatozoa (13). A similar substance has been obtained from several other sources such as culture filtrates, extracts from many species of invasive bacteria, snake and spider venoms, and

leeches. Since these "spreading factors" are closely associated, if not identical, with a group of enzymes that hydrolize the hyaluronic acid of the synovial fluid, vitreous humor and skin, they are called hyaluronidases.

Evidence has been found that the hyaluronidase activity of semen is of testicular origin, and, also that the gel surrounding the mammalian ovum in the Fallopian Tubes contains hyaluronic acid (31).

The question of why nature requires the production of enormous numbers of sperm cells for the fertilization of a single mammalian ovum has always been of interest. The fact that reduction of sperm cells to below 60 million per cubic centimeter rapidly leads to infertility of the individual has been somewhat of a mystery. Certainly, it would seem that even a low count of 40 million spermatozoa per cubic centimeter appears too large a number to miss fertilization of the ovum continuously, when considered statistically on the basis of numerical hits and misses. Apparently, it must be assumed that each sperm cell contributes something that aids in the fertilization process, and that a certain number of sperm must be present to provide a sufficiency of this substance. This substance could well be hyaluronidase, serving the function of liquifying the hyaluronic gel which surrounds the ovum. McClean and Rowlands (31) have demonstrated this for rat ova, and Fekete and Duran-Reynals (18) showed this for mice ova. The clinical application of this concept and these observations for the treatment of infertility would be tremendous. Kurzrok and his group (17, 20) have done some work along this line which is promising, but requires further substantiation. The author of this thesis is also interested in this phase of the problem.

Although hyaluronidase is derived from an aqueous extract of the

germinal epithelium of the testicle or the sperm cell, as is also the second or the inhibitory hormone of the testicle, no functional connection between the two has been noted to date.

THE INHIBITORY HORMONE OF THE TESTICLE

1. Theoretical Considerations Supporting Its Existence. -

The concept of a second hormone in the testicle was postulated from the observation that the lipoid soluble hormone (testosterone) derived from the interstitial cells did not fulfill or answer all the findings of gonadal relationship and gonad - hypophyseal relationship. To logically and chronologically develop the thesis of the presence of a second hormone it is necessary to first review the basic work in the literature from three standpoints: (1) The effect of castration, (2) The effect of castration in parabiotic animals and (3) The effect of destruction of the germinal epithelium of the testicle, either by x-ray, cryptorchidism, or by pressure atrophy from vas ligation.

a. Castration Effects.- The investigations of Steinach (55) and Moore (44) established that the main effect noted after castration of the male rat was the degeneration or atrophy of the seminal vesicles and the prostate gland. These castration effects were later used as a method of assay for the androgen hormone (testosterone) by Moore, Price, Gallagher, and Koch (45, 46, & 47).

Experimental studies have shown that gonadectomy in both sexes leads to hypertrophy of the anterior pituitary. This may be observed both histologically and in changes in the physiological function of the animal. Histologically, there is an increase in the size and number of basophile cells, which undergo vacuolization and development of signet

ring or "castration cells". The increase in physiological function can be shown by implantation of such altered hypophyses into normal immature animals or by parabiosis studies on gonadectomized rats and mice united with normal animals. The change in the gonads of the normal partner, and of the secondary sex organs of these animals is very definite. Fichera (10) found that castration in the rooster and other animals produced hypertrophy of the anterior pituitary. Biedl (2) noted this experimentally and in addition summarized the literature on this subject. Lehmann (19) and Evans and Simpson (7) also established this point beyond reasonable doubt.

Further experiments of this type lead to a significant discovery. Lehmann (19) injected a water soluble testicular hormone into castrated rats and prevented the usual expected histological changes in the pituitary gland. This was the first direct experimental evidence that there is an inhibitory hormone in the testicle. This extract did not prevent atrophy of the secondary sex organs of the castrate animal.

b. Parabiosis Experiments. -

Considerable information as to the interaction and secretory effects of the endocrine glands may be learned by joining two animals, such as rats, together in parabiosis. This may be accomplished by surgically suturing the incised skin down one side of the animal to the other and by keeping the animals in this apposition until healing takes place. This allows an intermingling and conjoint circulation of the tissue juices of the two animals although no direct contact exists between the individual circulatory system of either animal.

In 1921, Matsuyama (42) showed that when a castrated female rat was joined in parabiosis with a normal female rat, hypertrophy of the ovaries

and uterus of the normal rat resulted. This was confirmed by Yatsu (64), and by Goto (12). In 1929, Kallas (16) found that when a castrate infantile rat (either sex) was joined in parabiosis with a normal infantile rat, signs of precocious sexual maturity appeared in the normal rat. He also obtained the same effect by injecting blood from an infantile castrate rat into a normal rat (infantile) (16). Some years later, Fels (19) repeated the same experiment and found similar results. Fels ascribed these results to the physiological overactivity of the anterior pituitary gland of the castrate animal. It must be remembered that when Fels did his work, he had the advantage of the fundamental discoveries of Zondek and Ascheim (65) and Smith and Engle (56).

In 1930, Martins (37) noted that in a parabiotic pair of animals the accessory sex organs of the castrated animal atrophied, whereas the same organs in the normal partner hypertrophied. He also found that animals with a cryptorchid testicle showed an increase in Leydig cells and normal accessory sex organs. In 1930, Martins and Rocha (39) used infantile rats to demonstrate that they could prevent anterior pituitary hypertrophy when they injected testicular mush or saline extracts of the testicle into the castrated male partner of a parabiotic union with either a male or female normal partner. This was shown by the lack of precocious maturity in the normal partner. These authors repeated this work in 1931 (40) and concluded that there was a hormone in the testicle which inhibits the anterior pituitary, that this hormone is present in the infantile testis, and that this hormone was not the same as the androgenic, lipoid soluble hormone, since its injection did not prevent the atrophy of the secondary sex organs of the castrated partner. In 1931, they again repeated the work and concluded that the hormone which regulates the development of the

secondary sex organs is different than that which regulates the anterior hypophysis. Lower and Hicken (26) confirmed this work suggesting that the internal secretion of the anterior pituitary of the castrated partner caused hypertrophy of the sex organs of the normal partner.

McCullagh and Walsh (34) found that the hyperfunction of the anterior pituitary in the castrated partner of a parabiotic pair could be inhibited by a lipin soluble male hormone concentrate, and also by a testis mush, but that much more lipin material was required to produce this effect than testis mush. Furthermore, the lipin concentrate regenerated the seminal vesicles and prostate in the castrate animal, but the testis mush had no such effect.

The previous experiments using parabiotic animals showed that the hormone which regulates the anterior pituitary is not the same hormone which is responsible for the maintenance of the secondary sex organs.

c. Destruction of the Germinal Epithelium. -

Since it has been postulated that the inhibitory hormone of the testis is formed in the germinal epithelium, another approach to the problem of establishing its identity would be the removal of its source in the experimental animal and studying the effects of substitution therapy with water soluble testicular extracts obtained from other animals. Various technics have been devised, such as ligation or irradiation, to produce a selective atrophy of the germinal epithelial cells of the testis. Only a few references will be made to point out the possible presence of a hormone in the testicle which is inhibitory to the anterior pituitary but which does not control the activity and growth of the secondary sex organs. Ancel and Bouin (1) used rabbits to cause degeneration of the seminal epithelium by ligature and resection of the

ductus deferens. They noted that this procedure caused no change in the Leydig cells. Massaglia (41) repeated the work of Ancel and Bouin, but used the rooster instead of the rabbit. He ligated the ductus deferens and produced atrophy of the seminal epithelium without loss of secondary male sex characters. He then removed the entire testicle and noted hypertrophy of the anterior pituitary. However, he missed the significance of the lack of secondary sex organ atrophy and anterior pituitary hypertrophy until after the entire testicle was removed. Mottram and Cramer (48) studied the effect of irradiation on the rat testicle and observed that the tubular elements underwent degeneration while interstitial elements remained normal. Also, changes occurred in the anterior pituitary gland which simulated those seen in castration but no atrophy of secondary sex glands occurred. They concluded that one testicular mechanism controls the pituitary gland, while another is responsible for the maintenance of the secondary sex glands.

Evans and Simpson (7) noted that the changes which take place in the testes in cryptorchidism are the same as those which follow Roentgen irradiation. These are characterized mainly by a rapid degeneration of tubular elements during the first month, whereas the interstitial cells persist for a considerable period. Then, following destruction of the tubular elements, definite changes occur in the pituitary gland, identical with those occurring in castration. In other words, the cryptorchid testes elaborates enough male hormone to maintain the secondary sex organs, but the mechanism which controls the pituitary has failed. Witschi, Levine, and Hill (63) showed that when a male rat, which has been sterilized by x-ray one month previously, is joined in parabiosis with a normal female rat, there is continuous estrus in the normal rat. In spite of

this over activity of the anterior pituitary, the testes of the sterilized rat becomes 25% heavier and the accessory sex organs are distinctly enlarged. Since it is apparent that the pituitary became more active because of the destruction of the germinal cells, the conclusion is that a testis factor which controls the anterior pituitary resides in the germinal epithelium, and not in the interstitial tissue.

Martins (38) ligated the internal spermatic artery and caused degeneration of the seminal epithelium. He stated that the interstitial cells hypertrophied, but the anterior hypophysis presented the same appearance as following castration. His conclusion was that the germinal epithelium must elaborate some hormone, the lack of which causes the changes in the anterior pituitary.

Johnston (15) irradiated rat testes and destroyed the tubular elements. Prostatic hypertrophy resulted in some instances, presumably because of the increased activity of the anterior pituitary of the rat.

In 1932, McCullagh (32) came to the conclusion that the testicle elaborates two factors, one called androtin which maintains the secondary sex organs of castrated rats but which will not prevent hypertrophy of the anterior pituitary of the castrated rat, and a second hormone which has no influence on the secondary sex organs of the castrated rat but which will prevent hypertrophy of the anterior pituitary. The name of "inhibin" was given to the second of these hormones. McCullagh also proposed the concept of the dual endocrine function of the testicle as a cause of prostatic hypertrophy.

The suggestion of the dual endocrine function of the testes was enlarged by Lower (22). Briefly, the theory of this conception is that senescence produces degenerative changes in the germinal epithelium and

retardation of spermatogenesis. Then, in such case, "inhibin" elaborated by these cells would be decreased and, with the removal of the "inhibin" effect on the anterior pituitary, this organ becomes hypertrophied. In turn the overactivity of the hypertrophied pituitary stimulates the interstitial cells of the testicle to elaborate more lipoid soluble male hormone. This leads to prostatic hypertrophy.

McCullagh (33) and Lower, McCullagh, and Walsh (29) further brought out these points about the presence of the inhibitory hormone of the testicle, resident in the germinal epithelium, and its role in the production of prostatic hypertrophy.

CRITICISM OF THE THEORY OF THE INHIBITORY HORMONE OF THE TESTICLE

a. Role of Estrogen in the Male. -

Not all workers accept the theory that an inhibitory hormone is found in the testis. Nelson (52) and Nelson and Gallagher (53) are of the belief "that it is not necessary to hypothecate" another male hormone for pituitary control since sufficient estrogen is present in the male to exert an inhibitory effect on the pituitary of the castrate rat. They feel that the biological role of estrogen in the male, combined with androgenic substances, is sufficient to account for the natural control of the anterior pituitary body. However, the statement above in quotes (taken from Koch's review, 1937) is not acceptable in view of the facts. Similarly, Tornblom (58) came to the conclusion that there is an inhibitory hormone in the testicle, that it is resident in the germinal epithelium, but that it is estrogenic in nature. While it is well known that estrogen is present in the testicle, and that estrogen is inhibitory to the anterior pituitary, there is no proof that Tornblom's results were

based on an estrogenic hormone derived from the germinal epithelium.

b. Experiments Failing to Show Inhibitory Effect. -

Rubin (54) fed normal male rats a dessicated bull testes material and also used a sodium sulfate precipitated aqueous extract of the testicle for injection. Neither of these testicular preparations caused any effect on the testicle or secondary sex organs. On the basis of this experiment Rubin found the whole theory of "inhibin" untenable. He believed the good results of others to be due to changes in nutrition. A similar criticism has been voiced by Moore (43).

Nelson (51) has also shown that if cryptorchids are observed long enough, other structures such as the seminal vesicles and prostate gland show castration effects. He pointed out that while the pituitary was the most sensitive to changes in male hormone concentration, the seminal vesicles and the prostate can be maintained at lower levels of male hormone concentration. Therefore, in cryptorchidism changes in the pituitary are noted first.

CLINICAL APPLICATION OF THE "INHIBIN" THEORY

It is perhaps regrettable that clinical considerations should be entertained before the inhibitory hormone had been definitely isolated, proven, and assayed. A comparison could be drawn, without much difficulty between the Brown-Sequard episode and the present one. Without a method of assay, control is impossible and without isolation of the hormone there is no method of assay. Furthermore, the clinical conditions involved are of themselves difficult to control.

Lower, Engle, and McCullagh (25) treated 40 patients with prostatic hypertrophy by feeding them 60 grams of dried fresh testes daily for

three months. This was based on the work done by Myers, Vidgoff, and Hunter (49) to be discissed below. They considered 65% of these to be improved, judged on the disappearance of symptoms, although there was no reduction in the size of the enlarged prostate. Later, Lower (23) added 36 more similarly treated cases to the above series and claimed that of the total of 76 cases treated, 46 showed improvement judged by disappearance of symptoms. The patients also reported a feeling of well being. He mentioned that the group which showed improvement, perhaps would have improved without any treatment. If so, then too many cases were being operated on. I have no brief with him on that point. In 1936, Lower and McCullagh (27) reviewed these 76 cases. In 1937, Lower (24) reported a total of 151 cases of prostatic hypertrophy with 79% of the patients experiencing relief from symptoms. In 1940, Lower and McCullagh retold the same story (28). Also in 1940, Lower, Schlumberger, and Ferguson (30) gave a collective review of the prostate problem and its hormonal control. McComb and Pearse (36) treated 17 patients with "inhibin", finding that 7 improved, although there was no change in the size of the prostate.

Kutscher (18) used a non-androgenic extract of bull testes which contained "significant amounts of inhibin" in four patients. One had a Eunuchoidism and pituitary tumor, and the other three had fundic changes and impaired vision. These cases were treated from 17 months to 2½ years. The report states that visual acuity improved, fundic changes cleared up to greater or lesser degree and dark adaptation returned to normal. Emboldened by these results, he treated three other cases; luetic atrophy of optic nerve, optic atrophy associated with pituitary tumor, and retinitis pigmentosa. These cases also improved.

It is a little difficult to visualize why the extract was used for these conditions in the first place, and it is more difficult to visualize why the extract should produce the results reported in these types of cases.

Beckman (3) and Cassner (11) reported on the use of the inhibitory hormone extracts (aqueous extracts) on mammary cancer of the bitch. According to both reports the effects were quite considerable, resulting in a reduction in size and vascularization of the cancer.

WORK DONE AT UNIVERSITY OF OREGON

In 1930, I became interested in the aqueous inhibitory hormone of the testicle with Dr. Harold B. Myers, the late Professor of Pharmacology. Our first attempts were with feeding experiments. Normal rats were fed dessicated beef testis material for a period of time varying from 85 to 117 days. The amount given was 0.2 gram daily for five days per week. In 1933, Myers, Vidgoff and Hunter (49) reported that the testes, prostate, and seminal vesicles of normal male rats so treated were grossly, considerably smaller than in the control animals. The atrophic appearance of the sex glands was most striking in those animals that were fed the dessicated beef testis material over the longest period of time.

Histologically, the prostate and seminal vesicles of the experimental animals showed evidence of parenchymal reduction with all the characteristics of simple atrophy. The testicles of the experimental animals showed aspermia and evidence of degeneration of the spermatic cell, and the primary spermatocytes as well. The interstitial cells showed no recognizable change.

In 1936, Myers, Vidgoff, Schade, and Hunter (50) reported such feedings on 14 animals and found the same findings as regards the prostate, seminal vesicles, and testicles of the normal rats fed the beef testis material (Fig. I).

In 1938, Vidgoff, Vehrs, and Hill (62) made a crude extract of beef testes by extracting with alcohol, and then removing the fat soluble materials with benzene. The aqueous layer was evaporated and the residue taken up in propylene glycol. Each cubic centimeter of

the final solution was equal to 25 grams of the fresh testes material. Normal male rats were given 1 cc. of this solution subcutaneously daily for three weeks, and then sacrificed. No gross changes could be made out, but there was definite evidence of microscopic alterations in the sex organs of the male rats which received the extract. The normal control and the "propylene" control showed no such effects.

In an effort to further isolate the inhibitory hormone of the testicle in more potent solution, Vidgoff, Hill, Vehrs, and Kubin (60), made an extract according to a modification of a method suggested by Deutsch, Eggleton, and Eggleton (5). By this method, proteins are precipitated with sodium sulfate, and at the same time the material is concentrated. The method of preparation was as follows:

Frozen bull testes were obtained from the packing house. The tunics were removed, the pulp cut into small pieces, and then ground in a meat chopper. The resultant mash was then warmed to 32°C., and 1/3 its weight of anhydrous sodium sulfate was added. The mixture was stirred frequently for five hours at this temperature. It was then pressed through muslin by hand. The thick juice obtained was filtered under suction through an asbestos mat. Throughout this procedure the temperature was kept at 32°C., or a little better to prevent crystallization of the sodium sulfate. The clear filtrate was then cooled to 0°C., and the decahydrate crystals of sodium sulfate were removed. The filtrate was then extracted with benzene. The aqueous fraction was concentrated so that each cc. represented 25 grams of the original mash. The final solution also contained 4% sodium sulfate.

The results reported in this series of papers (59, 60, 61) were of three series of experimental animals. A fourth series of animals was

injected with an extract from which the remaining 4% sodium sulfate was precipitated with alcohol. The results showed that the active principle had been lost. Since a group of five animals was given 4% sodium sulfate subcutaneously for four to five weeks and showed no changes, the sulfate was not removed from the other series. White adult male rats, 5-6 months old, were chosen for the experiments. Injections were given daily for a period of 28 days. The results of this work showed the following: (1) The sex organs of the experimental animals were reduced in size and weight. (2) The prostatic lobes showed more changes than the other organs. Their weight was reduced 1/3 to 1/2 of the control. (3) The seminal vesicles were smaller and had less colloid. (4) The testicles of the experimental animals showed a consistent loss of weight. (5) Histological changes in the prostate and seminal vesicles were atrophic and degenerative in nature, and simulated the changes seen in castrate rats. (6) The histological changes in the testicle were atrophic and degenerative in nature and showed arrest of spermatogenesis. These changes were very similar to those seen in the hypophysectomized rat. (7) The pituitary gland of the experimental animals was decreased in weight. Histologically there was vacuolization and signet ring formation in the basophile cells. (8) The adrenals showed an increase in weight and hypertrophy of the fasciculate and reticular layer. These findings are illustrated in Figures II to VI.

According to McCullagh and Schneider (35) this extract proved more effective in depressing the estrus cycle of the white adult female rat than any employed up to that time.

NEW EXPERIMENTAL WORK

The above published results showed the effects of the sodium sulfate precipitated, aqueous extract of the testicle on the sex organs, pituitary, adrenal, and thyroid of the intact male rat. For the past year experiments were directed to show the effect of the extract directly on the pituitary, in order to accumulate further experimental proof and to definitely define the nature of the inhibitory effect on the anterior pituitary. In order to definitely classify the extract as a specific and separate hormone, which has an inhibitory effect on the anterior pituitary, the following criteria were decided upon as necessary:

1. The extract must prevent the physiological hyper-function of the anterior pituitary which occurs in the castrate animal.

2. It must be non-androgenic.

3. It must be non-estrogenic.

The last two criteria were felt to be necessary since those who criticized the entire concept of the inhibitory hormone, based their criticism not on the results mentioned heretofore, but that these results could be obtained by the presence of either androgen or estrogen in the extract.

Two sets of experiments were decided upon to fulfill these criteria. In one set, which would satisfy criterias 1 and 2, the castrated male rat preparation was used, testing the physiological function of the pituitaries of these animals by injecting a suspension of the pituitary into the immature female rat. Normally, the immature female rat of 23 to 25 days of age presents the following characteristics; the vagina is closed,

the ovaries weigh about 6 mgm., and show no evidence of follicles, the uterus is pale, avascular, with no fluid and weigh about 18 mgm. When the pituitary gland of a male rat, that has been castrated 21 days previously, is injected into such an immature female rat, the effect is one of precocious maturity. That is, the vagina is opened, the ovaries become injected, have follicles and corpora, and increase greatly in weight; the uterus becomes very vascular, increases greatly in weight and size and is filled with fluid. This precocious puberty denotes an increase in output of the pituitary gonadotropic factor, or hyperfunction of the pituitary.

The method was as follows. White rats of the Sprague-Dawley strain were used. The rats were adult, varying from five to eight months in age. It would be well to mention here that particular attention was paid to the nutritional needs of these animals, since one of the criticisms of this work was that our results could be obtained by a nutritional deficiency in the animals.

A series of male rats was castrated and injections of the sodium sulfate precipitated aqueous extract of the testicle were started the next day, and continued daily for 21 days. In one series a commercial aqueous extract, Androstine A (Ciba Company), was injected instead of the sodium sulfate precipitated extract which was made in the laboratory. At the end of 21 days, the animals were sacrificed. The pituitary gland, prostate gland, seminal vesicles, and adrenals were weighed and the gross appearance noted, as compared with the controls. The pituitary gland was then ground up and suspended in 3 cc of saline. This suspension was then injected into immature female rats, 23 to 25 days of age, twice

daily for three days. At the end of that time, the condition of the vagina was noted, and the ovaries and uterus examined and weighed.

An examination of Table I, which is a typical control series, shows the effect of castration on the prostate and seminal vesicles by weight. There is a gross atrophy of these organs, which is very marked in the three week castrate. The injection of the pituitary of these castrates into the immature female resulted in a very marked "maturity" reaction as shown by the increase in weight, and appearance with follicles and corpora in the ovary; increased vascularity, increased weight, and fluid in the uterus, and the open vagina.

Table II shows an example series done in the same way as the control series with the exception that the castrated animal received 1½ cc Androstine A daily for 21 days beginning the day after castration. From the gross picture and the results it can be seen that the picture is exactly the same as that found in the control series, within experimental limits of error.

Table III is a resume of a typical series of animals injected with our own sodium sulfate precipitated extract, 1 cc twice daily, started the day after castration of the male rat and continued for 21 days. As can be seen from the protocols, we had difficulty with the general health of these animals. In spite of this, the 21 day male castrates demonstrated the expected atrophy of the secondary sex organs, and their pituitaries caused a marked maturity reaction when injected into the immature females. Because of the illness of the animals the weights were not recorded.

Table IV is included to show a representative group of animals in which the pituitaries of the castrate male rats were titrated down to

50% and 25% of their weights before injection into the immature female test animals. The results in general were the same; the marked precocious puberty developed even after the dose of the pituitary gland was cut down. A finding on Table IV, and repeated in other animals, which I cannot explain, is that the immature female rats which received smaller titrations of the pituitary tended to show a greater maturity response than the test animals which received greater doses. This finding was also noted by Gassner at Fort Collins, Colorado (Personal communication).

The second experiment was done to satisfy criterion number three, or to show that the extract was non estrogenic. White adult female rats were injected with our extract for five consecutive days. The vaginal smear was followed before, during, and after injection twice daily for some time. Estrus occurs in the adult female white rat every four or five days. This can be determined by the microscopic picture of the smear, taken from the vagina by means of a swab. Any inhibition of the gonadotropic factor of the pituitary gland should delay the appearance of estrus or cause its disappearance. As a test for the inhibitory hormone of the testicle, this procedure leaves much to be desired because other factors will cause such delay in the estrus. However, for the purpose of demonstrating the absence of estrogenic effect, the experiment lends itself to our cause. The presence of estrin should cause an increase or the appearance of more or less continuous estrus. Table V shows a prolonged experiment. It can be seen that in some cases there was a delay in the estrus (designated as X) but in no case was there any increase in estrus.

SUMMARY

The results of this year's work disturbed us, at first. It was felt, as a result of the work, that there was adequate proof that the extract was non-androgenic and non-estrogenic. However, the same extract which caused such widespread changes in the intact male rat, failed to show any inhibition of the pituitary, which should cause the changes in the sex organs. This was shown by the hyperfunction of the pituitary of the castrate male rat. Gassner (personal communication) found this same situation, using a different approach to the problem. It is felt now that the testicle, or some system in the testicle, must be present before the inhibitory hormone of the testicle can be effective. Gassner's work also demonstrated that this substance is not testosterone. Moskins (14) intimated that this would be the case in his statement mentioned in the early part of this thesis. In further experimental work this possibility is to be investigated.

It is also contemplated to renew efforts to isolate the hormone. A new approach has been developed for extraction. The possibility that the hormone may be present in some other fraction of the testicle than the aqueous portion is to be thought of. There is some evidence, perhaps, that the phospholipins should be investigated (Koch - letter seen by the author).

Since the onset of this work, it has been difficult to convince workers that the concept developed in this thesis, that there is a hormone in the testicle other than androgen or estrogen which has marked inhibitory effect on the pituitary, is a valid one. Most of the criticism has been more or less non experimental theorizing.

What experimental work has been done, in the effort to disprove the concept, has been very flimsy. From the increased efforts by more workers today, and especially the recapitulation of some of the men who were loudest in their disapproval, it becomes evident that this field of work will be widened.

FIGURE I

In the figure are shown section of testes at equal magnification (X32) taken from litter mate rats, A, control, B, fed beef testis as described. These sections were chosen as representative of the changes observed in the 2 groups. Representative sections of prostate glands from C, control, and D, an experimental animal, at equal magnification (X70) are reproduced, also contrasts found in the seminal vesicles of the 2 groups, each magnified X 70, are shown in E and F.

(Reprinted from ENDOCRINOLOGY, 20:589, 1936

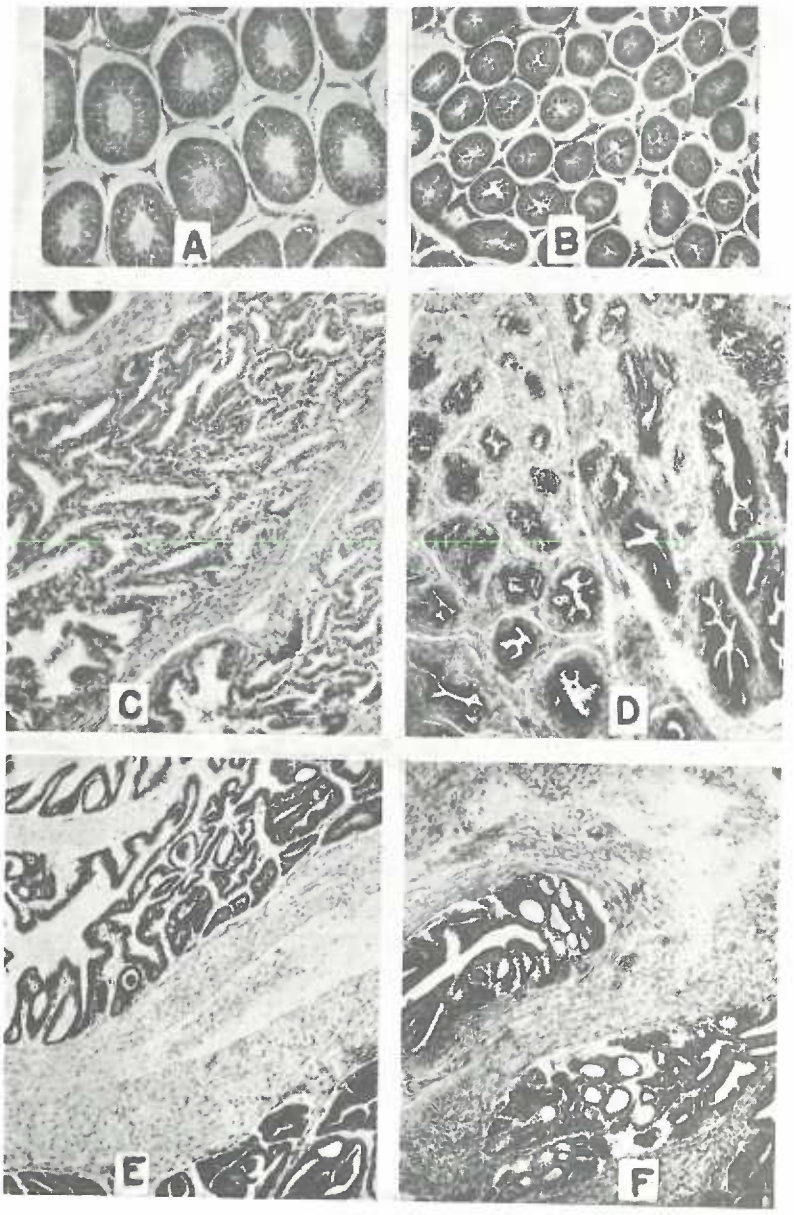
A
B
C
D
E
F

FIGURE II

Fig. 1. Anterior view of genital tract of rat. Control rat 74, weight 196 gm.; experimental rat 63, weight 194 gm. received 1 cc daily subcutaneous injection of the extract for 35 days.

Fig. 2. Anterior view of genital tract of rat. Control rat 57, weight 288 gm.; experimental rat 68, weight 280 gm. received 1 cc daily subcutaneous injection of the extract for 30 days

(Reprinted from ENDOCRINOLOGY , 25:591, 1939)

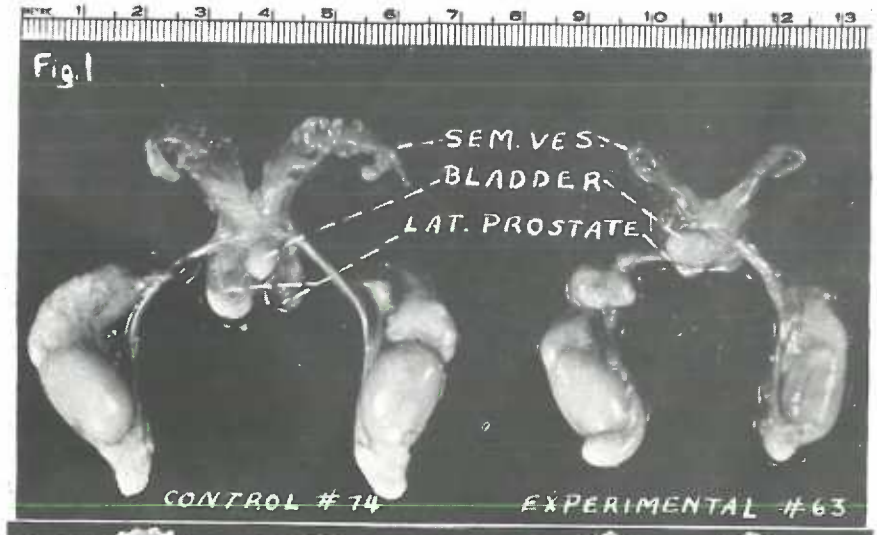
Fig. 1
SEM. VES.
BLADDER
LAT. PROSTATE
CONTROL # 74
EXPERIMENTAL #63

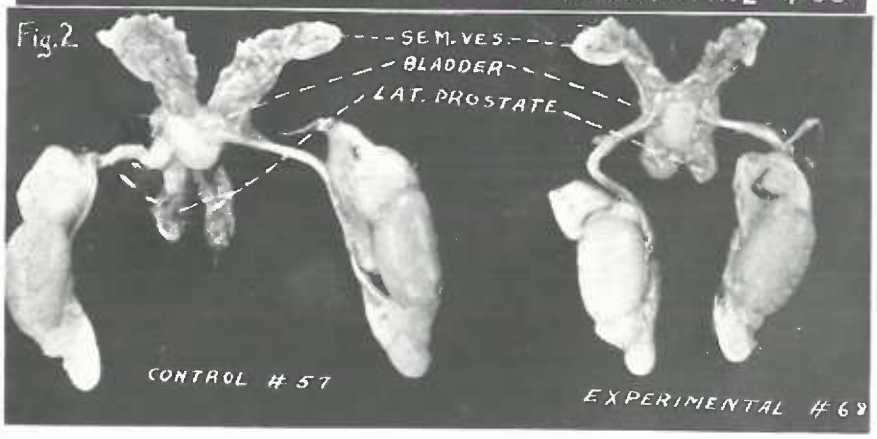
Fig. 2
SEM. VES.
BLADDER
LAT. PROSTATE
CONTROL # 57
EXPERIMENTAL #68

FIGURE III

Fig. 1. Photomicrograph of normal rat prostate

Fig. 2. Prostate of litter mate which received extract.

Fig. 3. Seminal vesicle of control (same animal as fig. 1)

Fig. 4. Seminal vesicle of experimental animal (same as fig. 2)

All magnifications X 84

(Reprinted from ENDOCRINOLOGY, 25:568, 1939)

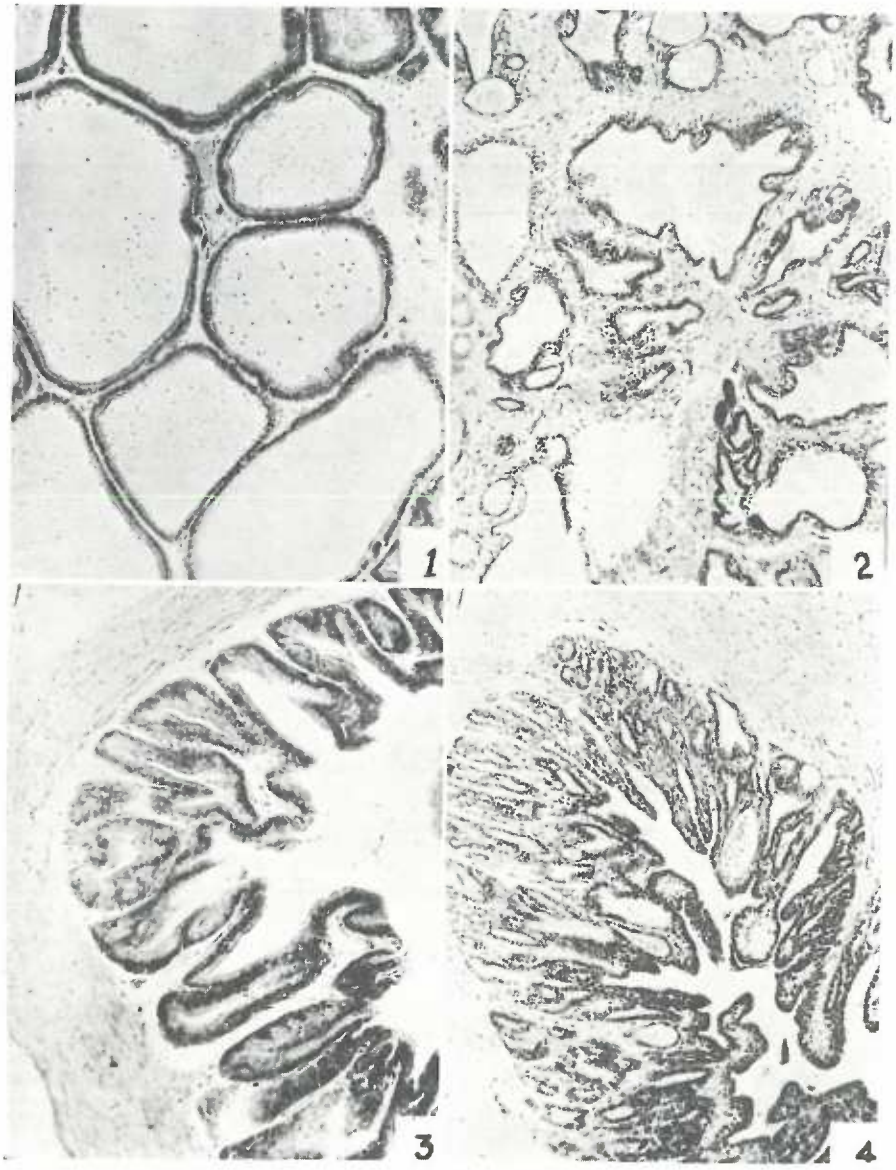
1
2
3
4

FIGURE IV

Fig. 5. Photomicrograph of normal rat testicle (same animal as fig. 1,3, in FIGURE III)

Fig. 7. Testicle of experimental rat showing second stage changes (same animal as fig. 2,4, in FIGURE III)

Fig. 9. Testicle showing third stage changes in the experimental rat

Magnification X 72.

Fig. 6,8, 10. Higher magnification of the above figures each one corresponding to the figure on the left. (X 172)

(Reprinted from ENDOCRINOLOGY, 25:568, 1939)

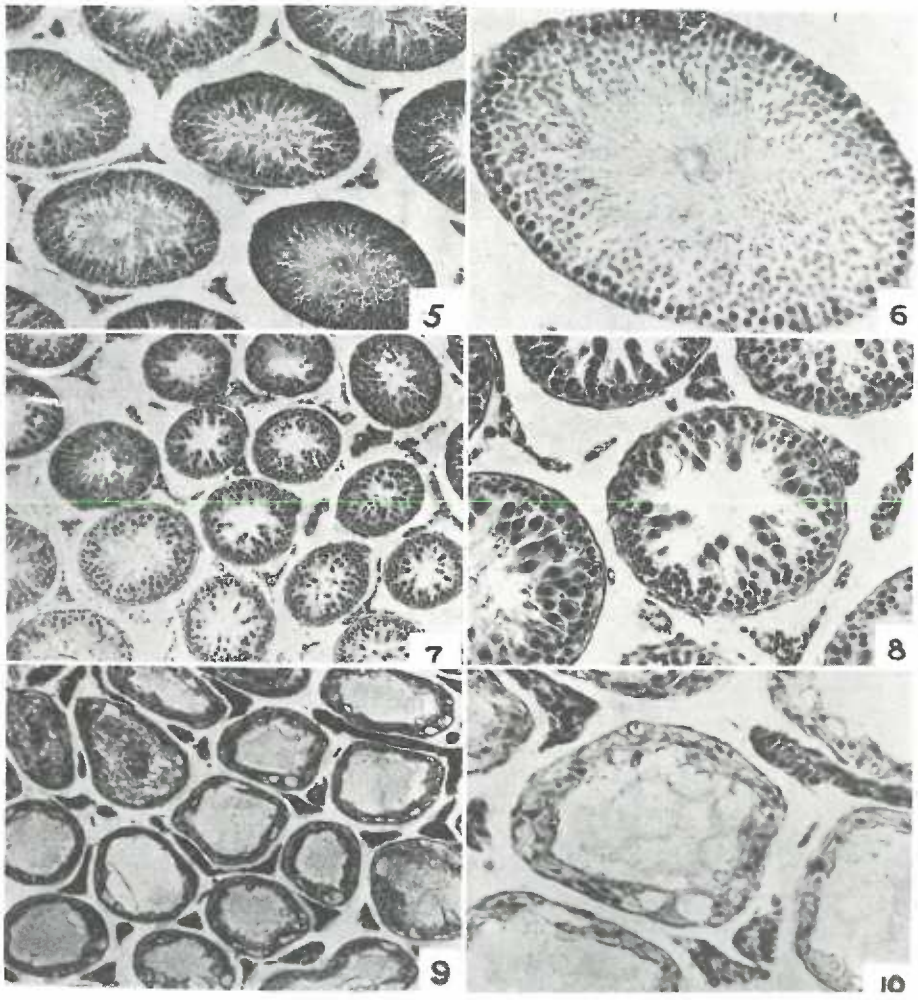
5
6
7
8
9
10

FIGURE V

Fig. 3. Section of anterior pituitary of experimental rat showing vacuolated cells (X 285).

Fig. 4. Control adrenal (X 50)

Fig.5. Experimental adrenal showing hypertrophy of the cortex (X 50)

Fig. 6. Control adrenal showing cells of the z. fasciculata (X 205)

Fig.7. Experimental adrenal showing hypertrophy of the cells of the z. fasciculata (X 205)

(Reprinted from ENDOCRINOLOGY, 26:656, 1940)

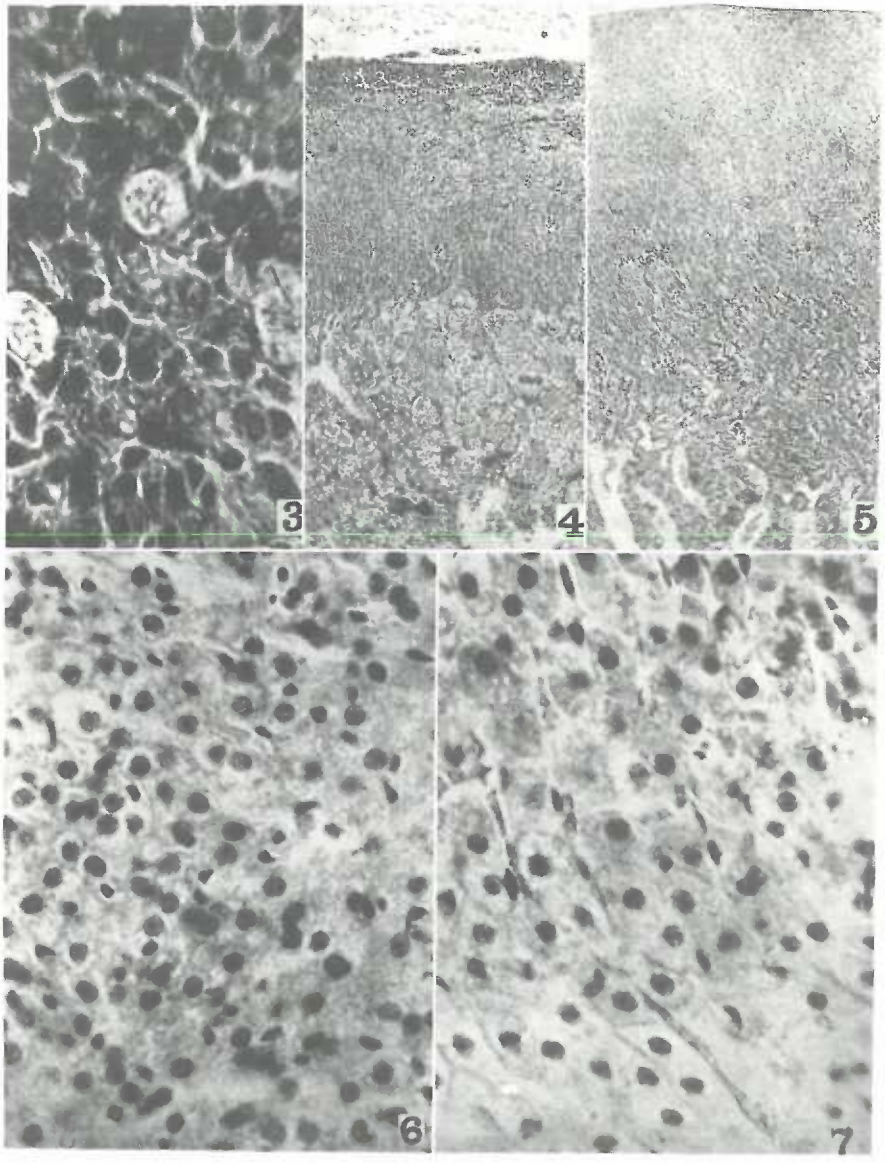
3
4
5
6
7

FIGURE VI

Fig. 1. Section of control thyroid

Fig. 2. Section of thyroid of rat which received 1cc of extract daily for 30 days (X67)

(Reprinted from ENDOCRINOLOGY, 26:656, 1940)

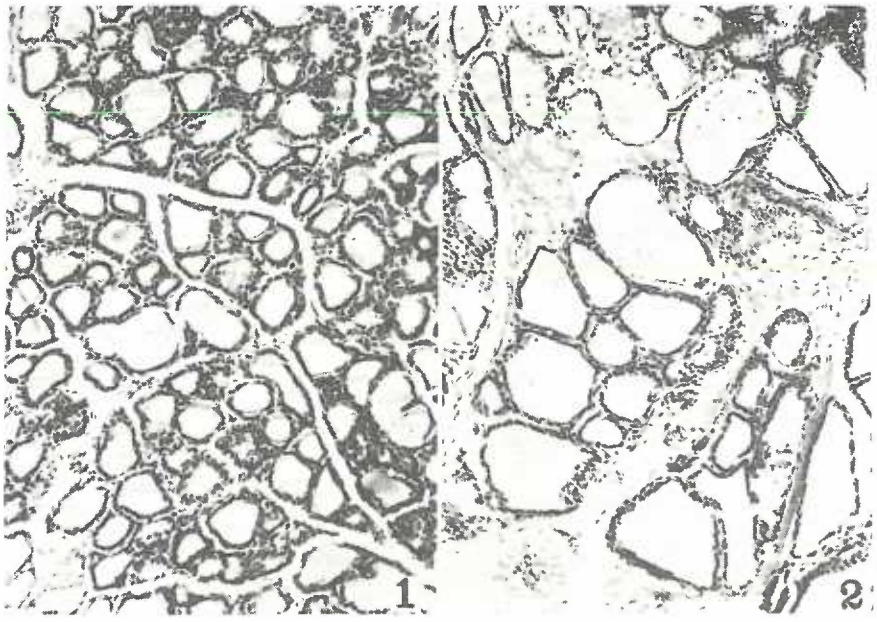
1
2

TABLE I

CONTROL SERIES OF CASTRATE MALE RATS

RAT NO.	WT. RAT BEGIN. GMS.	WT. RAT END GMS.	EXPERIMENT DURATION	WT. PITUITARY MGM.	WT. PROSTATE MGM.	WT. SEM. VES. MGM.	WT. ADRENALS MGM.	RESULTS - PITUITARY INJECTION INTO IMMATURE FEMALE RAT	CONDITION - ACCESSORY SEX ORGANS - CONDITION OF ANIMAL
1	320	330	3 weeks	10	403	185	36	Died	Atrophied
2	290	308	" "	16	70	150	41	Vagina opened - Ovaries 27 mgm. Follicles - Uterus 92 mgm. - No fluid.	"
3	340	370	" "	18	51	149	43	Vagina opened - Ovaries 55 mgm. corporahemorrhagicum - Uterus 93 mgm.	"
4	250	340	" "	11	67	132	44	Vagina opened - Ovaries 108 mgm. - Follicles - Uterus 92 mgm.	"
5	268	300	" "	10	38	140	38	Vagina opened - Ovaries 31 mgm. - Follicles - Uterus 134 mgm. - Fluid	"

TABLE II

ANDROSTINE "A" ON CASTRATED MALE RAT

RAT NO.	WT. RAT BEGIN GMS.	WT. RAT END GMS.	EXPERIMENT DURATION	WT. PITUITARY MGM.	WT. PROSTATE MGM.	WT. SEM. VES. MGM.	WT. ADRENALS MGM.	RESULTS - PITUITARY INJECTION INTO IMMATURE MALE RAT	CONDITION - ACCESSORY SEX ORGANS - CONDITION - ANIMAL
1	300	314	1½ cc. Androstine "A" Daily - 21 days.	23	127	210	54	Ovaries 8 mgm. No Follicles - Uterus not enlarged.	Atrophied
2	250	290	"	12	59	142	53	Vagina open. Ovaries 54 mgm. $\bar{c}$ follicle formation. Uterus 126 mgm. - Vascular $\bar{c}$ much fluid.	"
3	340	390	"	11	60	145	57	Vagina open. Ovaries 72 mgm. - Follicles Uterus 65 mgm.	"
4	250	338	" 27 days	10	156	173	49	Vagina open. Ovaries 54 mgm. - Follicles Uterus - 145 mgm. $\bar{c}$ fluid.	"
5	264	350	" 28 days	10	62	212	48	Vargina open. Ovaries 23 mgm. $\bar{c}$ Follicles - Uterus 150 mgm. $\bar{c}$ fluid.	"

TABLE III

Na_2SO_4 EXTRACT ON CASTRATED MALE RAT

RAT NO.	WT. RAT BEGIN. GMS.	WT. RAT END. GMS.	EXPERIMENT DURATION	WT. PITUITARY MGM.	WT. PROSTATE MGM.	WT. SEMINAL VES. MGM.	WT. ANDRENALS MGM.	RESULTS - PITUITARY INJECTION INTO IMMATURE RAT.	CONDITION - ACCESSORY SEX ORGANS - CONDITION - ANIMAL
1	155	188	1 cc. Ext. B.I.D. 21 days	10	120	145	47	Marked maturity reaction on ovaries and uterus.	Atrophied.
2	330	322	1 cc. Ext. B.I.D. 3 days	-	-	-	-	No change.	No change - 3 days died.
3	250	240	1 cc. Ext. B.I.D. 21 days	11	115	150	48	Marked maturity reaction on ovaries and uterus.	Atrophied.
4	308	274	"	10	125	160	50	"	"
5	144	138	"	9	120	140	46	"	Atrophied - Right lung consolidated.
6	300	288	" 14 days	11	130	155	47	"	Atrophied.
7	210	228	" 21 days	11	80	120	44	"	"
8	312	308	" 21 days	12	100	130	47	"	Right lung - white nodules. Atrophied -
9	164	160	" 21 days	10	80	100	45	"	Atrophied.
10	188	176	" 21 days	10	85	110	45	"	Atrophied - Rt. lower lobe lung consolidated.
11	356	248	" 16 days	12	?	?	50	-	Left lung consolidated. Large encapsulated mass in pelvis.

TABLE IV

Na_2SO_4 EXTRACT ON CASTRATED MALE RAT

RAT NO.	WT. RAT BEGIN. GMS.	WT. RAT END. GMS.	EXPERIMENT DURATION	WT. PITUITARY MGM.	WT. PROSTATE MGM.	WT. SEMINAL VES. MGM.	WT. ADRENALS MGM.	PITUITARY INJECTION INTO IMMATURE MALE RAT. FRACTIONIZATION 50% PIT.	25% PIT.	CONDITION - SEX ORGANS- CONDITION - OF ANIMAL
1	228	224	1 cc. Ext. B.I.D. 21 days	11	90	110	43	Mature reaction, Ovaries & Uterus 100 mgm.	Mature reaction, Uterus & Ovaries 160 mgm.	Atrophy Right lung consolidated.
2	232	200	" 21 days	12	100	130	45	Mature reaction, Uterus & Ovaries 210 mgm.	Mature reaction, Uterus & Ovaries 375 mgm.	Atrophy
3	160	158	" 21 days	10	70	100	42	Mature reaction, Uterus & Ovaries 150 mgm.	Died	Atrophy
4	214	208	" 7 days	7	120	150	40	Uterus - Slightly swollen Ovaries - No follicles.	Uterus - swollen and vascular Ovaries - follicles.	Atrophy - Died Pneumonia.
5	228	210	" 21 days	10	80	120	44	Died	Died	Atrophy
6	204	180	" 14 days	9	130	160	40	Mature reaction, Uterus & Ovaries 190 mgm.	Died	Atrophy

STUDIES OF VAGINAL CYCLE IN RAT

RAT NO. DAYS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1	0	0	0	X	0	0	0	0	X	0	0	0	0	X	0	0	0	0	X	0	0	0	X	0	0	0	0	0	X
2	0	0	0	0	X	0	0	X	0	0	0	X	0	0	0	0	X	0	0	0	0	0	X	0	0	0	X	0	0
3	0	X	0	0	0	0	0	0	0	X	0	0	0	0	0	X	0	0	0	0	0	X	0	0	0	0	0	X	0
4	0	0	X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	X	0	0	0	0	0	0	0	X	0
5	(X	0	0	0	0)	0	0	0	0	X	0	0	0	0	0	X	0	0	0	0	0	0	X	0	0	0	0	X	0

INJECTION

X = ESTRUS

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