# EFFECTS OF SEX HORMONES ON THE GONAD PITUITARY COMPLEX IN IMMATURE FOWLS

by

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TABLE OF CONTENTS

# INTRODUCTION

Sex hormones have been used for several years in the successful relief of menopause disturbances. It is believed that sex hormones used in this manner restore at least a semblance of the former balance between ovarian and pituitary hormones. Since the pituitary, at the menopause, in absence of inhibition which ordinarily exists in the presence of natural estrone, produces a superabundance of its gonadotropic hormone an imbalance can not be avoided when the ovary either gradually or more or less suddenly stops functioning.

The use of sex hormones in this connection has been one of the prominent contributions to clinical endocrinology. The exact mode of their action, however, is unknown since critical determinations have not been made to evaluate the gonadotropic activity of the pituitary both before and after artificial introduction of the hormones. The studies that have been made were concerned largely with mammals. Many conclusions have been reached but the premise of a gonad-pituitary balance needs further confirmation before final conclusions can be drawn.

Little, if any, investigation in this field has involved the use of birds. To determine, if possible, if a gonadal-pituitary balance exists in birds, experiments herein described were undertaken. It was hoped that these experiments would help to clarify existing questions concerning birds and would throw light on the problem as a whole.

Stilbestrol, a synthetically prepared female sex hormone of recent development, and testosterone propionate, also synthetic, were selected to study their effect respectively upon immature female and male chickens. Later it seemed advisable to use estrone, a naturally produced female sex hormone, so that its effect could be compared to that of stilbestrol.

Since several laboratories report that the gonadotropic hormone from the pituitary is identical in males and females, the pituitary implantations, during these experiments, were made into chicks of a different sex than those which had received the hormone.

## REVIEW OF LITERATURE

Working independently, Smith (1916) and Allen (1917) observed that removal of the pituitary from frogs resulted in a retardation of both growth and sexual development. Many attempts to explain the function of the pituitary gland followed. Evans and Long's failure (1921) to produce any change in rats by oral administration of beef pituitaries was overshadowed by their success (1922) when they were able to report that rats having been treated intraperitoneally with finely ground fresh anterior lobe of the hypophysis (pituitary) of beef were much heavier than their controls and their ovaries weighed twice as much. They also showed that similar effects were not produced by using the posterior lobe. Evans (1925) in summarizing the advances pointed out that the reports of Evans and Long (1922), of Smith and Smith

(1922) and others indicated that although the exact mechanism was not yet known, the normal function of the sex glands, thyroid and adrenal cortex were dependent upon the hypophysis.

Smith (1926a), using rats, demonstrated that daily anterior pituitary transplantations would restore atrophied genital organs which follow hypophysectomy to a normal or nearly normal condition. His success suggested that such transplantations might hasten the development of immature animals. Consequently, he made them from both male and female adults into young female rats and brought about changes characteristic of sexual maturity much earlier than they normally would have occurred. By using ovariectomized rats as recipients of the transplants Smith (1926b) showed further that no precocity in the development of the uterus or vagina was effected nor was the atrophy of these organs which follows the removal of the ovaries prevented. In the following year (1927a) he obtained similar results by making transplants into immature males. The testes were enlarged but failed to exhibit as marked an increase in weight as did the whole genital system.

Allen and Doisy (1924) produced early sexual maturity of the vagina and uterus by injections of follicular fluid from the ovary. Evans and Long (1922) had used pituitary transplants to induce estrus. Smith (1927b) demonstrated that the uterus and vagina in the absence of ovaries fail to respond to pituitary stimulation. He concluded that the hastening in the development of genital organs by pituitary transplantations was due to the presence of the follicular hormone, the formation of which had

been stimulated by the hormone in the transplant.

Smith and Engle (1927) showed that daily transplants of anterior pituitary tissue not only from mice and rats but also from cats, rabbits and guinea pigs into sexually immature mice and rats of both sexes induced precocious sexual maturity. They further transplanted pituitaries from immature animals to produce an earlier maturity and concluded this probably indicated that the hormone, though present but not normally liberated in young animals, was released after transplantation. In castration-transplantation experiments they observed that the hypophyseal hormone did not produce changes in the genital system of animals whose testes were removed. This finding together with that obtained earlier by Smith (1927) clearly indicated that the pituitary stimulation of the genital system was by way of the gonad. A reciprocal action of gonad upon the pituitary, suggestion of which had been made by Smith and Engle (1927), was indicated by Evans and Simpson (1929) who found that gonadectomy resulted in an increase of size of the pituitary and that the gonad stimulating power of pituitaries from castrate animals was much greater than those from non-castrated males. That the increased weight of the gland was due to a storage of the hormone and not a true hypertrophy was indicated by histological examination.

The dependence upon the presence of the gonad for the release of the gonad stimulating complex from the anterior pituitary was demonstrated by Engle (1929). He also confirmed the findings of Evans and Simpson and others who had shown that the

hypophysis of castrated rats is considerably more potent than that of the non-castrate. In 1931 Engle submitted more evidence to support his earlier idea and suggested that the oestrus hormone in the recipient may be the factor which caused the release of the gonad stimulating complex. Criticism had arisen regarding the use by himself and others (Allen and Doisy and Smith) of the term sexual maturity to describe the condition which resulted in animals which received pituitary transplants. Engle suggested that it better be termed, "exhibits certain phases of puberty". By injecting estrogenic hormone into castrated female rats Burch and Cunningham (1930) produced an increase in the amount of gonad stimulating hormone in the pituitary. Implantations were made into female mice to make this determination. By injecting an estrin into young females Meyer, Leonard, Hisaw and Martin (1930) found that those from the injected animals were much weaker in their gonad stimulating ability than those from the controls.

Seeing in the use of the then rather recently prepared chemically pure sex hormone preparations the possibility of clearing up the conflicting ideas which had arisen from purely surgical and allied methods of investigating the pituitary, Moore (1930) stated that the old idea of sex hormone antagonism (which had been more or less accepted since about 1912) had been definitely replaced. The newer hypothesis was indicated in more detail by Moore and Price (1930) who, two years later (1932), believing there was enough evidence (Golding and Ramirez, 1928, Evans and

Simpson, 1929, Engle, 1929, Leonard, Meyer and Hisaw, 1931, Spencer, Gustavson and D'Amour, 1931, Meyer, Leonard, Hisaw and Martin, 1932, and others) to show that their ideas were more than a mere working hypothesis concluded:

> Gonads function only when they are forcibly stimulated by certain secretions that are normally provided by hypophyseal activity. Hypophysis activity, on the other hand, is to some extent controlled by gonadal secretions, for when these gonad hormones are present in effective amounts hypophyseal activity is lowered. Injuries to the gonads follow administration of either sex hormone into either sex, and we interpret the mechanism as a hypophyseal involvement.

A series of investigations to determine the effect of estrogen administration were initiated by the Denver University group after Kunde, D'Amour, Gustavson and Carlson (1931) reported that both a smaller pituitary and smaller ovaries in which an arrest in the development of the follicles occurred after estrin was injected into immature dogs. To complete a preliminary report (1931) in which they had recorded a smaller increase in weight by rats injected with estrin (211 percent as compared to the controls' 282 percent) Spencer, D'Amour and Gustavson (1932) using large numbers of young rats found that the increase in body weight was substantially less in the injected animals. Measurements of the skulls and leg bones indicated that the injected animals were actually smaller. Histological examination revealed a few normal ovarian follicles and no corpora lutea. Doisy, Curtis and Collier (1931) reporting a depression of immature ova also confirmed the findings of Meyer et al (1930).

Following a demonstration by Nelson (1936) and Selye, Collip

and Thomson (1935) that injection of estrone into intact rats resulted in larger ovaries, Ellison and Burch (1936), in an effort to clear up the disagreement used several estrogenic substances. Acting upon the idea that weights were a better indication of pituitary function than was the ovary stimulating ability of transplanted glands they found that certain estrogenic hormones in sufficient amount would seemingly cause hypertrophy of the ovaries in normal animals. The increased size was due to the presence of many and larger corpora lutea. Mazer, Israel and Alpers (1936) showed that the duration of treatment is an important factor in the pituitary-ovarian response brought about by estrogenic substances. Large doses given for a long time to young animals caused ovarian degeneration while either single or multiple doses of the same size if given for but one week caused definite stimulation, resulting in corpora lutea formation in rats and mature follicles in rabbits.

Data regarding the effect of estrogens continued to accumulate. Allen, Hisaw and Gardner (1939) concluded that "estrogens seem to have little or no stimulating effect upon the ovarian follicles of normal animals. Many descriptions of ovaries following estrin treatment have reported inhibiting or depressing effects upon follicular development." In 1937 and 1938 Lauson, Heller and Sevringhaus had showed that small doses of estrogens did not materially depress the gonadotropic action of the pituitary gland. This finding together with that of others served to develop the present attitude which admits that the

mechanism by which the pituitary-gonadal interrelationship is maintained must be determined before any adequate explanation for the apparently conflicting results which have been obtained can be made.

With the increased availability of chemically pure androgenic substances there came a revival in efforts to determine more conclusively the effect of these compounds upon both gonads and pituitary. Moore and Price (1937) reported that the testes of young males treated with androsterone were lighter than those of their litter mate controls and that their pituitaries when transplanted into mice did not stimulate spermatogensis. The next year (1938) they obtained similar results with testesterone and testosterone propionate when physiological doses were given. However, when the dosage was increased there was less inhibition.

Hamilton and Wolfe (1938) demonstrated that immature rats which had received pituitary implants from immature rats injected with testosterone propionate had gonads which did not weigh as much as those from rats which had received normal pituitary implants but yet more than those from the animals which received no implants. This, they said, showed that the androgenic material could act to lessen the pituitary-gonadotropic substance by both suppressing its production and prevention of its release.

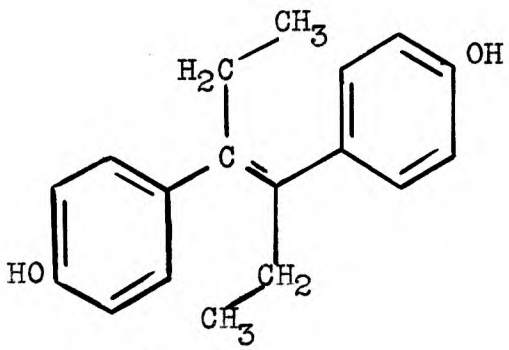
At present much disagreement exists as to whether or not the testes' size is always decreased by injection of androgenic materials. Many investigators: Korenchevsky, Dennison and Hall, 1937, Biddulph, 1939, Greene and Burrill, 1940, Mark and Biskind,

1941, and Selye, 1941, have reported a reduction in testis weight. Breneman (1937) and Bottomly and Folley (1938), working respectively with chicks and guinea pigs, have reported definite injury to the testes. However, there were the reports of Selye and Friedman (1941) and Shay, Gershon-cohen, Paschkis and Fels (1941a) that showed that very large doses stimulated the testes of rats. At the same time, the latter demonstrated that a much smaller dosage (3 mg as compared to 30 mg per week) resulted in decrease of testis weight, inhibition of sperm maturation and damage to interstitial tissue. Their conclusion was that the male hormone could, at the same time it was exerting an inhibitory effect upon the pituitary which in turn inhibits the testes, stimulate the testes directly. They suggested that during the first month the stimulating effect was not counteracted by the pituitary-gonad inhibition because at that age the gonadotropic function was not yet well developed. In later life the effect on the testes could be intermediate between direct and indirect inhibition. Very large doses were necessary to make the testes stimulating effect dominant. This led them to assume that the pituitary-gonadal inhibition is brought about by smaller doses, thus showing the pituitary to be more sensitive than the testes to testosterone action.

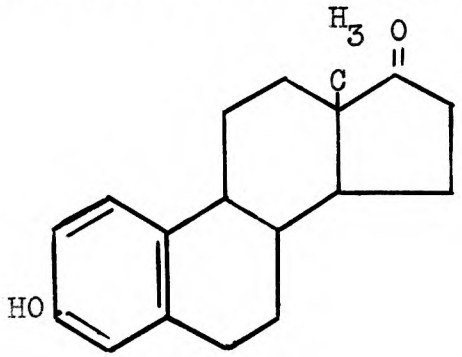
Rubinstein and Kurland (1941) demonstrated that very small doses (5 gamma per day for 10 days) led to a 17 percent increase in testes weight, stimulation of the germinal epithelium and no hindrance of the maturation of spermatozoa. Doses of 10 gamma

failed to show any variance in testis weight but those increasingly larger, up to 2.5 mg, produced progressively lighter testes resulting finally in a degeneration and sloughing of the germinal epithelium. They further showed that doses which were testes depressing when used for a short time failed to be when given for longer intervals. They explained that when the dosages were increased until they became massive the resultant large size of the various parts representing secondary sex characteristics would use a large amount of the injected hormone for their own maintenance. Thereby the amount of the hormone in the blood would be lowered, the pituitary inhibited to a lesser degree, and in turn, the testis spared.

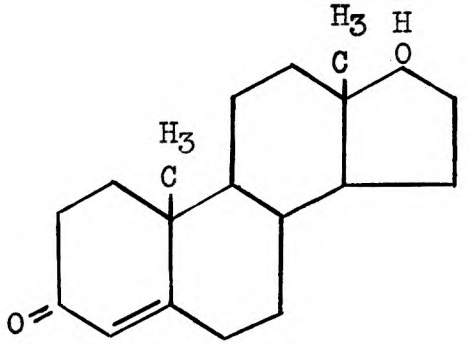
With the synthesis of stilbestrol (diethyl stilbestrol or 4;4'-dihydroxy-alpha;beta-diethylstilbene) by Dodds, Goldberg, Lawson and Robinson (1938) a whole new phase of estrogenic therapy was opened up. In an effort to show the relationship of stilbestrol to the natural hormone its formula was first written;



Striking resemblance had already been shown to exist between the formulas of estrone and testosterone viz.

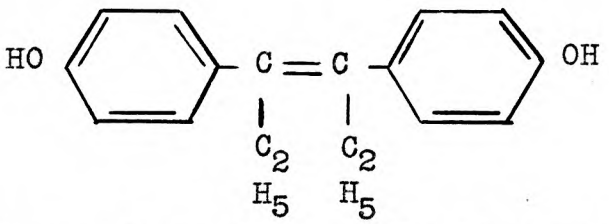



Estrone




Testosterone

Nevertheless stilbestrol was not organized around a sterol nucleus and its formula came to be written:




Very soon it was shown that stilbestrol was much more potent than the natural estrogens. Dodds, et al (1938) showed that by injection it possessed two and one-half times the activity of theelin. Sondern and Sealy (1940) reported that the potency was 65 to 80 times as much as theelin when it was given orally; by injection its action per unit was practically the same. Nevertheless, that stilbestrol fulfills and sometimes surpasses the therapeutic qualities of natural estrins, is effective by mouth, is easy to administer and costs comparatively little are advantages upon which most investigators have agreed. These facts were summarized by Payne and Shelton (1940).

Dodds, Lawson and Noble (1938) and Leighty and Wick (1939) used stilbestrol for sensitizing the rabbit, guinea pig and mouse uterus to progesterone. Noble (1938) reported that stilbestrol implanted subcutaneously would inhibit body growth and development of the genitalia of both male and female rats. Wellish, Baer and Macias (1940) found that stilbestrol inhibited both body and gonad growth of rats. It also markedly reduced the gonadotropic potency of the pituitary. Knowledge of the foregoing led to the conclusion that stilbestrol was "known to manifest essentially all the types of biologic activity characteristic of the naturally occurring estrogens" (Sevringhaus, 1940). A decrease in ovarian size and a marked retardation of body growth in rats was reported by Morrell and Hart (1941). Richards and Kueter (1941) reported that the inhibition of growth in young rats receiving stilbestrol could be overcome by administration of pituitary growth hormone. They suggested that the arrest of growth which followed stilbestrol treatment could be due to a decrease in the amount of food consumed by the animals. After the growth hormone was added there was an apparent stimulation of the appetite because more food was eaten. Suggestive of the early work done with follicular fluid, Lee, Robbins and Chen (1942), using the opening of the vagina as a criterion, showed stilbestrol to be 12 times as active as estrone by oral administration and 32 times as active when injected subcutaneously.

In contrast to the many reports regarding growth inhibition due to stilbestrol those for estrone are not so numerous. Zondek

(1936) induced retardation of growth in mice with estrone. Golding and Ramirez (1928) reported that it had no effect on body weight. Leonard, Meyer and Hisaw (1931) reported that rats injected with follicular hormone weighed slightly less than the uninjected ones. Mentions of body weight in testosterone reports appeared only incidentally. However, Rubinstein and Kurland (1939) reported that rats receiving this hormone became lighter than their controls. No loss of weight occurred in young male rats treated with testosterone propionate by Korenchevsky, Dennison and Hall (1937). Guinea pigs treated with the same hormone by Bottomly and Folley (1938) suffered no particular loss of weight. Shay, Gershon-cohen, Paschkis and Fels (1941b) found no weight increase difference between treated rats and controls. They explained that the administration of testosterone propionate to hypophysectomized rats showed that the hormone exerted its metabolic and growth effect by way of the pituitary. They summed up the situation by bringing out the comparison that estrone was known to inhibit the pituitary function, both gonadotropic and growth. Testosterone propionate at least allowed the release of growth hormone and possibly stimulated its production.

Very few have used birds in their experimental work. Juhn, Gustavson and Gallagher (1932) studied the factor of age in the reactivity of fowls to sex hormones. Lahr, Riddle and Bates (1941) investigated the effect of gonadotropic pituitary extracts on pigeons. Breneman's use (1937, 1938 and 1939) of the chick to show how comb growth is especially sensitive to male hormone

was the forerunner of his study made in 1941 of the growth of endocrine glands in the chick. Hamilton (1938) had produced in chicks a precocious masculine behavior by injections of testosterone propionate. This showed that the development of accessory reproductive structures and characteristics in chicks as in other animals was capable of stimulation by male sex hormone.

## MATERIALS AND METHODS

In the experiments reported in this paper, pure bred single comb White Leghorn chickens were used. They were kept indoors in clean metal batteries in a suitable, well lighted and ventilated room. Their food consisted of an adequate and well balanced diet and, like water, was before them at all times.

At the beginning of each experiment, members for the control and experimental groups were selected to be as nearly identical as it was possible to arrange them. Dosage of the hormone and the number of injections varied with the experiment. This information together with other pertinent facts is recorded in Table 1.

The stilbestrol used was dissolved in N/15 sodium hydroxide, the dilution calculated so that a 1/2 cc dose, either by intramuscular injection or orally, contained the desired amount of hormone. The estrone (theelin) and testosterone propionate were each dissolved in oil and prepared commercially in ampules.

The procedure in all the experiments was very nearly the same. When the hormone administration had been completed the

Table 1. Summary of method.

| Experiment | Hormone | Dosage (mg/da.) | Time (days) | Chicken age (mo.) | Sex | Number | | Method |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Injected | Controls | |
| I | Stilbestrol | 1.0 | 16 | 3 | F | 10 | 8 | Injection |
| II | " | 0.1 | 20 | 1 1/2 | F | 14 | 8 | By mouth |
| III | " | 0.5 | 20 | 1 1/2 | F | 8 | 8 | " |
| IV | Estrone | 0.25 | 20 | 1 1/2 | F | 10 | 10 | Injection |
| V | Testosterone | 2.0 | 22 | 3 1/2 | M | 6 | 6 | " |
| VI | " | 4.0 | 8 | 2 | M | 6 | 6 | " |

chickens were weighed and then killed two at a time. A sufficient number of chicks, some to receive pituitary implants and some to serve as normals, had been selected. The sex of these assay chicks, determined at the hatchery, was opposite to that of the donors.

The heads of the two chickens just killed were transferred to an operating table which was kept as clean as possible. By removing the comb, in cases where it was large enough to interfere, and then cutting through the dorsal part of the skull, pulling away the skin and breaking open the cranium, the pituitary in the sella tursica was exposed. By using suitable forceps the gland was removed and laid upon some still warm moist exposed muscle. In the same manner the other pituitary was removed and the two were then implanted into the breast muscle of an anaesthetized chick. A place for the implant had been prepared by making a small slit in the skin and then with a blunt forceps, spreading the muscle fibers apart. The two pituitaries were then inserted. To prevent loss of the implant a suture was made in both the muscle and the skin. As the implants were completed each assay chick was banded and its number together with that of the donors recorded. The chicks were allowed to recover from the effects of the anaesthetic and then were returned to the brooder.

Following the process of implantation, the gonads of both injected and control donor chickens were removed, weighed, fixed in Bouin's solution, dehydrated with dioxane, and via toluol were

imbedded in paraffin. They were then sectioned at eight microns, put on slides, and stained with alum haemotoxylin and triosin. A small drop of clarite was used over the mount and the cover glass was put into place. This technique was used for all tissues throughout the experiments.

The assay chicks were allowed to live for one week at which time they were killed. Their gonads were removed, weighed and fixed for histological study. Average gonad weights for all groups were later calculated.

Since male assay chicks were used to evaluate the effect of stilbestrol and theelin and female chicks for the testosterone propionate, the procedure varied. In the case of the testes, tubules were measured. A representative section from each was chosen. Using an ocular micrometer in a bi-ocular microscope with a mechanical stage, the total diameter and that of the lumen of 40 consecutively encountered tubules were measured and recorded. If there were not enough tubules in one section, another, some distance from the first, was selected and measurements were continued until the desired number was obtained. Similar studies although not so intensive were made of the gonads from the injected and control chickens.

In the case of the ovaries a total count of the follicles of a representative section from each was first made. Then the number of follicles over 50 microns and over 75 microns in diameter was determined. From these data the percent of each of these two sizes was calculated. Averages of all measurements

were determined in each group and tabulated so that comparison was facilitated.

When the estrone injected birds were being opened for gonad removal it was observed that their oviducts were much larger than those of the uninjected ones. Hence, they were removed, fixed and prepared on slides. An experiment to determine if the synthetic female hormone, stilbestrol, would have the same effect (it had not been noticed in previous experiments) was set up. A total of 2 mg (equivalent to 5 mg of estrone) in 20 days was given to three chickens of the same age group as had been used for the estrone experiment. Sections of the oviducts of both the injected and uninjected groups were prepared and examined.

## RESULTS

### Stilbestrol Experiments

In all cases except Experiment II the chickens treated with stilbestrol showed a greater average increase in body weight than did their controls. The ovaries of the injected birds consistently weighed less than those of the controls (Table 2).

With one exception the gonads of the assay chicks which had received no pituitary implants were lightest, those from birds which received implants from uninjected birds, heaviest, and those which received pituitaries from injected birds, intermediate.

Measurements of the tubules revealed that the average total diameter and average lumen diameter for the three groups were in the same relative order as were the gonad weights. When the per-

Table 2. Summary of results obtained in female sex hormone experiments.

| Hormone | Group | Avg. body wt. gain (g) | Avg. gonad weight (g) | Avg. gonad wt. of assay (mg) | Avg. tubule diameter (microns) | Avg. lumen diameter (microns) | Lumen-tubule diameter ratio (%) |
|---|---|---|---|---|---|---|---|
| Stilbestrol I | Injected | 211.17 | 0.339 | 56.8 | 43.1 | 7.05 | 16.4 |
| | Controls | 110.1 | 0.375 | 76.75 | 44.563 | 10.914 | 24.5 |
| | Normals | | | 45.0 | 41.781 | 6.290 | 15.1 |
| Stilbestrol II | Injected | 214.21 | 0.181 | 25.6 | 48.179 | 6.857 | 14.2 |
| | Controls | 223.65 | 0.204 | 27.2 | 48.646 | 8.427 | 17.3 |
| | Normals | | | 24.5 | 44.661 | 3.563 | 7.97 |
| Stilbestrol III | Injected | 234.57 | 0.183 | 28.0 | 47.922 | 8.094 | 16.9 |
| | Controls | 223.65 | 0.204 | 27.2 | 48.646 | 8.427 | 17.3 |
| | Normals | | | 24.5 | 44.661 | 3.563 | 7.97 |
| Estrone IV | Injected | 250.9 | 0.181 | 21.4 | 45.363 | 8.150 | 17.0 |
| | Controls | 209.25 | 0.195 | 26.0 | 47.281 | 9.047 | 19.1 |
| | Normals | | | 21.0 | 47.587 | 4.913 | 10.3 |

centage of the whole tubule occupied by the lumen was determined the same order was evident. The lumens of the tubules in the testes of chicks that received normal implants had opened more than those of the chicks that had received implants from injected birds. The lumens of those of the normal chicks were still smaller (Plate I).

When examined both grossly and microscopically, the oviducts from the injected birds were very similar to those of their controls in both size and cellular organization (Plate II).

## Estrone Experiment

In comparison with their controls, the chickens which were injected with theelin had a greater average increase in body weight but their gonads weighed less.

With the exception of the average total tubule diameter of the normals, the assay chicks showed the same gonad weight, tubule diameter and lumen percentage relationships as did those in the stilbestrol experiments.

The oviducts of injected birds showed a much greater degree of development than those from the uninjected controls (Plate III).

## Testosterone Propionate Experiments

The variation between the gonad weight of chickens which received the male sex hormone and that of their controls was very evident for in the older birds (Experiment V) they weighed about 1/23 as much. The difference was not so obvious in Experiment VI

## EXPLANATION OF PLATE I

Fig. 1. Photomicrograph of section of testis from assay chick which received pituitary implant from uninjected chickens.

Fig. 2. Photomicrograph of section of testis from assay chick which received pituitary implant from injected chickens.

Fig. 3. Photomicrograph of section of testis from assay chick which received no pituitary implant.

PLATE I

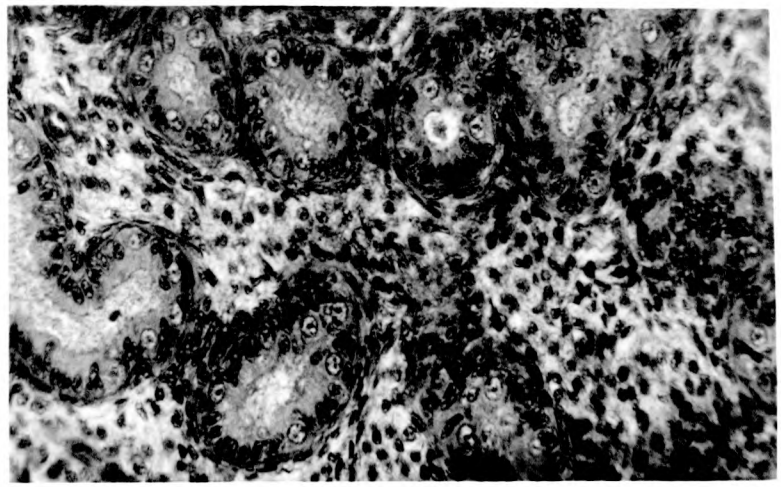


Fig. 1.

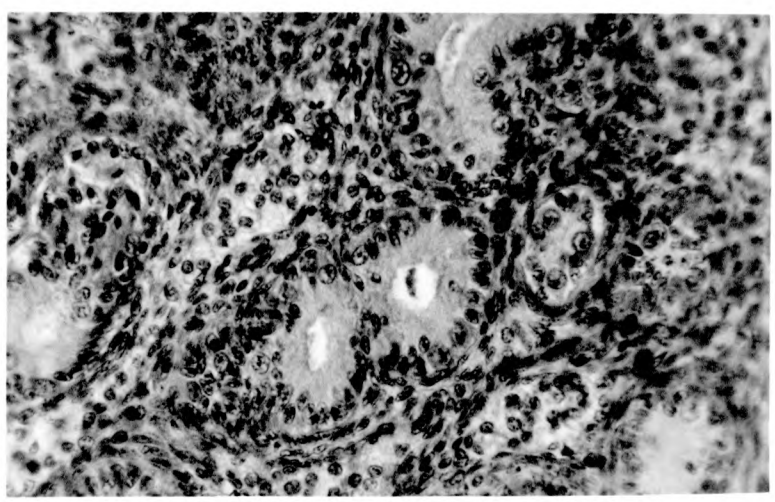


Fig. 2.

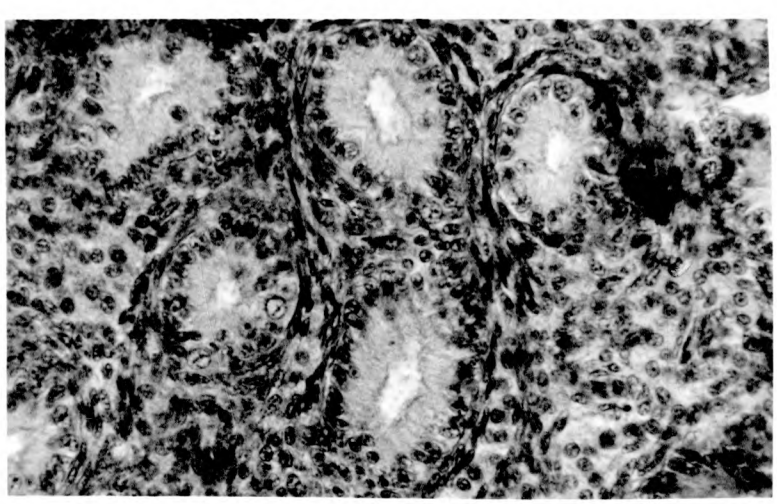


Fig. 3.

## EXPLANATION OF PLATE II

Fig. 1. Photomicrograph of section through oviduct of uninjected chicken.

Fig. 2. Photomicrograph of section through oviduct of chicken injected with stilbestrol.

PLATE II

Fig. 1.

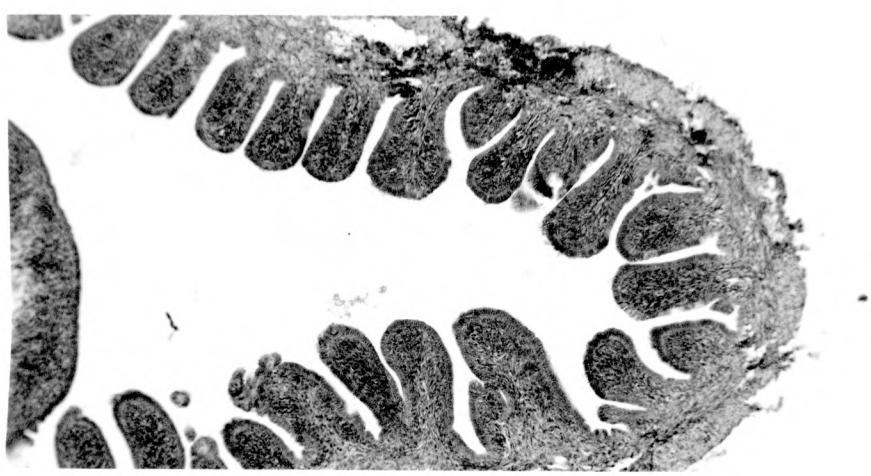


Fig. 2.

## EXPLANATION OF PLATE III

Fig. 1. Photomicrograph of section through oviduct of chicken which received estrone.

Fig. 2. Photomicrograph of section through oviduct of normal chicken of the same age.

PLATE III

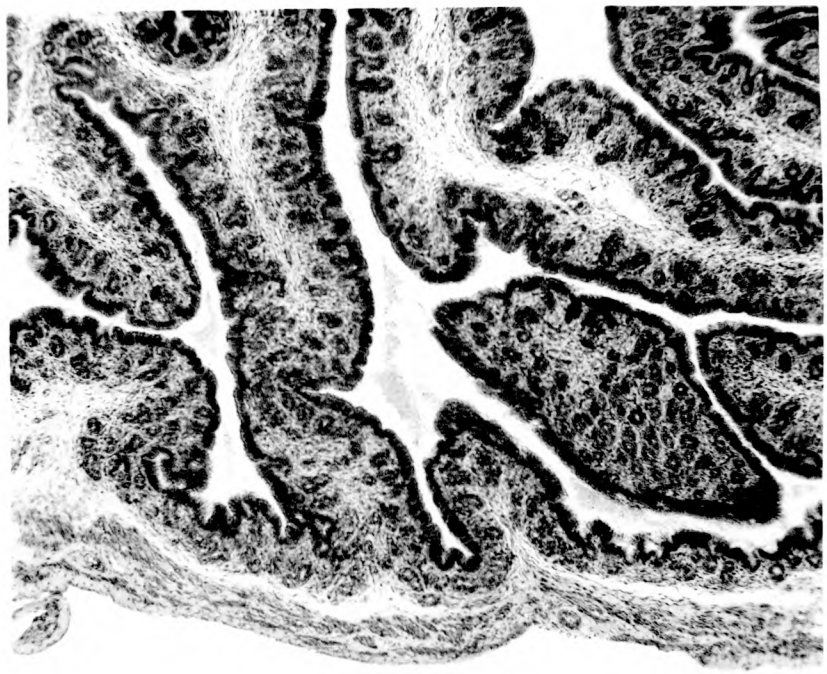


Fig. 1.

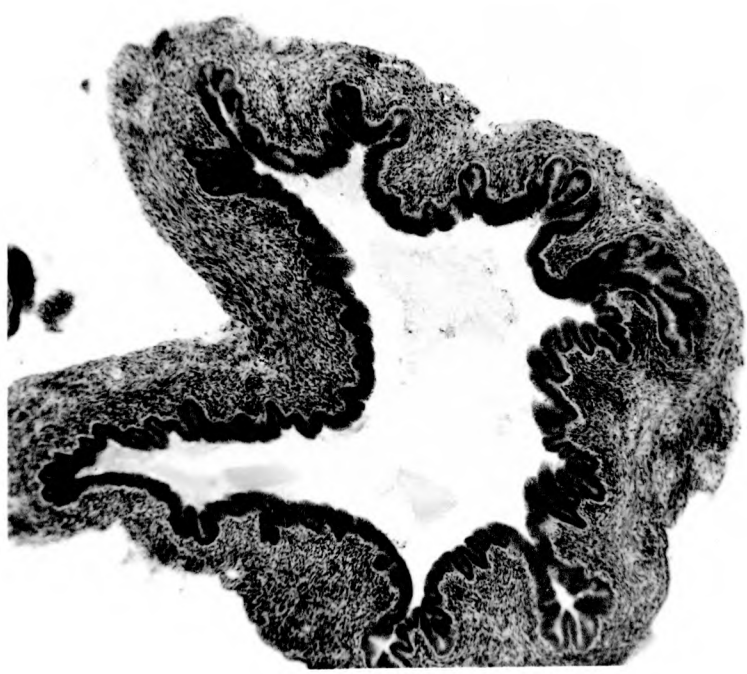


Fig. 2.

since the birds were younger with correspondingly smaller gonads (Plate IV). Average increase in the body weight of the injected chickens was greater in both cases than that of the uninjected ones (Table 3).

Contrary to the results from stilbestrol and theelin the gonads from assay chicks which received the pituitary implants from injected chickens weighed more than those from the other two groups. Counts revealed that the greatest percentage of larger follicles (Table 3) were found in the ovaries of assay chicks which received implants from uninjected chickens. The follicles were even smaller in the chicks which received implants from injected chickens than those of the normal group.

Histological examination of the gonads of the injected birds in Experiment V revealed tubules which were much smaller than their controls, had much more interstitial material and showed some shoughing of cells into the lumen. The tubules from the normal birds were approximately 150-180 microns in diameter (compared with 50-90 microns in the injected birds) had very little interstitial tissue and had mature spermatozoa in the lumens (Plate V).

The tubules of the control birds in Experiment VI were larger than those of the injected ones and the cells appeared to have many nuclei--suggestive of beginning spermatogenesis.

## EXPLANATION OF PLATE IV

Fig. 1. Photograph showing difference between testis of chicken which received male sex hormone (left) and that of uninjected chicken of same age (right). Experiment V.

Fig. 2. Photograph showing difference between testis of chicken which received male sex hormone (left) and that of uninjected chicken of same age (right). Experiment VI.

PLATE IV


Fig. 1.

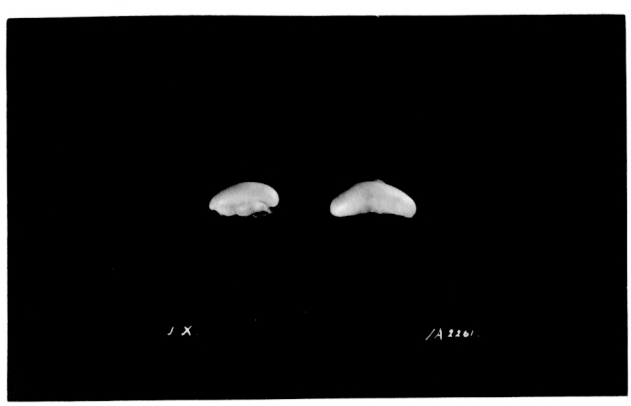



Fig. 2.

Table 3. Summary of results obtained in male sex hormone experiments.

| Hormone | Group | Avg. body wt. gain (g) | Avg. gonad weight (g) | Avg. gonad wt. of assay (mg) | Follicles over 50 microns (%) | Follicles over 75 microns (%) |
|---|---|---|---|---|---|---|
| Testosterone V | Injected | 506.5 | 0.356 | 39.3 | 14.29 | 5.58 |
| | Controls | 410.83 | 7.104 | 33.0 | 27.25 | 10.20 |
| | Normals | | | 22.0 | 14.74 | 6.24 |
| Testosterone VI | Injected | 132.5 | 0.205 | 44.3 | 10.28 | 3.20 |
| | Controls | 62.67 | 0.272 | 23.0 | 20.47 | 8.22 |
| | Normals | | | 22.0 | 14.74 | 6.24 |

## EXPLANATION OF PLATE V

Fig. 1. Photomicrograph of section of testis from chicken which did not receive male sex hormone.

Fig. 2. Photomicrograph of section of testis from chicken which received male sex hormone. (Same age as that in Fig. 1.)

PLATE V

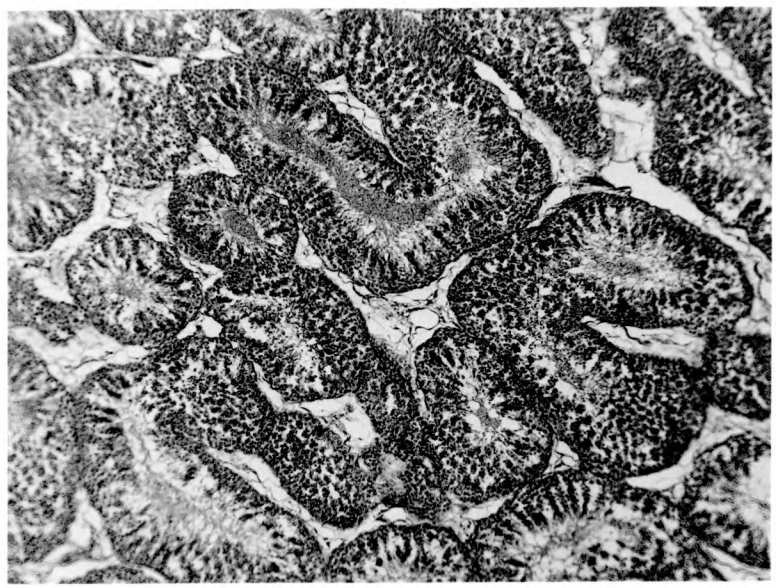


Fig. 1.

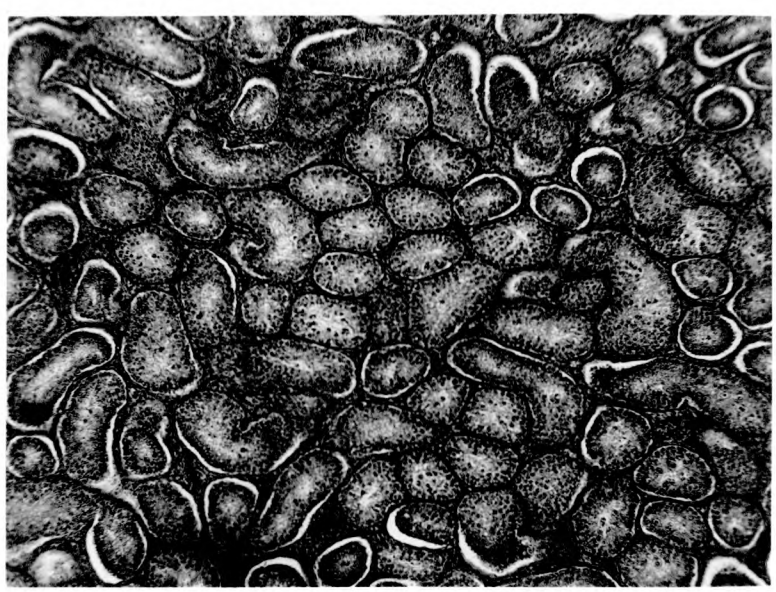


Fig. 2.

## DISCUSSION OF RESULTS

The loss in gonad weight observed in these experiments following sex hormone treatment, is in agreement with the results obtained in other laboratories using mammals. Some have observed an increase in gonad weight following male sex hormone administration but the dosage used by them was greater than that used here.

The greater than normal increase in body weight following female sex hormone administration is at variance with the results of others especially those who have used stilbestrol. However, several investigators have concluded that estrone had no apparent effect on growth. Therefore, further investigation may reveal that it is a matter either of length of time or total dosage or both that could be responsible for the differing results. Perhaps by recording body weights at frequent intervals a clue to the situation would be obtained. There is also the possibility that the chicken, or even the whole class to which it belongs reacts differently than other animals.

Since it has been suggested by many that the male sex hormone, testosterone propionate, might stimulate the production of growth hormone the results here obtained are not particularly unusual.

An examination of the assay chicks' gonad weights herein obtained during female sex hormone experiments would give particular satisfaction to those who have advocated the use of

the assay animal's gonad weight as the sole indication of the gonadotropic activity of pituitary transplants. The gonads of chicks which received pituitary implants from normal birds were the heaviest. Those from chicks which received implants from injected birds were lighter, thereby indicating an inhibition of gonadotropic activity of the pituitary following stilbestrol and estrone treatment. Furthermore, a comparison of the weight of gonads of chicks which received implants to the weight of those from normal chicks showed that some gonadotropic stimulation had occurred following implantations. Only one exception, which could perhaps be accounted for by the small number in the assay group (the sex had been determined incorrectly at the hatchery so several had to be discarded) interfered with a consistent sequence among the groups in each experiment.

The tubule measurements in all stilbestrol groups exhibited the same sequence as did the gonad weights. This fact might lead to the conclusion that, after all, gonad weights were sufficient in the evaluation of gonadotropic activity of implanted pituitaries. Examination of the results of the tubule measuring in the estrone experiment showed that the tubules in the testes of normal chicks were larger than those of the assay chicks which had received implants. This alone necessarily would mean that the implanting of even normal pituitaries did not result in increased gonad development. However, when the ratios existing between the lumen diameter and total tubule diameter were determined it was obvious that for their size the lumens had not

opened as completely as they had in the testes of chicks which received implants.

If the gonad weights of assay chicks were used as a criterion of the extent of pituitary inhibition by male sex hormone these results would indicate that not inhibition but definite stimulation was the effect upon the pituitaries of chickens treated with this hormone. The gonads of assay chicks which received the pituitary implants from injected birds were even heavier than those which had received implants from normal birds. This must surely indicate that some factor other than gonadotropic activity is involved.

Although this situation existed in the relative gonad weights the counts of follicles revealed that inhibition of the gonadotropic activity of the pituitary had resulted from male sex hormone treatment. Indeed the percentage of larger follicles was even less in the ovaries of the assay chicks that received the pituitary implants from injected donors than it was in the ovaries of the normal chicks. Perhaps the small size of the groups was responsible for these variations but they are consistent in both the proportion of follicles over 50 and over 75 microns in both experiments.

The failure of stilbestrol to stimulate the development of the oviduct to as great a degree as did estrone is suggestive that further investigation should be made. Not until special care in selecting the various regions of the oviduct for comparison is exercised, different relative dosages of the two hormones

used and, perhaps, length of treatment varied can any conclusion be made. However, these results seem to indicate the existence of a difference not previously reported in the effect of stilbestrol and estrone in birds.

## SUMMARY

Stilbestrol, estrone, and testosterone propionate were given to immature chickens to determine the influence of these hormones, not only upon gonad development but also upon the gonadotropic function of the pituitary. The latter was accomplished by implanting the pituitaries from both injected and uninjected fowls into chicks. The gonadotropic influence was determined by testis tubule measurements and ovarian follicle counts, depending upon the sex.

The following results were obtained:

1. Greater than normal gain in body weight occurred in all injected birds with the exception of those receiving the smallest dosage of stilbestrol.

2. The gonads of chickens that had received injections of the hormones were significantly smaller than those of normal birds. Histological examination revealed advanced stages of degeneration in the testes of birds which had received the male sex hormone.

3. Pituitaries of normal chickens when transplanted into chicks cause an acceleration of gonad development.

4. Pituitaries of chickens treated with stilbestrol or

estrone, when implanted, also stimulate gonad development in chicks although to a lesser degree than pituitaries from normal fowls.

5. If gonad weight alone is used as a criterion, pituitary implants from chickens injected with testosterone propionate seem to result in greater gonad weight than those from normal birds.

6. If resultant follicle development is used as a criterion, the gonadotropic activity of pituitary implants from chickens injected with testosterone propionate is less than that of those from normal chickens.

7. Gonad weights of assay chicks are not adequate indicators of sex hormone-pituitary inhibition.

8. Measurements of testicular tubules and counts of follicles in the gonads of assay chicks reveal more accurately the exact comparative degree of stimulation or inhibition.

9. Stilbestrol apparently stimulates development of the oviduct of immature chickens only slightly as compared to the marked stimulation by estrone.

10. A new method was used in these experiments to evaluate gonadotropic activity. Its essential features were the transplantation of pituitary glands into assay chicks, followed by testes tubule measurements or ovarian follicle counts made on the gonads of the chicks. Judging by the consistent results, this appears to be a valid method.

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