THE IMMATURE FOWL GONAD AS AFFECTED BY GONADOTROPIC AND MALE HORMONES

by

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INTRODUCTION

In this laboratory in 1937, Groody undertook to compare the results from injection of commercial extracts of anterior pituitary substance and anterior pituitary-like hormone from the urine of pregnancy, on the gonads of immature fowls. It was his purpose to make both histological and weight augmentation studies. As far as could be determined from the literature no histological work on this problem had been done up to that time. Groody obtained data from a small number of experimental animals which showed that, although both hormones were potent, they were different in the degree of stimulation produced.

The present experiments were undertaken with the thought of adding to the limited data already obtained and to gather further information by varying, somewhat, the conditions of experiment. It is the purpose of this paper to set forth the facts derived from further comparison of the effects of these hormones on the immature fowl gonad.

REVIEW OF LITERATURE

That the anterior lobe of the pituitary is a major factor in controlling the normal function of the gonads, and that pituitary extracts and pituitary-like extracts are potent gonad-stimulating agents when injected into experimental animals, has long been known. However, at the present time much work from various laboratories is giving results which

show that the hormones from different sources are not so nearly alike as was thought earlier, that the action is to a great extent dependent on the dosage used, and that the effects of a single hormone vary in different test animals. Some little data has been gathered which points toward the pituitary gonadotropic hormone containing two or more factors.

In implantation work, it has now been demonstrated that the condition of the donor, in certain species, at the time the tissue is taken, is to a great extent the controlling factor when resulting stimulation is considered. Also it is reported that the extract from the pituitary glands of different forms seems to differ in potency. Among the pituitary-like hormones in use, the potency seems to vary with the source and the time of taking the material.

Apparently much of the present disagreement can and is being composed by more extensive researches on hormones from different sources and in varying doses.

Investigations on Mammals

Three general methods have been used in attempts to stimulate growth or function of the testes of male mammals: implantation of fresh pituitary material, injection of extracts of the anterior lobe of the pituitary, and injection of whole pregnancy urine or an extract from it.

Smith and Engle (1927) reported that the implantation of fresh pituitary tissue into young rats and mice brought about an increase in the weight of the male reproductive organs, exclusive of the testes, that

was proportionally greater than that of the testes. Using bovine pituitary extract Johnson and Sayles (1929) were able to produce this same effect. A growth of the reproductive organs, exclusive of the testes, was produced in rats by the injection of whole pregnancy urine (Engle, 1929; Bourg, 1931). Tubule proliferation occurred in the testes of ground squirrels following pituitary implants (Johnson and co-workers, 1934), and in rats following pituitary extract injections (Engle, 1932). Injection of anterior pituitary extract into mice did not produce tubule proliferation (Johnson and Hill, 1930). Engle (1932), using rats, Engle (1932) and Aberle and Jenkins (1934), using monkeys, and Baker and Johnson (1936), using sexually inactive ground squirrels, were able to bring about a proliferation of the testis tubules by injection of a pregnancy urine extract. Moore (1936) found that the sensitivity of rat testes to several gonadotropic hormones decreases with age, there being little effect after maturity.

In general, all of the methods used are able to cause stimulation in growth of the male reproductive organs or tubule proliferation.

Since the effect of the pituitary substance is more pronounced in female mammals than in males, more comparative work with the gonadotropic hormones has been done using the female as the test animal. Evans and Long (1921, 1922) recorded general ovarian growth in rats injected with anterior pituitary extract. Follicle stimulation, as a result of whole gland implants, has been noted in immature mice (Engle, 1929), rats

(Evans and Simpson, 1929), and rabbits (Hill and Parkes, 1931). Evans, Meyer and Simpson (1932) found that immature rat ovaries were capable of a limited amount of growth stimulation with a pregnancy urine extract, while a follicular stimulation was reported in mice (Engle, 1929), rats (Evans and Simpson, 1929), and rabbits (Siegmund, 1930; Friedman, 1930). In rats the effect was less than that obtained with whole pituitary implants. Leonard (1934) was able to stimulate ovarian weights in rats with both pregnancy urine extracts and anterior pituitary extracts, but obtained growth stimulation with a combination of the two extracts which was greater than the combined results of the two separate injections. Cole (1936) using serum from pregnant mares reported that ovarian stimulation as measured by follicle size varied with the dose.

Smith, Engle, and Tyndale (1934) showed that some maturation changes must be undergone before immature rat ovaries will respond to gonadotropic stimulation. Age was found to be a major factor in the response of immature rat ovaries to mare gonadotropic hormone (Saunders and Cole, 1936).

Kido (1937) was able to demonstrate the placental origin of human anterior pituitary-like hormone by transplanting small pieces of tissue from the human chorion to the orbits of rabbits and so inducing ovulation and corpus luteum formation.

Schmidt (1937) reported that in the case of guinea pigs the time of removal of the pituitary from the donor in implantation experiments was

the major factor in the results obtained. The pituitaries taken during the interoestrus period were the most effective, while those removed during oestrus had little effect on the ovary of the recipient. No lutinization or maturation of follicles was observed from implantation of female pituitaries, but male glands gave both effects. Aron (1932, 1933) found that the size and time of dosage of the gonad stimulating hormone from the anterior pituitary determine the effect on guinea pigs.

Katsman, Nelson, and Doisey (1937) reported the continued implantation of rat pituitaries into female rats for periods as long as nine months with no diminution in the effectiveness of the implants. The ovaries were still large, no evidence of gonadotropic inhibitory substance was observed, and the serum of these rats augmented the response in other implanted rats. Adult female rats treated by McPhail (1933) with pregnancy urine extracts and anterior pituitary extracts for long periods showed inhibition of the vaginal cycle and a marked decrease in fertility.

Ovulation has been produced by anterior lobe implants in female mice (Engle, 1929), and in rabbits by injections of anterior lobe extracts (Bellerby, 1929; Jares, 1930; Hill and Parkes, 1931; Snyder and Wislochi, 1931). Pregnancy urine extracts have been reported to cause ovulation in ferrets (Hill and Parkes, 1930), and rabbits (Friedman, 1929; Winter, 1931; Hill and Parkes, 1931; Snyder and Wislochi, 1931; Wolfe and Ellison, 1932). Three cases have been reported in which extracts of pregnancy urine have failed to cause ovulation: in mice

(Engle, 1929), in rabbits (Friedman, 1930), and in guinea pigs (Jares, 1931). Anterior pituitary hormone has been used to produce lutinization in mice (Johnson and Hill, 1930), in rats (Johnson and Sayles, 1929), and in rabbits (Hertz and Hisaw, 1934). Pregnancy urine extracts have been reported to attain the same results in mice (Engle, 1929), and in rabbits (Winter, 1931; Wolfe and Ellison, 1932).

In general, it can be stated that whole pituitary implants, anterior pituitary extracts, and pregnancy urine extracts are able to cause general growth of the ovary, lutinization, follicle stimulation, and ovulation in female mammals. Some stimulation has been produced with blood serum from pregnant mares.

Loeb (1933) and others have some evidence that there may be two anterior pituitary gonadotropic hormones, one causing enlargement of follicles and the other causing the destruction of these follicles. Different species seem to possess these two hormones in varying ratios.

Investigations on Male Birds

The similarity in action of the anterior pituitary hormone and the pregnancy urine derivative on mammals is not extended to avian forms, according to the findings of various workers. Administration of anterior lobe hormone has brought about a great increase in testis growth in immature ring doves (Riddle and Flemion, 1928; Riddle and Polhemus, 1931), in immature domestic ducks (Schockeart, 1931), and in cockerels (Domm and Van Dyke, 1932a; Schockeart, 1933; Groody, 1937). Riddle (1931)

found that the pigeon testis was a very sensitive test medium for the anterior pituitary gonadotropic hormone, but that an extract from the urine of pregnant women did not give a comparable response. Similar failures of pregnancy urine extracts have been reported in immature ring doves (Riddle and Polhemus, 1931), in immature domestic ducks (Schockeart, 1931), and in cockerels (Schockeart, 1933). Riddle and Polhemus (1931) reported a regression of the testes of doves upon the administration of the pregnancy urine hormone. Evans and Simpson (1934) reported that the effect of various gonadotropic hormones on the immature pigeon gonad varies with the source of the hormone. This test animal was very sensitive to anterior pituitary hormone, less sensitive to mare serum, and insensitive to pregnancy urine extracts. Byerly and Burrows (1938) showed that one of the most rapid activators of the newly hatched chick testis is that from the serum of mares in early pregnancy.

Contrary to its action on mammals, anterior pituitary extract failed to give any increase in the interstitial cell mass in male ducks (Schockeart, 1931), and cockerels (Schockeart, 1933). Pregnancy urine extract was likewise inactive. Stimulation of accessory structures of cockerels has been noted as resulting from anterior pituitary hormone (Domm, 1933; Schockeart, 1933), but not from pregnancy urine fractions (Riddle and Polhemus, 1931; Schockeart, 1933). Breneman (1936) was able to augment greatly chick testis weights with whole pituitary extract, pregnancy urine, and mare serum. Groody (1937) obtained similar results with pregnancy urine extracts.

Domm (1931a) subjected immature male fowls to repeated homeoplastic hypophyseal implants and noted an increase in the size of the testis tubules and precocious secondary sex characters.

Summarizing the work cited on male birds, it might be stated that while the anterior lobe produces testicular growth, tubule enlargement, and accessory structure growth, with the two exceptions noted, the pregnancy compounds do not bring about these manifestations.

Investigations on Female Birds

Repeated homeoplastic hypophyseal implants by Domm (1931b), resulted in increased growth of immature fowl ovaries, but no histological changes were noted. The female secondary sex characters were precocious and the oviducts were enlarged. Domm and Van Dyke (1932b) using hebin were able to obtain similar but more marked results and in addition noted indications of follicular enlargement.

Mare serum, whole pituitary extracts, and pregnancy urine extracts were shown by Breneman (1936) to cause augmentation of ovary weights in chicks. Evans and Simpson (1934) elicited differential weight responses in immature pigeon ovaries with various gonadotropic hormones. Asmundson, Gunn, and Klose (1937) using an extract from the serum of mares in early pregnancy were able to demonstrate enlarged follicles in the ovaries of their older immature fowls. Stimulation of the secondary sex characters and increases in ovary weight were noted in all cases.

Others (Riddle and Flemion, 1928; Schockeart, 1931; Riddle and Polhemus, 1931; Groody, 1937) have reported the growth of the ovaries of immature fowls as a result of injection of anterior pituitary hormone. Riddle and Polhemus (1931) using pigeons, and Pearl and Surface (1915) with hens, have noted the failure of this extract to effect ovarian growth. Follicular stimulation by anterior pituitary extract has been recorded in ducks (Schockeart, 1931) and in hens (Schockeart, 1933; Groody, 1937). Some controversy still exists as to the effect on the process of ovulation.

Pregnancy urine or its extracts inhibited the growth of dove ovaries (Riddle and Polhemus, 1931). If excessive amounts are injected, the ovaries of sparrows react but slightly and mainly in degenerative processes (Witschi, 1935).

Male Hormone Investigations

The combs of chicks, mature hens, and capons, as well as the bill color of male English sparrows, have long been used as test objects for standardizing male hormone extracted from testes or from urine. A study of the histological and growth changes in the ovaries of immature fowls injected with male hormone was undertaken. It is believed that this is the first work of this kind that has been reported.

Smith (1912) reported that an extract from the testis of fowls had no effect on the secondary sex characters of females. Growth of comb, wattles, and ear lobes of the capon, and comb growth in bilaterally

castrate hens injected with bull testis extract, was noted by McGee, Juhn, and Domm (1928). Gallagher and Koch (1935) were able to reproduce growth increments in capon combs, using the same dosage after each regression. English sparrow capon bill color was found by Keck (1932) to be a very sensitive test object for male hormone. Witschi (1935, 1936) noted the same fact using male, female, and castrate English sparrows and other finches. Danforth and Fisher (1935) were unable to effect masculinization of the plumage or eye-color in the female Brewer's blackbird with a testicular extract. According to the authors it may be that this failure was due to a lack of linkage of plumage and eye-color to hormone control.

Greenwood, Blyth, and Callow (1935) found the effect of androsterone definite and predictable on capon combs. Callow and Parkes (1935) noted very rapid growth of capon combs with large doses of androsterone, and that size once produced, could be maintained with small doses.

Oreton and Oreton-B stopped the growth of the testes in five and ten-day old chicks immediately, but growth was resumed as soon as the injections were stopped (Breneman, 1937). The testes of these chicks were 90 per cent greater in weight than those of controls at the age of one month. Comb growth was directly correlated with testis weight.

Greenwood and Blyth (1930) observed that persistent testicular grafts in normal hens inhibited to varying degrees the primary and secondary functions of the ovary. Some hens did not lay, others gave fewer

eggs than normal, the head furnishings of the male were assumed, the pelvic bones were at times insufficiently sprung for egg laying, and in some the differentiation of the oviduct was below normal.

No effect on the normal ratio of males to females was observed by Kozelka and Gallagher (1934) when male hormone was injected into chick embryos. Dantchakoff (1935 a and b) using X-ray in addition to male hormone was able to cause sex inversion in chick embryos, so that testes were developed from ovaries.

Hamilton (1937) was able to masculinize the reproductive tract of female rats, rabbits, puppies, and monkeys by injections of male hormone.

MATERIALS

The test animals used in all of the experiments were immature pure-bred single-comb white Leghorn fowls. The gonadotropic hormones used were the commercial products of Parke, Davis and Company, Antuitrin and Antuitrin-S. In a private communication Parke, Davis and Company states that Antuitrin is prepared from the anterior lobe of the pituitary of cattle, and Antuitrin-S from human pregnancy urine exclusively. The male hormone used was testosterone propionate in oil, containing 4.113 mg. of pure material per cc. This was a mixture of Peranderen (Ciba Pharmaceutical Products, Inc.) and Oreton (Schering Corporation).

METHODS AND PROCEDURE

In all cases the hormones were administered by intra-muscular injections. Body weights and comb measurements were taken at the beginning

of each experiment and at the time of sacrificing each group. In all cases adequate controls were kept. The experimentals and the controls were kept in the same pen and received the same care and handling. The pens used were indoors and limited in size. Vitamin D was added to a balanced mash diet.

In an attempt to parallel the experiment by Groody previously mentioned, one group of twenty-five chickens was put on experiment at thirty-six days of age. These will be known as Group I. Ten (3 males and 7 females) of these birds were injected with 0.5 cc. of Antuitrin-S daily; eight (all females) received 0.5 cc. of Antuitrin daily; and seven (3 males and 4 females) were used as controls and injected daily with 0.5 cc. of 0.8 per cent saline solution. All chickens received eighteen injections and were killed at fifty-six days of age, three days after the last injection, to allow time for the last injection to become effective.

A second group of thirty chickens, fifty-four days old, were used in a similar experiment. These will be known as Group II. Eight (four each of males and females) were injected every second day with 0.5 cc. of Antuitrin-S; twelve (six males and six females) received the same amount of Antuitrin; and ten (six males and four females) were used as controls and received no injections. Each of the experimental fowls received thirteen injections and all, including the controls, were killed when eighty-two days old.

Eleven female chickens, forty-eight days old, were used in the male hormone experiment: six experimentals and five controls. Those on

experiment were injected daily for fourteen days with 0.3 cc. of male hormone. The controls received a like amount of 0.8 per cent saline solution. To allow the last injection to become effective, the animals were not sacrificed until they were sixty-four days old.

To eliminate all weight errors possible, small vials having a capacity of approximately 1½ cc. and containing only enough Bouin's or Heidenhain's "Susa" fixative to cover the tissue well, were weighed just before sacrificing the chicks. The tissues were removed immediately, placed in the vials, and the whole reweighed at once.

In preparing the tissues for histological study the sections were stained with Ehrlich's hematoxylin and eosin.

Two slides were made from each ovary and testis, and a single typical section on one of these slides was used for data. In the case of the males, the thickness of the walls of one hundred testis tubules was measured with an occular micrometer (Figs. 3 and 4). All of the tubules encountered in a sweep of the section with a mechanical stage were measured, and sweeps were continued until one hundred tubules had been measured. In the ovarian sections (Figs. 1 and 2) counts were taken of all follicles appearing on the section, of all follicles one hundred micra or over in diameter, and of all follicles two hundred micra or over in diameter. All data was computed to four significant figures with a calculator.

RESULTS

Group I. The chickens injected with Antuitrin showed the greatest number of large follicles (Table I), with 20.93 per cent one hundred micra or over in diameter and 9.55 per cent two hundred micra or over in diameter (Fig. 2). In those chickens injected with Antuitrin-S, the corresponding figures were 18.11 per cent and 7.03 per cent. The controls of this group were found to have 13.40 per cent of the ovarian follicles one hundred micra or over in diameter, and 7.76 per cent two hundred micra or over in diameter.

Two measurements were taken on the testis tubules: the diameter of the lumina and the thickness of the tubule wall. No males were injected with Antuitrin. The fowls injected with Antuitrin-S had an average wall thickness of 16.96 micra and a lumen diameter of 10.57 micra (Table I). The controls showed an average tubule wall thickness of 18.77 micra and an average lumen diameter of 11.66 micra. By both measurements the injected chickens showed some retardation in tubule growth.

The female chickens injected with Antuitrin showed an average gain of 40.82 per cent in ovary weight over the controls. Of those injected with Antuitrin-S, the females averaged 40.63 per cent less in ovary weight than the controls, and the males were 5.20 per cent below the control average in testis weight (Table I).

Comb measurements were taken; the measurement used was the sum of the length and the height. The female chickens which received Antuitrin

developed combs which exceeded the combs of the controls by 27.00 per cent. The variation in the case of the females treated with Antuitrin-S probably is not significant, since the experimentals exceeded the controls by only .38 per cent in comb size. The males, on the other hand, averaged 31.95 per cent smaller in comb size than the control chicks.

Body weight augmentation was not noted in either group.

Group II. The effects of the two hormones on follicle size in this group very closely paralleled those in Group I. The fowls injected with Antuitrin showed 25.44 per cent of the total follicles to be one hundred micra or over in diameter and 14.54 per cent to be two hundred micra or over in diameter (Table II). Antuitrin-S gave 19.68 per cent of follicles one hundred micra or over in diameter and 9.89 per cent two hundred micra or over in diameter. The corresponding figures for the controls were 12.55 per cent and 6.67 per cent.

If the results in groups I and II are combined, we have:

Percentage of follicles one hundred micra or over in diameter

Antuitrin	22.55
Antuitrin-S	18.88
Control	12.77

Percentage of follicles two hundred micra or over in diameter

Antuitrin	11.34
Antuitrin-S	8.44
Control	6.95

These figures in each case closely approximate the ratio of 4:3:2.

The thickness of the testis tubule wall in this group gave no data of significance (Table II). The birds treated with Antuitrin had

tubules with an average wall thickness of 18.23 micra, those injected with Antuitrin-S averaged 18.39 micra, and the controls averaged 17.57 micra. When lumen diameter is considered a greater difference is noted. The birds injected with Antuitrin averaged 13.30 micra in lumen diameter, those injected with Antuitrin-S averaged 9.73 micra, and the control group, 9.21 micra. For the Antuitrin group this is an increase of 44.41 per cent over control size.

There seemed to be little consistency between the groups when gonad weight changes were considered (Table II). In Group II the males injected with Antuitrin gained 57.34 per cent in testis weight over the controls, and the Antuitrin-S group gained 23.14 per cent. The females treated with Antuitrin gained 14.71 per cent in ovary weight, and those injected with Antuitrin-S gained 26.47 per cent.

In the Antuitrin group, males gained 11.82 per cent and females 16.03 per cent in comb size over the controls. Comb growth did not seem to be significant in either males or females injected with Antuitrin-S. The males were 1.60 per cent above and the females 1.46 per cent below the controls in size.

Male Hormone Group. This group of chicks gained on the average 123.05 per cent in comb size over the control group (Fig. 5). The injected chicks in this group, however, fell below the average ovary weight of the control chicks by 5.91 per cent.

In the injected chicks, 13.56 per cent of the follicles were one hundred micra or over in diameter and 4.35 per cent were two hundred

micra or over in diameter (Table III). The controls showed 18.88 per cent of the follicles one hundred micra or over and 6.34 per cent two hundred micra or larger. Since the apparent retardation is but 1.68 times the probable error, it is not considered significant.

TABLE I.

Group I. All chicks received eighteen daily injections of 0.5 cc. of material. All were put on experiment at 36 days of age and killed when 56 days old.

Number and Sex	Injected Material	Initial Body Wt. (grams)	Final Body Wt. (grams)	Wt. Lt. Gonad (grams)	Wt. Rt. Gonad (grams)	Testis Tubule Lumen Diam. (micra)	Testis Tubule Wall Thick. (micra)	Follicles 200 micra or over	Follicles 100 micra or over	Total in sect.
B62 M	Antui-trin-S	110.2	241.2	.0461	.0254	9.05	17.90			
B65 F	"	91.7	150.6	.0500*				-	-	-
B66 F	"	128.7	272.6	.1265				38	78	351
B71 F	"	150.5	283.4	.1321				26	88	625
B72 F	"	128.8	240.0	.0855				21	54	290
B77 F	"	121.4	227.8	.0830				24	72	453
B79 F	"	135.0	243.0	.1181*				-	-	-
B86 M	"	102.8	182.4	.0133	.0078	13.77	14.86			
B87 F	"	164.8	339.6	.1310				28	61	230
B88 M	"	153.0	206.6	.0277	.0145	8.92	18.13			
B67 F	Antui-trin	135.4	254.0	.1482				18	66	424
B68 F	"	149.1	288.5	.0258				30	62	366
B69 F	"	157.2	311.0	.2131*				-	-	-
B70 F	"	124.0	229.9	.1202				35	110	509
B74 F	"	106.1	199.0	.1895				42	90	325
B89 F	"	126.5	269.1	.2523				20	47	314
B90 F		111.5	194.5	.0545				32	48	164
B91 F		127.7	262.8	.2296				62	101	399
B63 M	Saline Solution	132.8	257.1	.0386	.0385	14.45	17.96			
B75 F		155.5	293.4	.1154				46	70	622
B76 F		122.3	256.1	.1092				27	56	318
B81 M		136.0	267.3	.0427	.0389	11.35	19.65			
B83 M		139.4	283.6	.0385	.0301	9.17	18.73			
B85 F		149.0	281.3	.0983*				-	-	-
B92 F		120.3	250.3	.1150*				-	-	-

*Tissue lost in histological preparation.

TABLE II.

Group II. All chicks received thirteen injections of 0.5 cc. of material. The injections were given on alternate days. The experiment began when the chicks were 54 days old and all were killed at 82 days of age.

Number and Sex	Injected Material	Initial Body Wt. (grams)	Final Body Wt. (grams)	Wt. Lt. Gonad (grams)	Wt. Rt. Gonad (grams)	Testis Tubule Lumen Diam. (micra)	Testis Tubule Wall Thick. (micra)	Follicles 200 micra or over	Follicles 100 micra or over	Total in sect.
B112 M	Antui-trin-S	232.3	452.0	.0655	.0681	11.60	20.00			
B113 M	"	276.5	556.5	.0852	.0952	6.71	19.66			
B121 M	"	179.5	334.3	——*	.0424	10.87	15.50			
B127 F	"	216.1	463.5	.1957				49	102	358
B129 F	"	325.3	533.3	.1798				57	100	513
B137 F	"	172.0	329.2	.1277				41	86	290
B138 F	"	247.0	474.2	.1641				40	84	729
B108 M	Antui-trin	231.2	530.6	.1289	.1026	20.81	17.93			
B109 M	"	258.5	359.0	.0547	.0598	12.41	18.33			
B114 M	"	198.3	363.5	.0711	.0595	11.47	20.00			
B122 M	"	281.0	485.1	.1494	.1122	10.38	17.80			
B125 M	"	246.4	422.8	.0979	.0749	11.43	16.66			
B126 F	"	293.2	458.7	.2552				54	83	502
B128 F	"	213.0	328.9	.1106				29	68	240
B133 F	"	221.3	320.0	.1162				29	69	370
B135 F	"	230.5	417.1	.0536*				-	-	-
B140 F	"	249.5	456.8	.1779				53	79	102
B142 F	"	201.0	324.5	.1991				39	58	189
B107 F	None	273.9	369.0	.1172				34	70	521
B110 M	"	225.0	332.5	.0478	.0450	12.71	15.66			
B115 M	"	254.7	441.3	.0412	.0477	3.05	18.53			
B116 M	"	289.1	552.2	.0909	.0692	9.35	18.26			
B120 M	"	252.8	404.2	.0435	.0074	8.90	17.06			
B124 M	"	215.5	354.9	.0899	.0962	12.03	18.33			
B131 F	"	227.0	359.2	.1673				85	155	759
B139 F	"	212.4	311.7	.1134				42	92	622

*Tissue destroyed in histological preparation.

TABLE III.

Male Hormone Group. All fowls received fourteen daily injections of 0.3 cc. of material each. All were forty-eight days old when experiment began and sixty-four days old when killed. The controls received saline solution.

Number	Status	Initial Body Wt. (grams)	Final Body Wt. (grams)	Ovary Wt. (grams)	Follicles 200 micra or over	Follicles 100 micra or over	Total in sect.	Initial Comb Measure (length plus height)	Final Comb Measure (length plus height)
B 93*	Experimental	224.4	330.6	.0744	15	51	420	24	67
B 94	"	216.5	301.0	.0945	19	45	220	22	72
B 100	"	233.4	346.3	.1297	11	39	171	25	76
B 101	"	302.5	402.0	.1639	20	63	465	26	84
B 102	"	247.2	342.6	.0916	15	50	601	24	84
B 104	"	195.5	296.5	.1046	17	63	323	20	64
B 97	Control	185.5	254.8	.0899	33	60	289	22	27
B 98	"	235.6	330.5	.1449**	-	-	-	27	38
B 99	"	228.1	307.4	.1366	9	55	230	23	33
B 103	"	238.3	330.7	.0816	23	73	351	22	33
B 106	"	276.9	382.2	.1326	16	55	406	28	36

*Bird crowed several times on day of killing.
**Tissue lost in histological preparation.

SUMMARY

1. Immature female fowls injected with Antuitrin and Antuitrin-S showed a greater number of large ovarian follicles than did control fowls of the same age. Antuitrin had a greater stimulating effect than did Antuitrin-S.

2. Two methods of measurement used on the testis tubules of the males in these same groups showed little significant difference between experimental and control chickens.

3. Testosterone propionate is an active agent in stimulating the development of secondary sex characters typical of males in immature female fowls. The retarding action of this agent on follicular development is not considered significant. The small number of birds may be a factor, however.

4. Body and gonad weights were not significantly influenced by the material injected.

5. Antuitrin has an activating effect on comb growth in the immature fowl. Antuitrin-S seems to be without effect.

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PLATES

Explanation of Plate I:

Figure 1. Photomicrograph of a section of ovary of a normal immature female fowl, B106, sixty-four days old.

Figure 2. Photomicrograph of a section of ovary of an immature female fowl, B91, injected with Antuitrin. Fifty-six days old. (Magnification not same as Figure 1.)

PLATE I

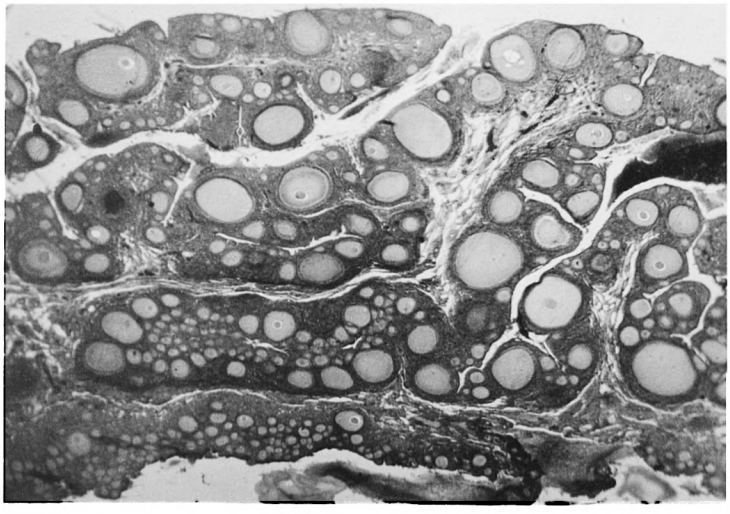

Figure 1

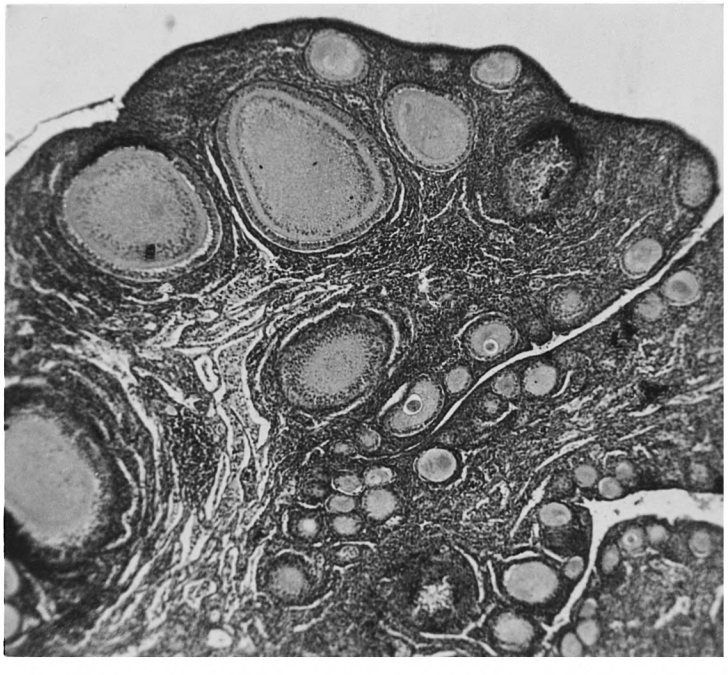

Figure 2

Explanation of Plate II:

Figure 3. Photomicrograph of a section of the left testis of a normal immature male fowl, B81, fifty-six days old.

Figure 4. Photomicrograph of a section of testis of a normal mature male fowl. (Magnification not the same as Figure 3.)

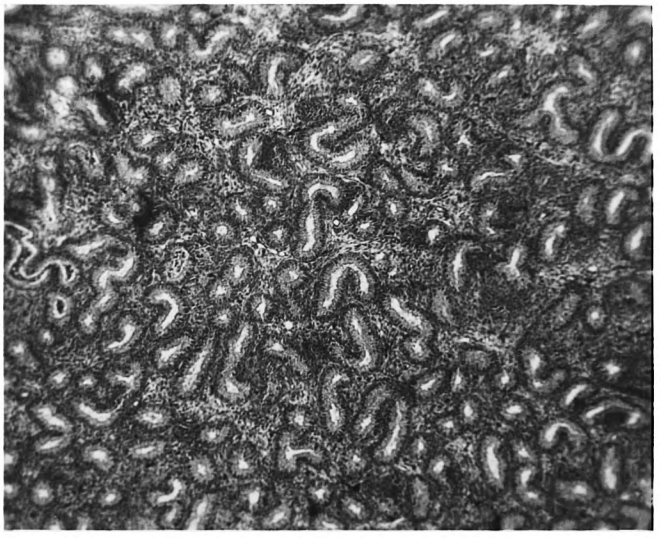

Figure 3

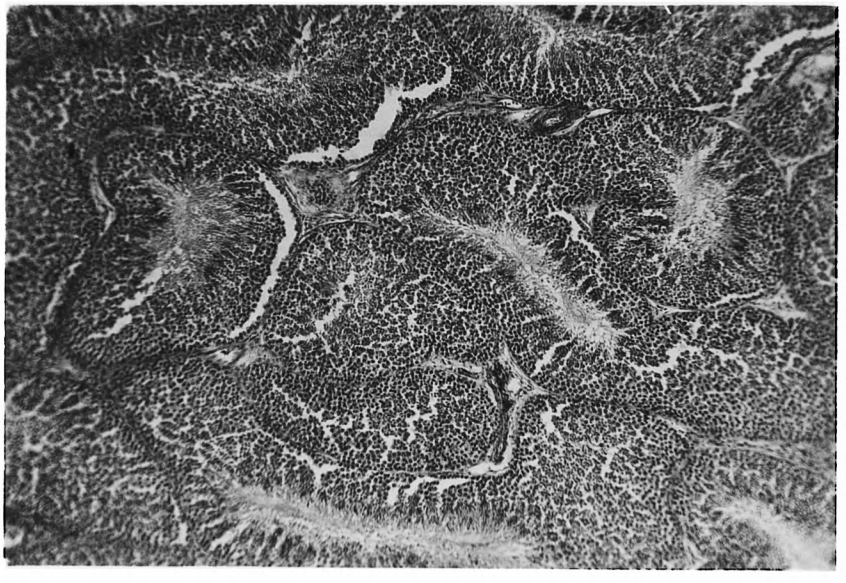

Figure 4

Explanation of Plate III:

Figure 5. Necrotic heads of immature female fowls, B103 and B105, of the same age and brood showing head furnishings. Left bird injected with male hormone, right control. (Approximately natural size.)

PLATE III

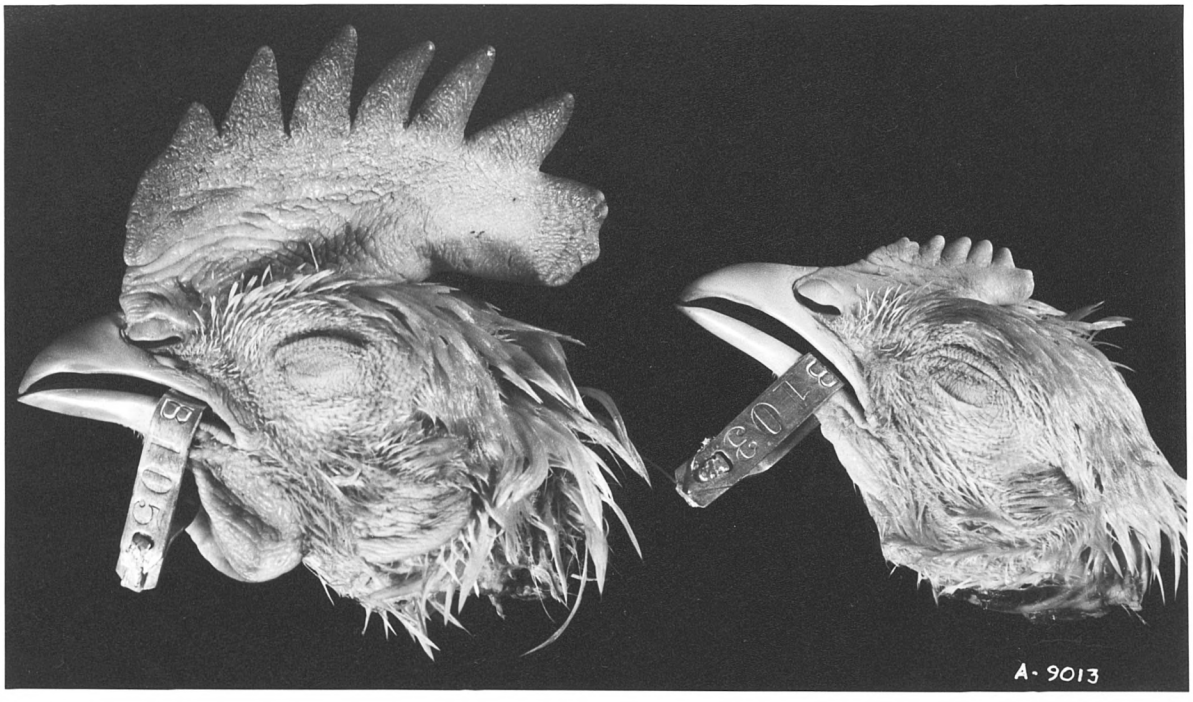

Figure 5