THE RELATIONSHIP OF VITAMIN E TO PITUITARY GLAND FUNCTION

by

MILTON RICHARD SNOW

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INTRODUCTION

An association between the pituitary gland, the hypophysis, and its effect on growth and sexual development has been recognized for a considerable length of time. Many investigators using numerous methods of procedure have presented information concerning this field, but not all results in published material are in agreement. Because of this variance in known information, additional investigation is needed. The purpose of this paper is to extend the work already done in connection with the pituitary gland in its relationship to vitamin E. The function of the pituitary gland with which this paper is concerned is the specific effect of the gland upon the female genital system of rats; that is, the gonadotropic effect of the pituitary gland.

REVIEW OF LITERATURE

During the past few decades in which this type of research has been done many different kinds of procedures have been employed. The type of donor of the pituitary gland varied as did the experimental animals which were to receive the extract of the gland.

The most common donor found in the review of the literature for this research was the chicken used by Domm and Van Dyck (1932), Herrick (1944), and Meyer, Mellish and Kupperman (1938). Other donors consisted of rats used by Smith (1926), Smith and

Engle (1927), and Engle (1929); bovine used by Evans and Long (1922) and Smith and Engle (1927); frogs used by Adams and Granger (1938) and Adams and Tukey (1938); sheep used by Forbes (1937); horses used by Krizenecky and Podhrasky (1926); doves used by Riddle and Flemion (1928); guinea pigs used by Smith and Engle (1927) and Schmidt (1937); calves used by Sisson and Broyles (1921); mice used by Smith and Engle (1927); cats used by Smith and Engle (1927); and rabbits used by Smith and Engle (1927).

The most common recipient was the rat which was used by Cahen and Ardoint (1936), Diakov and Krizenecky (1933), Engle (1929), Evans and Long (1921 and 1922), Goetsch (1916), Gusman and Goldzieher (1941), Herrick (1944), Meyer, Mellish and Kupperman (1938), Nelson (1933), Sisson and Broyles (1921), Smith (1926), Smith and Engle (1927) and Wallace (1939). Other recipients which were used as experimental animals were mice used by Adams and Tukey (1938), Adams and Granger (1938) and Smith and Engle (1927); guinea pigs used by Klinger (1919) and Schmidt (1937); chickens used by Domm and Van Dyck (1932); alligators used by Forbes (1937); rabbits used by Iscovesco (1913); frogs used by Krizenecky and Podhrasky (1926); and doves used by Riddle and Flemion (1928).

Some of the earlier work with the pituitary gland was accomplished by feeding the gland to the experimental animal. Goetsch (1916) tried two phases of feeding: first, a dried powdered pituitary extract was made up of both the anterior and

the posterior lobes. This whole pituitary substance appeared to be somewhat toxic to the experimental rats after a short period of feeding. The rats lost their appetites, lost weight, exhibited increased peristalsis, mild enteritis, muscular tremors and weakness of the hind limbs. However, over a longer period of feeding, 25 to 40 days, the experimental animals showed considerably more growth of the ovaries, tubes, and cornua of the uterus than did the control animals. The endometrium of the experimental rat became thickened like that of a pregnant animal. The increased vascularity was not limited to the endometrium but was present throughout the entire reproductive system. Second, rats were fed only the anterior lobe of the pituitary gland. The experimental animals were much more vigorous and showed greater body growth than their controls. There was an earlier development and a more active genital system in the experimental rats. Since no toxic effects were noticed in the second group, Goetsch proposed that possibly the posterior lobe was responsible for such a toxicity. Each rat in the second experimental group had two pregnancies in seven months as compared to no pregnancies in their control group.

Sisson and Broyles (1921) also fed the anterior lobes of the pituitary to experimental animals. These experimenters fed the pituitary of calves to albino rats which were three weeks of age. On observing the animals and their controls, no difference could be seen in their activity, fur condition, skeletal development, nor external sexual characteristics. The gonadal

weight, body weight, and the reproductive organs, in general, appeared the same when studied under the microscope.

Evans and Long (1921) fed cattle anterior pituitary gland at the rate of one-half gram per rat per day. This feeding was begun when the rats were 21 to 23 days of age, but no significant difference could be observed between the rats receiving the gland substance and those acting as their controls, that is, no difference in age of maturity, general body growth, or length of subsequent oestrous cycles was observed.

Krizenecky and Podhrasky (1926) by feeding horse pituitary glands to frogs found that metamorphosis was speeded up and also an increase in the general rate of growth resulted. Diakov and Krizenecky (1933) fed anterior pituitary substance to rats, and the hormone present caused a precocious sexual maturity with an advance ripening of the follicles and hypertrophy of the labiae vaginalis, uterus and tubes. Large doses of anterior pituitary substance, taking 160 to 180 hours to react, were required to obtain these results.

Valle (1937) introduced a way to permit the observation of the anterior material when fed orally to pigeons. This was done by making a small window in the crop which enabled him to observe some of the first modifications of the substance in the crop.

Positive results from the oral administration of pituitary substance were probably brought about through the use of excessive doses, while it seems probable that the negative results

were caused by insufficient doses and lack of standardization of dosage. The inconsistency of results through the feeding method led to the use of injections.

Another method of administering the pituitary substance was transplanting or injecting. Even the results found when the injection method was used were not entirely consistent. Many authors reported their work being done with transplants, implants, transplantations, or merely injections; while some said specifically that the pituitary substance was injected intraperitoneally, subcutaneously or intramuscularly.

In contrast to the results of oral administrations of anterior pituitary substance, Evans and Long (1921) found that by intraperitoneal injections a greater rate of growth was produced in the experimental animals. Although greater growth was produced in the test animal, the sexual development was retarded and in some cases was delayed to the extent that the oestrous cycle was lengthened considerably and finally inhibited entirely.

Evans and Long (1922) continued their experiments with intraperitoneal injections. Previously they had been getting negative results with oral and intraperitoneal methods. In later experiments with intraperitoneal injections they produced a definite amount of general body growth in rats. These experimental animals weighed twice as much as some of the control animals. Not only was there a greater amount of fat stored on the experimental animal, but the skeleton was larger and heavier than the littermate control. The most significant difference was the

result found when the ovaries were inspected. In all instances the ovary weight doubled in the treated animal, and a larger number of corpora lutea were reported. Also in connection with this experiment Evans and Long tested a substance made from the posterior lobe. They found the animals could not tolerate large doses and the amount that could be injected was not sufficient to produce any sharp differences between the experimental and the control animals.

While Evans and Long found a decided growth of the ovaries in the experimental animal, Forbes (1937), by injecting a four month old alligator with sheep pituitary substance, found a significant growth of the ovaries and an even more decided hypertrophy of the testes. In repeating the same experiment with an 18 month old alligator the same results were found only with a greater acceleration of the development of the sexual apparatus. The accessory sex structures were also greatly stimulated. Schaefer (1933), as reported by Forbes (1937), stated that when male garter snakes, Thamnophilis sirtalis and T. radix, are hypophysectomized, atrophy of the testes results.

Adams and Granger (1938) found that by injecting large amounts of fresh frog anterior pituitary substance intramuscularly into the hind legs of mice a stimulation of the reproductive tract of the immature female resulted.

Domm and Van Dyke (1932) by using subcutaneous homeotransplants of pituitary gland have observed precocious sexual development in chickens. The response of head furnishings of

White Leghorn cockerels proved greater in the experimental birds than in the controls.

Diakov and Krizenecky (1933) with excessively large oral doses of hormone from the anterior lobe of the pituitary produced precocious sexual maturity in the experimental animal. The same substance when given by subcutaneous injection produced the same results in less than half the time and with smaller doses than were used in oral administrations.

Gusman and Goldzieher (1941) produced a marked increase in ovarian weight in immature female rats by injecting a pituitary extract subcutaneously.

Very few attempts have been made to try to give the pituitary substance to one animal from another animal of lower class. Adams and Tukey (1938) gave daily injections of frog anterior pituitary substance to immature mice in adequate amounts, but no sexual maturity was produced.

Klinger (1919) implanted a fresh emulsion of whole anterior lobe into immature guinea pigs but did not notice any influence upon the growth when compared with the control animal.

Riddle and Flemion (1928) injected acid and alcohol extracts of bovine pituitary into doves with negative results. The poor results may have been due to inadequate dosage. However, when an alkaline extract of bovine pituitary was injected there resulted an increased weight of the testes from 50 to 100 percent. There was a slight weight increase found in some of the female experimental birds. With daily homeo-transplants of

dove pituitaries there was produced an increased growth in the testes of immature doves with a less marked change found in the female. Riddle and Polhemus (1931) reported that alkaline extracts of anterior pituitary contained much maturity principle.

The most common result when a pituitary substance was administered by simple injections or transplantations was a general precocious sexual development as found by Smith (1926) (1927), Smith and Engle (1927), and Meyers, Mellish and Kupperman (1938). Smith (1926) found that with daily homeo-plastic pituitary transplants he could restore a genital system which had atrophied because of hypophysectomy. He then found that he could induce precocious sexual maturity by transplanting the pituitary substance into immature rats. The hastened sexual development was evident by the opening of the vagina, congestion of blood, an extension of the uterus, and the formation of follicular and corpora cells. A precocious sexual development was induced as early as the weaning date, or at 22 days of life. It was also found that uterine contractions of experimental animals receiving pituitary transplants greatly exceeded those of control animals when measured with a kymograph. In another experiment Smith found that no part of the genital system of rats was stimulated by the pituitary glands of the guinea pig.

However, Smith and Engle (1927) in a series of experiments using transplants of anterior pituitary substance from mice, rats, cats, rabbits and guinea pigs have produced precocious sexual development in the immature female rat. In the male the

sexual development response was negative, but there may be a possible increase in libido. Weights of the ovaries and the uteri in the experimental animals were greater than the weights of the controls. In addition to the enlarged ovary there resulted an increase in the number of follicles and a development of follicular cysts. Another phase of precocious sexual development was worked out by Smith and Engle (1927). They found that the genital system of the female mouse was stimulated much faster by pituitary homeo-transplants than that of the female rat. Although the pituitary substance failed to produce precocious sexual development in the rat as reported by Smith (1926), the transplant into mice produced hastened sexual maturity. The pituitary transplant from rabbits hastened sexual maturity even more than transplants from guinea pigs or mice.

Meyer, Mellish and Kupperman (1938) found that male chicken pituitaries have more gonadotropic properties than do the female pituitaries. This was measured by the amount of ovary growth produced in immature female rats. Cahen and Ardoint (1936) used the enlargement of the uterus as a measure of the dosage of the stimulating hormone of the anterior pituitary gland.

Exner (1910) found that pituitary transplants were, in general, of decisive importance for only a short time. This may be explained from the fact that the implanted glands are soon resorbed, and the increased pituitary secretion exists for only a short time.

Moore and Price (1932) reported a reciprocal relationship between the gonads and the pituitary gland. Gonads were stimulated by a pituitary hormone and functioned only when they were forcibly stimulated by this hormone. Gonadal hormones lowered the influence of the pituitary gland. Removal of the pituitary caused regression of the gonads. Removal of the gonads caused abnormal pituitary activity.

Pencharz and Long (1934) experimented with removing different portions of the pituitary gland to see how it affected the birth of young. When a small portion of the gland was removed from the pregnant animal, normal parturition still occurred, producing normal offspring. When a large portion of the gland was removed, dead and living young were born after a prolonged pregnancy.

Smith (1932) found that when 30 percent or more of the pituitary gland was removed abnormal development resulted and fewer follicles were formed in the ovaries. When less than 30 percent of the gland was removed normal growth and development followed.

Engle (1929) stated that a valid sign of sexual maturity is the establishment of the vaginal orifice. In all cases when the pituitaries from gonadectomized animals were transplanted into immature rats or mice, a hastened establishment of the vaginal orifice resulted. Ovaries in animals receiving transplants from gonadectomized animals were four to nine times as great as those found in the animals receiving transplants from

normal animals. The increase in size of the ovary was due to the increased number of follicles. Therefore, the anterior pituitaries of castrated animals appeared to have more gonad-stimulating hormone than does a normal animal. The increased amount of gonad-stimulating hormone in castrate animals was possibly caused by a greater rate of secretion within the pituitary after gonadectomy. The pituitary of a gonadectomized animal has been reported hypertrophied; thus it would seem logical that the excess hormone could be stored in the elaborated cells. The anterior lobe appears to continue to produce the gonad-stimulating hormone even after castration, but most of the product is kept within the pituitary. Emery (1932), as reported by Nelson (1933), reported that there is evidence of minute quantities of gonad-stimulating hormone in the blood.

Homeo-transplants of pituitary substance taken from guinea pigs at different stages during oestrous were made by Schmidt (1937). When the donor was in oestrous the injection caused no stimulation. At five to six days after oestrous there occurred little or no follicle stimulation but a typical vaginal oestrous and some uterine proliferation. When taken from donors seven to 10 days after oestrous, there resulted growth of the follicle and a proliferation of the germinal epitholium. At 11 to 15 days there was maximum proliferation produced.

Wallace (1939) found that the mouse uterus was two to four times as sensitive to treatment as the rat uterus. The uteri of both species were more responsive than the ovaries of either

species. However the rat ovary was more sensitive than the mouse ovary.

With the exception of Riddle and Flemion (1928) and Adams and Tukey (1938) the investigators since 1922 have found positive results in their experiments with pituitary extracts injected into experimental animals. Riddle and Flemion attributed their negative results to the use of acid and alcohol extracts of bovine pituitary, and Adams and Tukey attributed theirs to the injection of pituitary substance from a lower class animal, a frog, into a mammal. Therefore the general conclusion can be drawn, in view of the experiments reviewed, that the injection of the pituitary extract into the experimental animal caused precocious sexual development.

This conclusion is not in variance to data given by Fugo and Witschi (1938) which stated that chick gonads developed normally to the age of 12 to 21 days, even though the pituitary primordium was removed from chick embryos which had incubated 33 to 38 days.

Failure to produce precocious sexual development with a vitamin E preparation was reported by Diakov and Krizenecky (1933). The preparations used were not named and no details were given by the Czechoslovakian company which supplied them. Fertility of the female rat was not destroyed but only suspended by the lack of vitamin E. Pituitary gonadotropins cannot replace vitamin E but they do aid in the beginning phases of embryonic development.

By keeping a group of Rhode Island red cockerels on an E-deficient diet Adamstone and Card (1934) found that at the end of the first year they were still fertile when allowed to mate. After two years they were tested again and showed a varying degree of fertility.

Rowland and Singer (1936) concluded that when there is a deficiency of vitamin E, the pituitary gland does not have the capacity to produce an ovulation-producing substance which will cause ovulation in the oestrous rabbit.

Müller and Müller (1936-1937) kept female rats on a vitamin free diet for 137-240 days and no morphological changes were found. When kept on an E-free diet for 230 days, however, the pituitaries of three rats showed castration cells. Stein (1935) found that no notable difference resulted from vitamin E deficiency in rats. Drummond, Noble and Wright (1939) found that vitamin E had no effect on the reproductive organs of immature or hypophysectomized rats.

Barrie (1944) found that there is a discoloration of the uterus in chronic deficiency of vitamin E. This discoloration cannot be cured by administration of vitamin E alone but the increased circulation during pregnancy is successful in removing the pigment.

Dam (1943) reported that two main symptoms of diseases are caused by vitamin E deficiency in chicks. These two are exudative diathesis and encephalomalacia. The former is characterized by plasma exuding from capillaries, massive accumulations

of subcutaneous fluid and a resemblance to edema of tissues or muscles; while encephalomalacia is characterized by uncoordinated movements of the legs, wings, and neck. The chicks would fall when trying to walk, and occasional tremors would occur.

Patrick and Morgan (1944) found that vitamin E-deficient chicks developed vitamin A deficiency, and the vitamin A storage in the liver was depleted.

Rats were fed an E-free diet by Biddulph and Meyer (1940), and a degeneration of the germinal epithelium and a decrease in the testes weight resulted. There was no effect on the weight or the cyclic function of the ovary in the female rats. An increase in accessory gland weight was found in rats which were deprived of vitamin E for about three months. By adding wheat germ oil to their diet for a short time the heaviest accessory glands of any were obtained. However, it was proved by Samuels (1931), as reported by Biddulph and Meyer, that exhaustion from lack of food would induce atrophy of the accessory glands.

Nelson (1933) found that vitamin E is necessary for normal pituitary and testes growth. In another phase of the experiment Nelson implanted the pituitaries from E-deficient male rats and produced an increase in weight of the ovaries and a decrease in weight of the uterus in females. When E-deficient females were used very little gonadal difference occurred.

Verzár (1931) with vitamin E containing extract produced an hypertrophy of the uterus of immature rats in three days by intraperitoneal injections. When the same substance was in-

jected subcutaneously the reaction was only one-fifth as much hypertrophy as when injected intraperitoneally. When 0.5 gram per day of the substance was administered orally there was hypertrophy produced in three cases. Vitamin E has a similar effect as the hormone from the anterior pituitary in that both produce hypertrophy in immature animals.

Urner (1931) deprived rats of vitamin E. They appeared normal, exhibited normal growth, estrous cycle, and breeding behavior. Pregnancy in all cases resulted in embryonic death and resorption.

Barrie (1937) and Valle and Junqueira (1947) used offspring of rats which had been fed an E-deficient diet through most of pregnancy. The offspring were poor in appearance and grew slowly. Valle and Junqueira found that when the offspring became pregnant all aborted at seven to 14 days after conception. Barrie gave her rats vitamin E and they returned to normal.

Bacharach, Allchorne, and Glynn (1937) assayed vitamin E from different sources testing for the type of implant which would permit a pregnant rat to give birth to live litters of young. They found that lettuce and wheat germ oil produced live litters at the rate of 100 percent and 87 percent respectively.

Patrick and Morgan (1943) found that chicks deprived of vitamin E did not grow as rapidly as those which received vitamin E. They worked out a daily requirement of 300 micrograms of dl alpha-tocopherol per 100 grams of ration.

Herrick (1944) reported an atrophy of the testes when

chickens were deprived of vitamin E in their diet. Chicks given vitamin E in their diet retained a normal development of the testes. When the pituitary of these birds were injected into immature female mice, the glands of the E-plus birds consistently showed greater gonadotropic activity than the glands of the E-deficient birds. The activity was measured by the degree of stimulation of the vaginal epithelium.

MATERIAL

For this experiment on the relationship of vitamin E to the pituitary gland 55 White Leghorn chickens were used. The chicks used were cockerels with the exception of eight females, the sex of which was indeterminate at the time of selection. The experiments were conducted in two different groups. The first group of 24 chicks were used in the fall of 1949, and the second group of 32 were used in the spring of 1950. Each group was divided into two parts, the experimental birds and the control birds. The chicks were given identical treatment. They were kept in wire batteries which facilitated cleaning and care. For the feed the following ingredients composed the basal ration.

Ingredient	lbs.	percent
Casein, dry	9	18
Salt mix	2	4
Corn starch	19	38
Rice	10	20

Yeast, dried Brewer's	5	10
Lard	2½	5
Fiber (ground up cellotex wallboard)	4	8
Cod liver oil		2

The chicks were fed and watered once daily. Enough basal ration was given at a feeding so that feed was constantly before them. Both groups received exactly the same diet with the exception of the experimental group which received vitamin E in the form of wheat germ oil (about one percent). The birds were fed for a period of six weeks. At the end of this time they were killed, and the pituitary glands were removed. The glands were made into an extract which was injected into white rats. After three injections over a period of five days, the rats were killed and a portion of the vagina immediately posterior to the bifercation of the uterus was removed and fixed in a solution of Susa fixative. The prepared slides were studied, and measurements of the endothelial lining of the vagina were made.

METHODS

Basal Diet and Feeding

The feed was hand prepared in this laboratory. Large tubs and other clean containers were used for the mixing. The casein and lard were mixed first and then the salt, yeast and rice were added in that order. Next the starch and the fiber and finally

the cod liver oil were added. The vitamin E in the form of wheat germ oil was added periodically, thus preventing any possible chance of oxidation. Both the experimental and the control chicks seemed in good health throughout the experiment, however, the E-deficient group appeared to be more eager for feeding time than did the group receiving vitamin E in their diet. Feeding of this special diet was started on the forty-fourth day of life and ended on the eighty-fourth day, thus permitting six full weeks of experimental feeding.

Both groups of chickens were fed essentially the same diet. In addition to the vitamin E in the form of wheat germ oil which was used exclusively in the first group, dl alpha-tocopherol was supplemented in the second one, thus assuring an abundant measure of vitamin E.

Removal of Pituitary

The chicks were killed in entire groups, that is, all of the E-deficient group first and then all of the group receiving vitamin E. After killing the chicks by cervical dislocation, the comb was clipped from the head. The skull was cut transversely just immediately posterior to the eyes with a large bone scissor. Usually this method permitted fairly easy entrance into the sella turcica from which the pituitary body was lifted out with either small scissors or forceps. Bleeding was usually held at the minimum, thus permitting quicker dissection.

Immediately after the pituitary glands were removed, they were placed in vials and were then quick frozen until the time when the experimental rats were old enough for injection.

Preparation and Injection of Extract

When the experimental rats reached the desired age an extract was made from the pituitary glands which had been removed from the chickens and stored at a low temperature. The glands were placed with a small amount of distilled water into a mortar and were ground with a pestle until thoroughly smooth fluid was obtained. Distilled water was used as the solvent at the rate of one cubic centimeter for every pituitary gland. The subcutaneous injections in the back were given every other day over a six day period to weaned rats which were about 22 to 25 days of age. The rats were given 0.2 cubic centimeter of pituitary extract per injection on the first, third, and fifth days. On the sixth day after the first injection, the rats were killed.

Dissection of Vaginae

The rats were killed in entire groups at a time by etherization. The vaginae and the horns of the uteri were dissected out and put immediately into Heidenhain's Susa fixative solution.

Slide Making Technique

The following procedure explains how the tissues were fixed and treated in preparation for embedding into paraffin blocks.

Tissue in fixative	24 hours
Wash in 70% alcohol I	1-3 minutes
Wash in 70% alcohol II	1-3 minutes
Dioxan I.	½ - 1 hour
Dioxan II.	2 hours
Dioxan 50% and paraffin 50%	1 hour
Paraffin I.	1 hour
Paraffin II.	1 hour
Embed	

After trimming the excessive paraffin from the tissue, the block was put on a wooden block and was sectioned with a microtome at six microns. The sections were stretched on slides with water and heat and cemented with egg albumin.

The following outline gives the procedure for removing the paraffin from the sections and staining of the first slides after the sections were thoroughly dried.

Xylol I, remove paraffin	5 minutes
Xylol II	3 minutes
100% alcohol	1-3 minutes
95% alcohol	1-3 minutes
85% alcohol	1-3 minutes
70% alcohol	1-3 minutes

50% alcohol	1-3 minutes
35% alcohol	1-3 minutes
Water, distilled	5 minutes
Mordant, 4% iron alum	1 hour
Wash, distilled water	3 minutes
Stain, iron haematoxylin	20 minutes
Rinse, tap water	momentarily
Destain, 2% iron alum	for desired coloration
Wash, in running tap water	10-30 minutes
Wash, distilled water	1-3 minutes
35% alcohol	1-3 minutes
50% alcohol	1-3 minutes
70% alcohol	1-3 minutes
Counterstain, triosin	to produce sharp differentiation
Wash 95% alcohol	2-3 quick dips
95% alcohol	30 seconds
100% alcohol	1 minute
Carbol xylol	5 minutes
Xylol	½ - 1 minute
Mount, gum demar or balsam	

The procedure for removing the paraffin from the sections and staining of the slides for the second group of animals varied from group I in the following respects: 1. Instead of using an iron haematoxylin stain, Delafield's haematoxylin stain was used. This eliminated the mordant as Delafield's has

the mordant in it and the mordanting and the staining takes place at the same time. 2. The progressive method of staining was used, therefore no destaining was required. 3. After staining, lithium carbonate was added to the rinse water to bring out the blueness of the stain. 4. Instead of triosin, eosin, which is a much faster and more powerful stain, was used as a counter-stain.

DATA AND DISCUSSION

When the chickens were selected for the experiment they were weighed and separated into two approximately equal groups according to weights. Feeding of the basal diet was begun immediately. At 12 weeks, the day they were killed, they were weighed again. Table 1 shows the results of the first group of chickens. Table 2 shows the results of the second group of chickens.

The chickens receiving vitamin E produced testes of greater weight than did the E-deficient chickens. This coincides with the reports of Biddulph and Meyer (1940), Nelson (1933) and Herrick (1944). Table 3 shows a comparison of the body weights, testes, and combs of the two groups of experimental chickens.

Patrick and Morgan (1943) reported that chicks raised on an E-deficient diet did not grow as rapidly as those which received vitamin E. This coincides partially with the findings reported in this paper. Since the weight of birds was not the primary

Table 1. Weights of the first group of chickens.

Wing band no.	Weight, grams: At 6 weeks	At 12 weeks	Average gain
	Vitamin E deficient		
1401	321	720	399
1402	488	1102	614
1403	383	830	447
1405	342	850	508
1408	450	766	316
1409	432	588	156
1410	366	890	524
1412	373	816	443
1413	400	990	596
1415	372	930	558
1419	370	978	608
1424	336	900	564
	Those receiving vitamin E		
1404	342	928	586
1406	380	818	438
1407	428	930	542
1411	370	689	328
1414	373	910	537
1416	364	906	542
1417	442	760	318
1418	400	920	520
1420	445	640	195
1421	321	840	519
1422	384	1064	680
1423	383	792	409

Table 2. Weights of the second group of chickens.

Wing band no.	Weight, grams: At 6 weeks	At 12 weeks	Average gain
	Vitamin E deficient		
1426	544	1256	712
1427	438	1096	658
1428	527	1218	691
1430	360	1018	658
1433	469	1010	541
1435	340	716	376
1436	470	1060	590
1437	454	1036	582
1438	510	1054	544
1439	450	814	364
1479	412	946	534
1480	490	1180	690
1481	425	962	537
1487	440	1040	600
1492	356	946	590
1494	283	820	537
1493	383	818	435
1488	440	1020	580
	Those receiving vitamin E		
1489	514	1154	640
1429	446	980	534
1431	524	1306	782
1432	350	830	480
1434	466	1230	764
1473	348	966	618
1441	480	1008	528
1440	456	1090	634
1483	508	1138	630
1484	454	962	508
1486	420	1178	758
1482	470	1090	620
1485	died	--	-
1491	410	984	574
1495	died	--	-
1496	340	924	584

purpose of this paper, further investigation was not pursued.

No significant conclusion can be drawn about the comb growth without further investigation.

Table 3. Averages of weight gained, testes weight, and comb weight of the two groups of chickens.

Birds	Average gain in weight per bird	Average weight of testes per bird	Average weight of comb per bird
		Grams	
		Group I	
E plus	467.833	.460	7.35
E minus	477.750	.292	5.55
		Group II	
E plus	618.442	4.962	19.762
E minus	567.772	4.600	21.131

Table 4 shows a comparison of weights of the two groups of rats used for injections.

Table 4. Weights of the two groups of rats.

Rats	Number used	Weight at start of experiment-25 days	Weight at end of experiment-31 days	Gain of weight
		Gm/rat		
		Group I		
E plus	9	37.88	39.88	2.00
E minus	9	37.88	50.00	12.12
		Group II		
E plus	18	39.66	53.00	13.34
E minus	17	38.88	47.06	8.18

Although the final gains in weight do not agree between the two groups, the rats appeared normal in every respect. The gain in weight of the E-minus group in group I does not appear to be too significant.

The vaginal epithelium was measured with an ocular micrometer under high power. Eight representative measurements were made on each slide, and an average measurement to represent that slide was calculated. These calculations were combined to get a representative picture of the average growth of the vaginal epithelium in both the E-plus and E-minus groups.

Table 5 shows a direct comparison between the growth of the vaginal epithelium of rats which received injections of E-plus pituitary and rats which received injections of E-minus pituitary.

Table 5. Comparison of the growth of the vaginal epithelium of E-plus and E-minus rats.

Rats	Number used	Average measurement of vaginal epithelium per rat Microns
	Group I	
E plus	17	100.735
E minus	18	75.260
	Group II	
E plus	18	59.165
E minus	17	25.285

Although the second group did not show as much growth in

the vaginal epithelium, there was a more marked difference between the E-plus and the E-minus groups than in the first group. This may have been caused by the added source of vitamin E used which was dl alpha tocopherol acetate, a distilled concentrate of mixed tocopherols.

RESULTS

There was more gonad-stimulating hormone in the pituitaries of the chickens fed vitamin E than in the pituitaries of the E-deficient chicks. This was proved by the evident difference in the production of growth in the endothelial lining of the vagina of the female rat. As can be seen from Table 5, the growth of the vaginal epithelium of the E-plus rats was 100.735 microns as compared with 75.260 microns in the E-deficient rats. This is approximately a 25 percent increase in growth in the rats receiving pituitary injections from chickens which received vitamin E in the form of wheat germ oil.

From Table 5, the decided difference can again be seen when the E-plus and the E-minus groups of the second experimental group are compared. The growth of the vaginal epithelium of the E-plus rats was 59.165 microns as compared with 25.285 microns in the E-deficient group of rats. This difference shows a decidedly greater amount of growth of the vaginal epithelium in the second group as compared with the first group of rats. As compared to the 25 percent increase in growth in the E-plus rats

of the first group, the second group showed approximately 50 percent more growth in the rats receiving pituitary injections from chickens which received vitamin E in the form of wheat germ oil and dl alpha tocopherol acetate, a distilled concentrate of mixed tocopherols. The difference in the percentages of growth between the two groups may have resulted because of the added source of vitamin E in the form of dl alpha tocopherol.

Not only did the vaginal epithelium of the E-plus rats show a greater increase in growth in the form of increased cells over the E-deficient rats, but the cells were also much larger in size. The cells of the E-deficient rats were not only smaller but showed little indication of induced growth.

SUMMARY

1. Over a period of six weeks, the general body weight of chickens was not significantly affected by a vitamin E-deficient diet.

2. There was more testes growth in White Leghorn cockerels which received vitamin E in their diet than in cockerels which were kept on a vitamin E-deficient diet.

3. There was more gonad-stimulating hormone in the pituitaries of chickens receiving vitamin E in their diets than in the pituitaries of E-deficient chickens. The effect of the gonad-stimulating hormone was measured by the amount of growth produced in the vaginal epithelium of rats receiving pituitary

injections from chickens which had been on a diet containing vitamin E compared to the growth produced in rats receiving pituitary injections from E-deficient chickens.

4. Although the reproductive tracts of the rats were not weighed, a noticeable difference was observed between the E-plus and the E-minus groups. The rats that received pituitary injections from chickens which were given vitamin E in their diet showed much hypertrophy of the genital system.

EXPLANATION OF PLATE I

Fig. 1. Photomicrograph of the vaginal epithelium from a rat that received pituitary substance from the E-deficient chickens.

Fig. 2. Photomicrograph of the vaginal epithelium from a rat that received pituitary substance from the chickens receiving vitamin E.

PLATE I

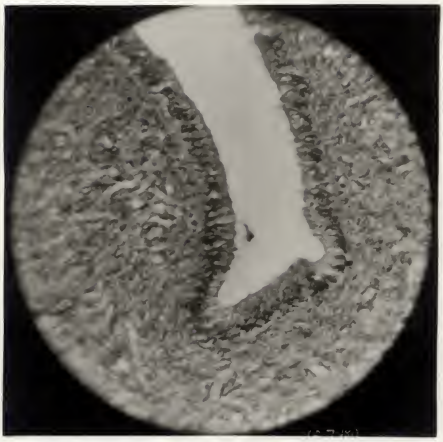

Fig. 1

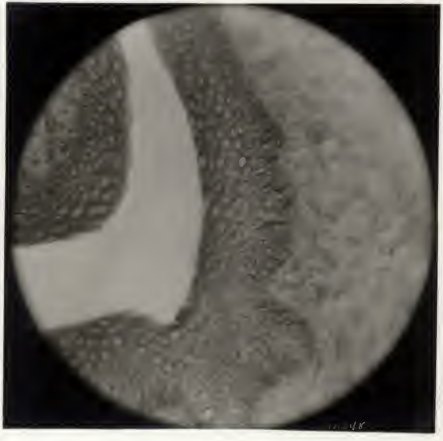

Fig. 2

EXPLANATION OF PLATE II

Fig. 3. Photomicrograph of the vaginal epithelium from a rat that received pituitary substance from the chickens receiving vitamin E.

Fig. 4. Photomicrograph of the vaginal epithelium from a rat that received pituitary substance from the E-deficient chickens.

PLATE II

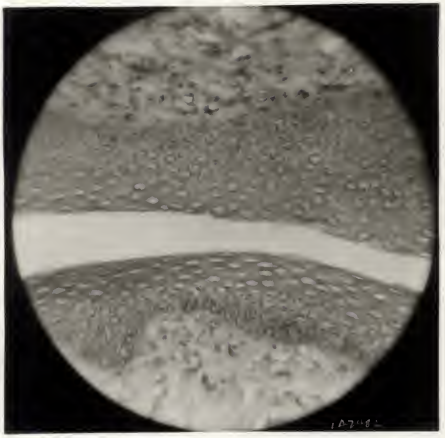

Fig. 3

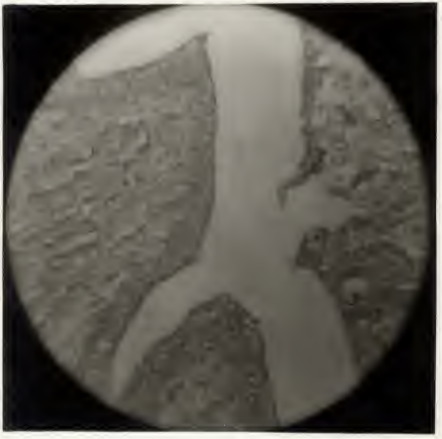

Fig. 4

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