THE EFFECTS OF DAILY INJECTIONS OF BOVINE ANTERIOR PITUITARY EXTRACT UPON THE DEVELOPING ALBINO RAT

by

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INTRODUCTION

The pituitary gland or hypophysis has been found to be a gland of internal secrètion only recently. It first became evident that there was an association between it and an increased skeletal growth which occurs in acromegaly and giantism, because these phenomena were accompanied by an enlargement of the gland. During the past 20 years experimental work on animals has shown that different parts of the gland do not have the same function. From the posterior lobe, which is nervous in origin, has been extracted a hormone known as "pituitrin" which raises blood pressure and stimulates contraction of the smooth muscles. No definite extract has been prepared on a commercial scale from the anterior lobe, as Robertson's "tethelin" has not been generally accepted as equivalent in action to the hormone or hormones elaborated in this lobe. At the present time there is much interest in the secretions of the anterior pituitary but there is still a lack of general agreement among the different investigators.

Early workers especially often obtained conflicting results. The function of the gland may be studied by attempting to produce a state of hypersecretion of its hormone by feeding, injection, or transplantation of the gland or

gland products. Different methods of preparation and of administration, as well as the difference in susceptibility and resistance of the various species of animals used, might very well lead to dissimilar results especially in the small number of animals generally used. These criticisms hold to some extent even today but in general more consistent results, obtained especially by injection and transplantation methods, are suggesting the possibility of determining the function of the gland and of securing an active extract of determinable potency.

ACKNOWLEDGMENT

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REVIEW OF LITERATURE

Relation of the Anterior Pituitary Body and Growth

Feeding. Schafer (1911), Aldrich (1912), Robertson (1916), Goetsch (1916), Marinus (1919), and Crofton (1924)

were among those who obtained an increased growth of the animals in general or in special cases by feeding either the dessicated or the fresh gland. Lewis and Miller (1913), Wulzen (1914), Sisson and Broyles (1921), Evans and Long (1921b), C. S. Smith (1923), and P. E. Smith (1927d) obtained either no advance in growth or else a retardation of growth by the oral administration of the gland. In feeding the gland to hypophysectomized tadpoles, Smith (1918) obtained normal growth. Working with metamorphosed salamanders, Uhlenhuth (1919, 1921) was able to produce true giants by the oral administration of bovine anterior pituitary. Hoskins and Hoskins (1920) concluded that feeding a preparation of the pituitary to normal or to thyroidectomized tadpoles brought about a precocious metamorphosis. Kriezenecky and Podhrasky (1926) did not produce results of any significance by feeding the gland to tadpoles.

Transplantation. Transplantation work has been slightly more successful in the results obtained. Clairmont and Ehrlich (1909), Exner (1910), and Shafer (1909, 1911) reported that transplants function for a time, showing the effects of the functioning through rapid growth and increased fat deposits in the experimental animals. Klinger (1919) could obtain no increased growth due to the transplants in the guinea pig. Smith (1926, 1927) showed that transplants into

pituitary-less rats repaired the growth curve, also effecting a partial repair of the adrenals, thyroids, and the sex glands. Allen (1928) has shown that boiled anterior pituitary glands transplanted into hypophysectomized tadpoles produce no acceleration of growth. Upon thyroidectomized tadpoles the implantations produced no increased growth above that of the normal tadpoles but did increase the growth above that of the thyroidectomized controls.

Injection. Experiments on the influence of an injected extract have been many. Cushing (1909), Franchini (1910), Klinger (1919), and Johns, O'Mulveney, Potts and Laughton (1927) found that injections of the extract of the anterior pituitary produced a retardation or did not accelerate the growth rate of the animals on which they were working. Iscovesco (1913) and Evans and Simpson (1925, 1926, 1927) were able to obtain positive results with most of their injection work, yet Iscovesco was unable to produce any growth in nearly mature rabbits and Evans and Simpson found two litters of rats which did not react even to large doses of extract of known potency. Positive results have been reported by Evans and Long (1921a) with rats, Robertson and Ray (1925) with mice, and by Coonfield (unpublished, 1927) with rats. Putnam, Teel, and Benedict (1928) obtained marked increase in growth with rats and dogs but were unable to produce any increase in growth with rabbits.

With hypophysectomized rats Smith (1926, 1927) showed that normal growth of young animals was restored by injections of the saline extract of the ox anterior pituitary, and that in case of the mature rats cachexia was avoided by the use of the extract. Flower and Evans (1925) showed by anterior pituitary injection experiments on thyroidectomized rats that the cretinoid growth dystrophy may be referable to the anterior pituitary body under-functioning, resulting from the absence of thyroid secretion, and growth resumption with thyroid therapy to the establishment of normal pituitary functioning. Evans and Simpson (1927) showed that normal and castrated males and females, under the influence of the anterior pituitary injections, developed into giant animals. Van Wagenen (1927, 1928) reported that 1 c.c. injection of the ox pituitary causes body length, tail length, and weight of the castrated males to be greater than that of the untreated castrated males. She also reported that injections of the extract increased the body length of the treated castrated males in proportion to that of the normal controls and raised the weight of the injected castrated males in most cases considerably above that of the controls. Body weight in respect to body length was greater in the injected castrated males than in the controls. Body length of the control animals and of the injected castrated animals (males) was about the same. Total length was greater due to differ-

ential growth response of body and tail.

Relation of the Sex Organs and the Anterior Pituitary

Gonadectomy and the Anterior Pituitary. Because of the great increase in the number of chief cells produced in the anterior pituitary during pregnancy it has been held for some time that some relationship exists between the gonads and the anterior pituitary. The development of the anterior pituitary studied clinically in connection with cases of eunucs and eunucoids also points to such a relationship.

Hatai (1913) by means of his experimental work demonstrated a direct relationship of the two glands. He found that in castrated rats the pituitary gland is 74 per cent greater in weight than in normal rats. He also says that after the removal of the sex glands it appears that when compensatory growth of the pituitary occurs there is no overgrowth or obesity. Both of these effects are noted if the enlargement does not occur. Weil and Gutman (1924) have also suggested that the relationship between the anterior pituitary and the gonads is reciprocal by their statement that removal of the anterior pituitary accelerated the growth of the testes to be 50 per cent heavier in one month than the testes of the control animals.

Feeding. Feeding of the pituitary gland to chickens

increased egg production on the fourth day after the first feeding and increased hatchability according to Clark (1916). From feeding the pituitary gland to rats Goetsch (1916) concludes that the anterior pituitary fed to young rats has a stimulating effect upon growth and upon sexual maturity and activity. The animals of his experimental group were more mature at two and one-half months than were the controls at seven and one-half months which had been fed posterior pituitary lobe tissue. At four months and one week the experimental female had a litter of young and three months later a second litter. The control female had small nipples and no sign of pregnancy. Marinus (1919) feeding pars anterior pituitary to experimental animals and pars tuberalis of the pituitary to the controls concluded that the feeding of the anterior pituitary gland produced larger animals that had heavier sex organs, which matured at the normal time.

Evans and Long (1921b) fed half-gram doses of the anterior pituitary gland daily to rats, beginning at the weaning date. They did not obtain any significant change in growth, sexual maturity, the oestrus cycle, or the opening of the vagina. When six to 10 grams of fresh anterior pituitary was rolled in barley and fed over a period of 20 oestrus cycles no change was noted. They were also unable to produce any result by using the dried commercial glands.

C. S. Smith (1923) concluded from his work that dessicated pituitary gland substance given daily to month-old white rats in 0.05- to 0.03-gram doses over a period of four to 16 weeks, plus plenty of green feed, had no effect on growth or gonad development. Smith (1927) reports that the feeding of the gland to hypophysectomized rats failed to restore the atrophied genital system, thyroids, or adrenal cortex.

Injection. From injection work with chickens Pearl and Surface (1915) concluded that the anterior pituitary extract injected did not cause any activation of the ovary in the sense of inducing ovulation at an earlier date than that at which ovulation would normally occur. The first outstanding research on the effects of the injected anterior pituitary extract was carried out by Evans and Long (1921a) and briefly reported by Dr. Evans in the Harvey Society Lectures (1923 –1924). Neutralized alkaline extracts which possess very potent principles or saline extracts of the anterior pituitary were injected daily into the peritoneal cavity of the experimental animals. This method of producing the hypersecretion of the anterior pituitary gave the following results. The oestrus cycle of the rat was lengthened or complete cessation of the cycle resulted. It developed that ovulation was delayed due to retention of the ova and the ovaries weighed twice as much as those of the normal animals. Many substantial corpora lutea were produced but

the uterus remained one-half its normal size. Histologically, the ovary showed much lutein tissue. The formation of the lutein tissue from the egg was demonstrated in unruptured mature follicles and in atretic follicles. Ripe normal follicles were invariably absent.

As reported by Walker (1924, 1925) intraperitoneal injections of fresh anterior pituitary extract into laying hens caused them to stop laying immediately and to remain sterile for one to four weeks after discontinuance of the injections. Post mortem examination revealed ovaries smaller than normal. No large ova were present. All except smaller follicles were undergoing atresia and the yolk mass was replaced by lutein tissue. Evans and Simpson (1925) reported that the resumption of rapid growth and the cessation of ovulation are reliable and characteristic indicators of the presence of the growth and sex hormones respectively. This conclusion was drawn after they had given injections of the anterior pituitary extract to litter mate sister rats that were between 90 and 150 days of age and with whom maximum growth and plateauing had already been attained before the beginning of the experiment. They reported (1926) that treated male rats became giants the same as the treated females with one exception in 10. Seven cases of diminuation in sex response were obtained by the fifth month. This was evidenced by the refusal of the treated males to copulate

with females in active oestrus. One exception in this was obtained resulting in the production of a normal litter. Autopsy of the seven cases showed great relative and considerable absolute diminuation of the testes by weight and by the small size of the tubules but with no evidence of degenerative change. Crafts and Flower (1925) attempted to determine whether the effect of the extract produced primary germ cell injury as results seem to indicate from the work of Evans and Long (1921a) who reported that cessation of ovulation was due to the retention of the ova and the conversion of the follicle wall into lutein tissue. They sought to learn if spermatogenesis was suspended by the anterior pituitary therapy. They found repeatedly that fertility was retained by males which had undergone for months the same daily dosage with the extract which in their sister rats had led to cessation of ovulation.

Teel (1926a,b) obtained typical decidual cell response to injury of the uterine mucosa in animals in which the ovaries were present following daily injection of the anterior pituitary extract. He concludes, "Provided that this decidual cell reaction is indicative of the ability of the uterus to implant the egg it would appear that the cause of the delay in implantation in injected animals is not due to a lack of sensitivity of the uterine mucosa membrane on day five and six." He also reports that injections of the fluid

in mature rats caused delayed implantation by 13 or 14 days. Corpora lutea remained at the time of birth of the young and either prevented birth or the lack of follicles prevented birth. Term embryos died and were expelled one or two days later. Evans and Simpson (1927) reported very sensative and very refractory litters were found. They found two litters exhibiting complete absence of response even with massive dosage of the hormone. Normal growth was not disturbed but accelerated growth or ovulation disturbance could not be induced by repeated trials with various levels of dosage using extracts of marked potency.

It then appears that injections of the anterior pituitary extract, as prepared by Evans and Long, produce a cessation of ovulation and an increased production of lutein cells which persist for a long time even in cases of pregnant females. That the extract does not injure the primary germ cells is evident by the virility of the males after periods of long injection and by the production of healthy normal rats in cases where copulation has been produced.

Transplant Work. By the transplant method, finely cut pieces of fresh glands are implanted by means of a glass cannula into the muscle tissue of the treated animal's leg. From here it is readily absorbed into the blood stream. Smith (1926) reports that hypophysectomy results in complete growth regression of the sex apparatus with accompanying

structural modifications. Daily transplants into pituitary-less rats of fresh anterior pituitary glands bring about a normal growth rate. In addition, transplants (intramuscular) affect a partial repair of the adrenals, thyroids, and the sex glands. Smith and Engle (1927) report that with young rats and mice the general response to anterior pituitary transplants from mammals is as follows: (1) Vaginal introitus is established. (2) The vaginal smear shows cornified cells or cornified and nucleated epithelial cells (stage just preceding ovulation). (3) The uterus is distended and hyperaemic. (4) Many follicles are evident. However, if autopsy is postponed one day the following results will be observed: (1) The uterus is rugose and muscular. (2) Numerous corpora lutea are present in the ovary and are larger than those in the preovulation stage. (3) The vaginal smear shows leucocytes plus epithelial plus cornified cells (post estral stage). They also report for mice 10 days of age that sexual maturity may be reached in five days. Mice 17 days of age are mature in 36 hours. Usually three transplants are necessary. This is in marked contrast to the average of 100 mice that were allowed to mature normally at 35 days of age. With rats, five daily transplants are necessary to accelerate maturity if begun at 21 days. With the hastening of sexual maturity goes the increase in weight of the ovaries, which is due not to an

actual increase in the size of the follicles but to an increase in the number of follicles maturing at one time.

True superovulation was obtained for it is reported that as high as 48 ova were found in one horn of the uterus of one mouse. The fewest number found were 14 in the right tube and eight in the left. For 100 normal mice the greatest number found in one tube was seven. The largest litter from normal mice was 14. The uterus in its development and weight approached that of normal animals at the time of their maturity. The increase in the vagina was no less profound. An interesting point is that in spayed animals the vagina and the uterus are not affected by transplants. Transplants from immature animals produced sexual maturity after three days of transplants. Transplants from senile rats produced sexual maturity in three days. They conclude that for the female response to anterior pituitary transplants there is a great variability of weights of the resulting sexual systems but the time necessary for maturity is not variable. The effect on immature males was marked in that 10-day treatments increased the size of the penis and the accessory glands, but slightly effected an increase in spermatogenesis. A two-day treatment of mature females with the transplants increased the weight of the ovaries and the uteri, the increase in the uterus always being less marked than in the case of the ovary. Fifteen to 35-day

treatments failed to produce any effects in mature male rats. Their work on pituitary-less rats and mice confirmed the work of Smith and also added the fact that ablation of the thyroid or of the adrenal gland did not hinder the compensatory effect of the transplants upon the genital system of the female and male rat. They also pointed out the fact that the simultaneous injection of the extract as prepared by Evans and Long prevented the restoration of the genital system in the hypophysectomized rats by the transplants. They conclude from the above facts that the relation between the gonads and the anterior pituitary seems to be reciprocal.

The papers of Smith (1927a,b,c) confirm the work of Smith and Engle (1927) and especially emphasize two points: (1) Sexual maturity and puberty are synonomous but the time of mating may come much later than sexual maturity. (2) The hastening of maturity of the uterus, vagina, and ovaries is dependent upon the presence of the follicular hormone which has been stimulated by the transplants.

Engle (1927) has shown that there is no mutual antagonism of the testes and ovaries. Rats into which gonads of the opposite sex had been transplanted did not present a normal histological appearance of the gonads except when the normal supply of the anterior pituitary secretion was supplemented by that from implanted anterior pituitary glands.

He further reports (1928) that tubal ova following transplants may number 20 to 50 in a single uterine horn. The intact ovary in a normal rat produced four or five ova. If one ovary be removed the number of ova produced in the other will be double the usual number of ova to each ovary. However, he states that in cases of maximum response due to anterior pituitary transplants, in which the weight of the ovary exceeds that of the control many times, the follicles become cystic and are not normal in size. Moreover, under such conditions ovulation is not observed to occur. He, too, concludes that a mutual interaction between the pituitary and the gonads does exist.

Summarizing briefly the results of transplants upon the gonads and their accompanying organs we find (1) an increase in the weight of ovaries due to an increased number of follicles being produced; (2) super-ovulation (20 to 50 ova to each tube); (3) a corresponding increase in size and development of the uterus and vagina indicated by the early establishment of the oestrus cycle; (4) partial repair of the gonads and genital systems in hypophysectomized rats, which is inhibited in its usual repair by the simultaneous injection of the extract of the gland as prepared by Evans and Long; and (5) maximum response to the transplants results in cystic ova and the cessation of ovulation.

PURPOSE

The purpose of this work was to study the effect of the bovine anterior pituitary extract upon the growth of the developing rat from the fourteenth day of life through the period during which a plateauing of the growth curve normally occurs, to study the effect of the injected extract on the sex organs, and to see if an extract prepared from glands removed from the cattle about 24 hours before extraction was as potent as that prepared from fresh glands.

MATERIALS AND METHODS

In this experiment anterior pituitary glands from which the extract was made were obtained from the Armour Packing Company of Kansas City. The glands were there removed from the heads of the cattle and chilled over night. These were packed on ice, shipped by express from Kansas City at 10:30 a.m., and arrived at Manhattan at 2:15 p.m., still cold, except in one case. The anterior lobes were shelled from the capsules under asceptic conditions, weighed and then rinsed with a very dilute solution of alcohol to remove excess blood.

The entire process of preparing the gland extract has been worked out from suggestions given us by Dr. Putnam of

the Harvard Medical School, who has since published his methods (Putnam, Teel and Benedict, 1928). Because of certain variations from his method the one used in this work will be described fully. The glands were ground in a mortar with sufficient sterile sand to facilitate complete crushing and pulverizing of the glandular material. This was then placed in a volume of 0.2 Normal solution of sodium hydroxide equal in weight to the mass of the glands used. This was allowed to stand for three to 48 hours. Then it was emptied into a cloth strainer and allowed to drain for five to 24 hours. The glandular material and the sand in the cloth were then placed under pressure to obtain the remaining free fluid. The filtrate was then neutralized with a 0.2 Normal solution of acetic acid and a fine precipitate of proteins resulted. Using litmus paper and phenol red of a known value as indicators the pH value of the extract was established at 7.0 to 7.2. The fluid was then filtered through glass wool and filter paper or only through coarse filter paper, which was frequently changed as the precipitate soon clogged the pores of the paper. The resulting fluid was a light clear red. It was measured and sufficient sodium benzoate was added to make a 0.5 per cent solution. This kept very well in the refrigerator until all used up. All the steps which required any length of time were carried out in the refrigerator, the temperature of which averaged

approximately 8° C. A new supply of the extract was prepared approximately each week. Albino rats from our stock were used in all the experimental groups reported upon except for one pair of black rats of Group VIII.

Littermate rats were weighed at 14 or 15 days of age and divided into two groups with the animals of similar weight and sex paired against each other, using the lighter one of each pair as the experimental animal except in one case. Beginning at this time the experimental animals were injected daily intraperitoneally with 0.2 c.c. of the extract and the dosage increased gradually so that by the time the majority of the group weighed 100 grams apiece each animal was receiving an amount of extract equal to approximately 1.5 grams of the beef anterior pituitary gland. More than 1.5 c.c. was not given at one time. This daily injection continued until the animals were 150 days old. The entire litter was then autopsied to study the internal conditions to make certain that no abnormal condition had been produced which might have influenced the development of the animal. The ovaries and the uterus were removed and fixed in Bouin's fixing fluid. The testes were removed and measured before being fixed. Control animals were injected with a 0.9 per cent solution of sodium chloride equal in amount to the fluid given the experimental animals. Weights were recorded every five days for each rat. At the autopsy

measurements were taken of the body length from the tip of the nose to the anus, tail length (if tail had not been injured) from the anus to the end of the tail. To get a measurement of a group of long bones of the animal the entire left hind leg was measured from the acetabulum to the tip of the claw on the third toe. All measurements were made three times and averaged to obtain the value recorded.

RESULTS

Effects of the Extract on Growth

The first effect of the extract upon the growing rat was the increase in rate of growth. Among a group of normal rats the individual rats grow at varying rates through different periods of time and crossing of the lines of growth is the usual result. With the rats injected with the anterior pituitary extract, however, a tendency to grow at an increased rate was manifested after some days of injections. (See Tables I and II.) This effect of the extract in accelerating the growth rate was shown in a study of the growth curves by the fact that the average length of time which it had taken for all the female experimental animals of a litter to become heavier than all the sister controls was 47.1 days. The corresponding length of time for the male experimental rats was 62.8 days. The crossing of the

growth curves in the graphs for litters 11 and 12 (figures 1 and 2) are typical of the results obtained in all the experimental groups.

The averages of the weights for the animals given in Tables I and II are for all the animals used in the experiment. Due to the fact that in Table I the experimental rats IX-2 and IX-5 had no controls and X-5 was subnormal in its development due to injury and because XII-11 was pregnant from the 101st day to the 125th day the averages slightly exaggerate the results obtained. The second set of averages in Table I is for all females of the experimental group which have control animals. The growth of the control females also appears greater than it actually was because numbers VII-2, VII-7, and XII-6 each produced a litter of young about the 125th day. Most of their increase in weight during the 21 days preceding this date was due to the development of the embryos. Eliminating rats IX-2, IX-5, and X-5 and using the estimated normal weights of any females that were pregnant a study of the table and especially of the growth curves made for all the animals showed that the experimental female rats did not cease their rapid growth rate as early as the control females. The point at which the control females failed to average a gain of one gram a day was about the 110th to 115th day. For instance, after 110 days their average daily gain was about 0.5 of a gram.

The time at which the experimental females did not average a gain of one gram a day was about the 120th to the 125th day. From the 120th day to the 150th day their average daily gain was about 0.7 of a gram.

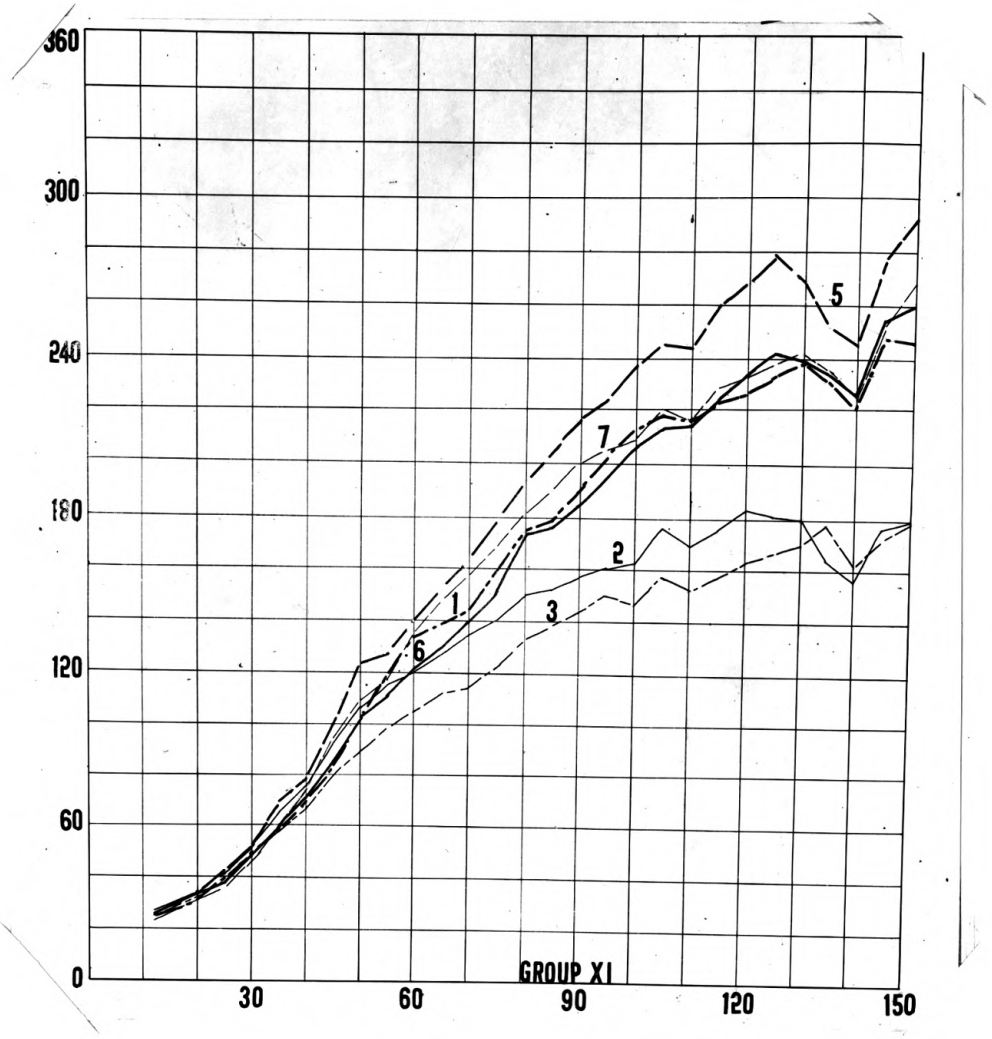

Fig. 1.-- Drop in growth due to insufficient feed which was corrected by increasing quantity of ration fed.

No. 5, experimental male. No. 7, control male. Nos. 1 and 6, experimental females. Nos. 2 and 3, control females. Abscissa -- days. Ordinate -- grams.

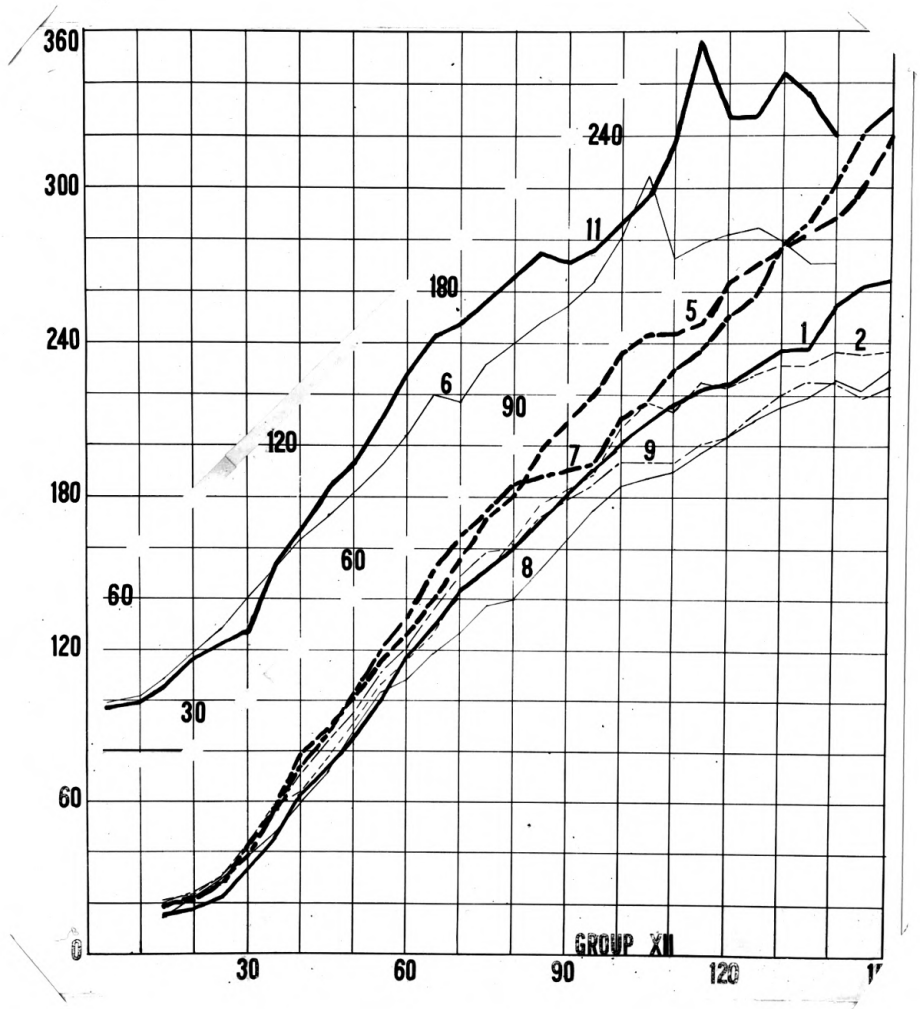

Fig. 2.-- No. 11, experimental female. No. 6, control female. Nos. 5, 7, and 1, experimental males. Nos. 2, 8, and 9, control males. Abscissa -- days. Ordinate -- grams.

Table I.-- Weights of females taken every five days from 15th to the 150th days of life. (Weight in grams.)

Group and No.	Days: 15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	105	110	115	120	125	130	135	140	145	150
Experimental Females																												
VII 2	18	22	27	36	44	45	60	70	81	88	98	103	113	120	134	154	173	180	187	194	211	218	224	237	235	231	237	238
VII 7	19	22	26	34	46	61	76	84	98	108	117	132	141	149	160	177	184	193	202	210	222	227	241	243	248	255	257	262
IX 2	23	34	43	52	61	76	93	113	118	128	138	155	173	186	213	221	---	237	248	259	268	276	280	282	280	283	289	279
IX 5	24	35	45	52	65	72	93	109	127	139	146	145	152	166	191	199	---	218	228	238	248	257	257	266	277	270	266	268
X 2	23	32	39	57	68	84	106	121	140	153	159	171	196	198	200	213	222	223	226	229	239	241	244	249	250(d)	241(d)	252	259
X 5	18	26	32	42	46	60	69	88	105	119	130	147	158	166	169	182	198	202	203	204	210	211	213	214	211(d)	198(d)	219	215
X 6	26	35	43	57	67	86	110	129	145	161	171	185	200	211	225	235	245	247	253	250	259	259	255	264	261(d)	259(d)	286	278
XI 1	25	33	38	49	58	70	83	103	120	135	139	145	160	176	179	190	204	213	218	217	222	226	232	238	230(d)	221(d)	248	247
XI 6	27	33	38	48	60	70	85	102	111	122	129	139	151	173	175	185	195	206	214	215	224	234	242	239	234(d)	225(d)	254	262
XII 11	18	20	25	36	43	48	74	88	104	114	132	147	162	168	177	---	195	192	197(c)	207(c)	217(c)	240(c)	277(c)	248	248	263	254	239
Average	22(a)	29	36	46	56	67	77	101	115	127	136	147	161	171	182	195	202	211	218	222	231	239	247	248	247	245	256	255
	22(b)	27	34	45	55	66	84	100	114	126	135	146	160	171	179	192	203	208	214	217(c)	228(c)	235(c)	245(c)	245	244	242	256	256
Control Females																												
VII 4	17	20	24	37	46	60	72	80	92	104	115	127	137	142	150	160	166	169	173	185(c)	195(c)	206(c)	248(c)	200	208	213	206	211
VII 11	19	22	27	33	40	54	70	83	101	112	102	111	122	129	139	153	154	161	162	167	181(c)	192(c)	221(c)	200(c)	dead at full term			
X 7	25	35	44	63	71	80	102	112	129	142	153	163	175	185	187	197	209	213	226	232	237	241	237	250	254(d)	239(d)	254	258
XI 2	27	35	39	50	65	75	92	107	115	120	127	135	140	150	153	157	160	162	177	169	176	184	181	180	164(d)	156(d)	177	181
XI 3	26	32	39	49	58	66	79	89	99	104	112	115	123	134	139	143	151	146	157	153	158	164	167	169	178(d)	161(d)	173	180
XI 6	19	23	30	39	48	60	72	84	94	102	112	124	140	138	152	---	167	176(c)	185(c)	201(c)	225(c)	195	199	203	204(d)	200(d)	192	192
Average	22	28	34	45	55	65	81	92	105	114	120	129	139	146	153	162	169	171	180	184	195	197	209	200	199(d)	194(d)	200	204

(a) Average for all experimental females used.
(b) Average for experimental females having controls.
(c) Weight taken during the period of pregnancy.
(d) Low weights due to insufficient feeding for several days.

Table II.-- Weights of males taken every five days from the 15th to the 150th day of life. (Weight in grams.)

Group and No.	Days 15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	105	110	115	120	125	130	135	140	145	150
Experimental Males																												
VIII	10	18	21	26	34	42	61	62	74	85	99	109	108	112	107	130	149	157	169	182	207	211	214	227	227	230	238	235	237
IX	6	19	30	37	49	65	79	96	117	136	154	158	171	189	199	225	235	---	254	267	280	293	307	319	325	344	346	343	338
X	3	22	31	39	54	68	79	91	115	139	158	168	199	223	248	243	261	290	299	290	287	312	324	321	324	322	311	347	357
XI	5	27	34	43	53	69	79	100	123	128	140	153	163	179	194	205	217	223	236	245	245	260	268	279	268	252	246	279	293
XII	1	15	19	23	34	45	62	74	86	102	117	130	144	152	160	172	---	190	202	210	216	222	225	231	239	239	254	263	264
XII	3	21	24	28	40	51	62	75	94	109	121	136	146	161	163	167	---	170	177	181	187	187	187	187	189	192	201	207	197
XII	5	19	23	29	44	60	80	89	103	116	127	140	158	173	182	200	---	221	237	243	243	247	264	270	278	283	289	301	302
XII	7	19	22	29	39	58	75	87	104	121	133	153	165	175	185	189	---	193	211	217	230	237	250	258	278	287	303	321	331
Average	20	25	32	43	57	72	84	102	117	131	143	157	170	180	179	215	206	223	229	237	246	255	262	266	269	274	287	290	
Control Males																												
VIII	9	20	24	31	40	51	61	74	84	96	110	120	129	137	140	145	164	176	188	198	189	200	209	221	227	233	244	247	253
XI	7	24	31	36	46	59	74	94	109	117	135	148	157	168	182	191	201	205	209	220	217	228	232	237	242	235	223	254	269
XII	4	16	20	28	37	49	61	60	72	88	98	103	116	125	130	145	---	150	162	162	161	167	167	172	182	188	191	188	192
XII	8	19	23	30	38	48	62	75	88	104	108	120	128	139	140	153	---	174	184	187	191	198	203	210	216	220	226	223	231
XII	9	21	24	30	42	57	70	84	95	111	122	136	150	160	162	174	---	186	194	194	193	202	205	214	220	225	226	220	224
XII	2	22	25	29	43	58	65	79	91	106	117	126	143	151	164	173	---	189	207	213	214	226	223	228	232	233	237	237	237
Average	21	25	31	41	54	66	78	89	94	115	126	137	147	153	164	183	180	190	195	194	204	207	213	203	222	223	228	233	

The striking effect of the injections was that in every case the experimental animals, lighter than their controls at 14 days of age, were at the end of the experiment (150 days) heavier than their litter mate controls of the same sex. Examples of this fact are readily seen in the two growth charts (figures 1 and 2). Although considerable significance must be attached to the fact that all the experimental animals were heavier than their controls yet it has seemed worth while to treat the results biometrically. For the female groups the following significant results were obtained. For the experimental females (seven animals) the standard deviation was 11.5 and the probable error of the mean was plus or minus 2.95. For the controls (five animals) the standard deviation was 30.1 and the probable error of the mean was plus or minus 9.14. The actual difference divided by the error of the difference gave the figure 5.43 in favor of the experimental females. For the eight males who were also heavier than their seven littermate controls the figure of 3.44 was obtained when the actual difference in weight of the two groups was divided by the probable error of the difference.

The increase in weight was due to general increase in muscular and visceral development. The abdominal muscles were very heavy. The organs of the viscera were correspondingly heavier and larger than in the smaller controls. That

a decided increase in the length of bone was not the primary factor in the increased weight of the experimental animals was shown by measurements of body length and the measurements of the leg bones (Table III). These measurements averaged only slightly greater in the experimental animals than in the controls.

Table III.-- Autopsy report at end of 150 days.

Number	Sex	Length (centimeters) Body	Tail	Leg	Parasitized	Peritoneum	Uterus Testes
VII* 2	F	20.15	Short	10.50	Heavy	Normal	Red
VII* 7	F	20.25	17.70	9.65	Heavy	Normal	Thick
VII' 4	F	19.65	Short	9.65	Medium	Normal	Thick
VIII* 10	M	20.60	19.30	10.30	Light	Normal	Normal
VIII' 9	M	21.30	16.90	10.10	Heavy	Normal	Normal
IX* 5	F	21.20	20.10	10.50	None	Normal	Slender,red
IX* 2	F	21.50	20.50	10.71	Light	Normal	Slender,red
IX* 6	M	22.80	20.60	10.90	None	Normal	Normal
X* 7	F	21.06	18.90	10.53	Light	Normal	Slender,red
X* 2	F	20.61	19.40	10.20	Medium	Normal	Slender,red
X* 3	M	23.21	20.50	10.09	None	Normal	Normal
X' 5	F	19.78	18.50	9.95	None	Normal	Slender,red
X' 6	F	21.13	19.20	9.80	Medium	Normal	Slender
XI* 1	F	20.50	18.03	9.80	None	Normal	Slender
XI* 6	F	21.20	17.50	10.03	Heavy	Normal	Slender
XI* 5	M	21.80	18.40	10.58	None	Normal	Normal
XI' 2	F	19.00	16.03	9.10	Heavy	Normal	Slender
XI' 3	F	18.45	15.80	9.60	None	Normal	Slender
XI' 7	M	22.63	17.00	9.83	Light	Normal	Normal
XII* 1	M	20.50	18.00	10.10	Heavy	Normal	Normal
XII' 2	M	22.20	16.70	10.30	Medium	Normal	Normal
XII* 3	M	21.00	15.90	10.20	None	?	Small
XII' 4	M	20.50	17.00	9.40	Heavy	?	Normal
XII* 5	M	23.90	17.60	10.50	Heavy	Normal	Normal
XII' 6	F	20.00	15.90	9.80	Heavy	Normal	Thick, red
XII* 7	M	23.20	18.10	10.95	Medium	Normal	Small
XII' 8	M	21.20	15.90	10.20	Light	Normal	Normal
XII' 9	M	21.20	16.60	10.10	Heavy	Normal	Normal
XII* 11	F	20.50	18.00	10.10	Heavy	Normal	Thick, red
Averages:							
Treated:	F	20.62	18.74	9.94			
Control:	F	19.65	17.08	9.73			
Treated:	M	22.45	18.55	10.42			
Control:	M	21.54	16.68	9.96			

*Animal treated with extract. 'Control animal.

Note.-- X'5, the intestine was inflamed and nearly constricted off. VII'4, XII'6, XII*11, produced one litter each. VII'11 died with a full term litter at 130 days so not included. X'3, XII'3, XII'4 not included in results due to abnormalities.

The body lengths of the females were treated statistically and a value of 0.099 was obtained when the actual difference was divided by the probable error of the difference. As the actual difference in body length between the two male groups was less than that found for the females it was very evident that no significant growth of bones of the body had occurred in the group. The leg measurements revealed very little difference between the length of leg among the experimental animals and the controls. The difference of the averages for the male groups was 0.46 centimeters and for the female groups it was 0.21 centimeters, figures which are not able to show whether any real significance exists or not because of the small number of animals used.

Effects of the Extract Upon the Genital System of the Female Rat

With the young female rats the opening of the vagina occurred about the same time that it was found among the control females. The effect of the injections on the number of litters produced was quite noticeable as three litters were produced among the control females and one among the treated females. The ratios of three to five for the controls and one to nine for the experimental females do indicate that the treated females were less fertile than the

control animals. A point of interest is that like the treated females the experimental males were not sterile for the treated pregnant female came from a cage which contained only treated rats.

The ovaries of the animals were observed macroscopically at the time of autopsy. Observations revealed that the ovaries of the treated females were larger than the ovaries of the control females not excepting the control females that had been pregnant. Several of the ovaries from the treated and control females were sectioned and stained. Microscopic examinations showed that the increase in size of the ovaries was due to numerous corpora lutea. In almost any section of the ovary from a treated female 10 or more corpora lutea were present. Sections of the control ovary showed three or four as a usual number for each section. The corpora lutea of the treated animals were usually not so large as those of the control females. A very important fact is that large normal follicles were very seldom found. The ovary was characterized by a scarcity of any type of normal follicle. Both small and large follicles were usually in a state of degeneration. This was evident by the atretic condition of the ova in the follicles. Perhaps the most important fact is that in several of the corpora lutea degenerating egg cells were imbedded in lutein tissue which nearly filled the corporus luteum.

Some indication of the manner in which this change from the follicle to this type of corpus luteum was produced has been gained by a study of the sectioned material. In some ovaries large follicles were found which were conspicuous because the discus proligerous had broken down or was not evident at all. In the follicular cavity, however, was found a very large cell which appeared to be partially divided into several units whose complete separation had not taken place. Scattered throughout the cell appeared dark staining bodies resembling nucleoli. The stratum granulosum had undergone a change so that most of the cells in that region were lutein cells.

Table IV.-- Dimensions of the testes.

(Given in centimeters.)

Number	Right testes: Length	Width	Thickness	Product	Left testes: Length	Width	Thickness	Product
Experimental Group								
VIII 10	1.95	1.15	0.90	2.02	1.90	1.05	0.91	1.81
IX 6	1.90	1.00	0.90	1.71	1.95	1.09	1.00	1.71
X 3	1.70	1.05	0.87	1.55	1.65	1.10	0.85	1.54
XI 5	1.80	1.10	0.90	1.78	1.70	1.10	0.80	1.50
XII 1	1.80	0.90	0.80	1.29	1.90	1.50	0.80	2.28
XII 7	1.35	0.90	0.80	0.97	1.40	0.90	0.70	0.95
XII 5	2.00	1.20	1.00	2.40	2.00	1.20	1.00	2.40
XII 3	1.40	0.90	0.80	1.01	1.60	0.85	0.70	1.37
Average	1.49	1.02	0.87	1.59	1.48	1.10	0.84	1.69
Control Group								
VIII 9	1.80	1.00	0.83	1.48	1.90	0.60	0.82	0.94
XI 7	1.80	1.10	0.90	1.78	1.80	1.10	0.90	1.78
XII 4	1.75	1.10	0.85	1.54	2.00	1.05	0.90	1.89
XII 2	2.00	1.10	0.90	1.98	1.95	1.10	0.90	1.93
XII 8	1.80	1.10	0.80	1.58	1.70	1.00	0.85	1.44
XII 9	1.90	1.10	0.80	1.67	1.95	1.05	0.90	1.84
Average	1.84	1.08	0.84	1.67	1.89	0.98	0.88	1.63

A later stage showed the lutein cells having more than half closed the cavity of the follicle. The ovum as in most atretic follicles was divided into two or three cells and lay in the fibrillar tissue of the center. Some corpora lutea were present whose cells nearly closed the cavity of the structure, yet inbedded in the cells was the degenerating ovum.

The uterus of the experimental animal had not increased in size nor undergone any development different from that found in the control females which were not pregnant (Table III). Cross sections of the fallopian tubes showed a small lumen which was more nearly closed by the mucosa layer than it was in the control animals. Usually no extensive vascularization in the uterus of the experimental animals occurred.

The Effect Upon the Male Genital Organs

The effect on the testes and other sex organs of the male was also studied. No difference in the degree of scrotality was evident. Heavier experimental males did not develop a scrotum as early as the control males. The other parts of the genital system did not appear to be any different in size from those of the controls. To see if the daily injection of the extract had any effect upon the testes each

one was measured for length, width, and thickness. These measurements were then multiplied together to get a value indicative of the total volume of the organ (Table IV). The figures were then treated statistically and a value of 0.02 in favor of the controls was obtained when the actual difference, 0.01, was divided by the probable error of the difference, .3614. This shows that up to 150 days of age the effect of the extract upon the male gonads had not manifested itself in any acceleration of development.

Potency of Extract With Method of Preparation

The extract prepared from glands which had been removed from cattle about 24 hours showed that it was potent because increased weight in the experimental animals was produced by the daily injections, no difference being noted in the effect of different batches. That the principle which affects the sex organs was also present was shown by the histological picture of the ovaries. From handling the material week after week it can be said that to keep an extract usable it must be kept cold, the filtration process must not be delayed too long as the fluid held in the filter paper with the extract may putrefy and refiltering will not help it. Sodium benzoate is valuable in extracts which are over a week old as it tends to cause any new precipitate to settle out.

Precipitates which form in extracts that lack the sodium benzoate tend to remain in suspension in the liquid. Refiltering of unputrefied extracts in which precipitates form aids in the prolongation of the usefulness of the extract. Such refiltered extracts produce results no less marked than the fresh extract.

DISCUSSION

The absence of a significant increase in bone growth in the treated rats of this experiment is not contradictory to the work of Evans (1923-1924) in whose experimental rats there was an overgrowth of the skeleton, of most of the viscera, and also an accumulation of fat, because most of the skeletal overgrowth could have taken place in his experimental animals after the 150th day, the time at which my experiment stopped.

Apparently an excess of the hormone of the anterior pituitary is not strikingly effective in the production of the long bones before the 150th day but tends to produce a general increase in growth. Excessive skeletal growth beyond this time appears to be made possible by a delay in the complete ossification of the long bones in the anterior pituitary injected rats. This ossification occurs at about 150 days in the normal female rat and at about 200 days in the normal male.

To one examining the results of the autopsies (Table III), parasitism of the animals by tapeworms might appear to have been a deciding factor in the results. This, however, was not the case. In Group XI, Control 2 was heavily parasitized and yet was heavier than No. 3 which was not infested at all. Experimental animal 6 of the same litter was heavily infested and yet was heavier than any of the other females of that litter. Table III shows other cases which clearly indicate that the effect of the parasitism is negligible.

If the anterior pituitary is responsible for normal growth in animals the question naturally arises as to what stops normal growth at the time of sexual maturity. In considering this question it is necessary to refer to the two distinct hormones of the extract of the anterior pituitary reported by Evans (1923-1924). One stimulates growth and the other the formation of lutein tissue, or in small doses, according to Smith (1927c) hastens sexual maturity, producing oestrus and super-ovulation, but in maximum doses arrests oestrus and stimulates the precocious formation of lutein tissue in the follicles (Engle, 1928).

The growth principle produces increased body weight and visceral and skeletal overgrowth (Evans, 1923-1924). Overdevelopment of the long bones occurs because ossification of the epiphysis and paraphysis is inhibited by the growth

principle of the anterior pituitary. That this inhibition is not produced by the sex principle of the anterior pituitary is shown by the fact that immature female rats, injected with the growth principle alone, developed into true giants possessing, however, normal sex organs (Evans, 1923-1924). This fact is also indicated in our work by the overgrowth of a pregnant experimental female whose growth was not retarded as was the case of the control pregnant female because the supply of anterior pituitary extract was sufficient to prevent ossification. That growth stasis might be caused by the presence of the sex hormone of the anterior pituitary seems improbable since in this case of hypersecretion it was not present in sufficient quantity to even completely suppress ovulation.

The absence of growth stasis and normal sexual activity in the experimental animal suggests that in normal rats the simultaneous appearance of these phenomena occurs possibly because the supply of the growth principle is stopped at the time of sexual maturity, but this does not seem likely because of the promotion of growth by injections of extracts from glands of animals which are no longer growing. This suggests that growth cessation must be produced by a counteracting harmone developed at the time of sexual maturity probably formed in the gonads such as, for instance, in the follicular fluid in the ovaries. The follicular fluid has

been shown by Allen, Doisy, Frances, Gibson, Robertson, Colgate, Kountz, and Johnston, (1924) to cause the development of the oviduct, uterus, and vagina and it would be interesting to determine whether it has an inhibitory effect on growth.

One of the problems relating to the effect of the anterior pituitary on the female sex organs is the inhibition of normal development and functioning of these organs in my experiments as well as in those of others using large doses of concentrated extract as opposed to the stimulation of these organs to super-ovulation and early oestrus by the use of smaller doses usually administered as homeotransplants. Because of this it has appeared that the sex principle has been modified or completely changed in the extract as compared to the transplants.

Upon analysis the differences in the results are not as great as they first appear. Normally lutein tissue is not produced until after ovulation and this case does not seem to be an exception for the egg begins to develop before the lutein does. It can then be said that the formation of lutein tissue indicates maturity as well as does ovulation in the transplant treated rats. Evans (1923 -1924) states that even in suckling rats the ovaries can be forced to produce lutein tissue by injections of the extract, therefore, the ovaries of the injected rats may mature as early as the

ovaries which are influenced by the principle from the transplants. Transplants also cause the oestrus cycle to be established early in the treated animal. Teel (1926b) has shown that in young injected female rats deciduomata may be caused by injury to the uterine mucosa, a condition which is not attainable in normal virgin rats. The only point at which the two types of results have not been related has been super-ovulation among the transplant treated animals and the production of lutein tissue in the ovaries of injected animals. Engle (1928) working for maximum response results by the transplant method has obtained histological pictures of the ovaries and uterus similar to those obtained by the injection method and, therefore, has shown the proper relationship of the two types of results. The injection methods using larger doses have been obtaining maximum response results (lutein tissue formation probably following transient follicle formation) while the transplant method using small doses has been obtaining less than the maximum hypersecretion responses from the ovaries and uterus (follicle formation, super-ovulation and enlargement of the uterus).

The effects upon the size of the testes noted in the experimental males are of interest. Why is not an effect produced on the male gonads as easily as on the female gonads? An examination of the weight chart and also of the

one dealing with the size of the male gonads shows that the heaviest animal has the smallest set of reproductive organs. The average of all the male treated animals shows a slightly smaller value for the size of the experimental male gonads than for the size of the control male testes. This indicates the possibility that because of the hardiness of the male rat a much larger amount of the extract is necessary to cause any effect upon the male genital system than is necessary to affect the female genital organs.

CONCLUSIONS

1. Daily injections of bovine anterior pituitary extract accelerated the growth rate of young rats.

2. Experimental female rats did not cease their rate of rapid growth as early as did their controls.

3. Treated rats at 150 days of age were significantly heavier than untreated littermate rats.

4. Increase in weight was due to general increase in muscular and visceral development.

5. In the treated rats no significant increase in the length of long bones occurred within the 150 days of the experiment.

6. The opening of the vagina was not delayed by the injections of the anterior pituitary extract.

7. Treated females were less fertile than the control females.

8. The increase in the size of the ovaries of the experimental females was due to the large number of corpora lutea present.

9. Normal follicles were seldom found.

10. Some corpora lutea contained degenerating ova.

11. No marked change in the uterus due to the injected extract was evident.

12. The development in the size of the testes was not affected by the injections of the extract.

13. Extracts prepared from glands which had been chilled for 24 hours were potent as they affected the body growth and the sex organs of the treated animals.

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