THE EFFECT OF ANTERIOR PITUITARY EXTRACT ON THE DEVELOPING ALBINO MOUSE

by

ROBERT TOWNER HILL

B. S., South Dakota State College, 1928

A THESIS

submitted in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

KANSAS STATE AGRICULTURAL COLLEGE

1929

TABLE OF CONTENTS

INTRODUCTION

Since late in the nineteenth century abnormalities of the pituitary have been associated with giantism and certain other body disorders, the most prominent of which was the disease known as acromegly. Acromegly is characterized by a great increase in the size of the lower jaw, hands and feet, and a spacing of the teeth. Certain internal disorders might accompany this disease, such as a broken rythym in the menstrual cycle and mental disturbances, which were sometimes improved by pituitary therapy.

While clinical observations have continued, workers of the past twenty years have turned largely to experimentation on small animals to determine the function of the pituitary. Various extract and transplant methods have been tried and entire or partial hypophysectomy has been accomplished by different means of operative procedure. While the results have been contradictory they have indicated that the anterior lobe of the pituitary is related to growth and to sexual development. The extract relationships remain to be worked out for the most part and this has led to the present work.

PURPOSE

The purpose of this work was to find the effect of an alkaline extract (subsequently neutralized with acetic acid) of the anterior pituitary on the developing albino mice between the ages of 20 and 150 days. It was desired to find the period of life when the extract caused changes in size and histological appearance, if any, of the gonads of both male and female mice, and to find the increase, if any, in the body weight and growth of the long bones of the body.

REVIEW OF LITERATURE

Relation of Anterior Pituitary to Growth and Reproduction

Feeding of pituitary substance. Schäfer (1909, 1911) fed rats anterior lobe substance in comparison with posterior lobe and pars intermedia substances. Anterior lobe material fed gave only slight increase in growth for a short time. Aldrich (1912) fed dogs anterior pituitary material and found no effect on the gonads or growth. Lewis and Miller (1913), believing that acromegly and giantism were produced by hypersecretion of the pituitary, fed the separate lobes of the gland but obtained no results. Marinus (1919) fed anterior pituitary to rats and found that there was an increase in the size of the testes of the male to about twice the normal which he states was due to

hyperplasia of the tubules. Pregnancy was not affected and the histology of the ovaries was not noted. By forcing salamanders to eat nothing but anterior lobe substance Uhlenhuth (1919) produced giants and maintained growth after the normal growth curve had plateaued and also produced a much more rapid growth during the early life of the individual when the growth curve of the controls was also on a rapid increase. He concluded, however, that, while growth can be produced in salamanders, the evidence that giants can be produced by the same method among the warm blooded vertebrates is still insufficient. Hoskins and Hoskins (1920) found that anterior pituitary tissue fed in the desiccated state caused some increase in the growth of hypophysectomized tadpoles but did not affect metamorphosis. Evans and Long (1921) were unable to change the oestrous cycle of the rat or the sex apparatus in any way by the feeding of either fresh or desiccated anterior lobe substance. Sisson and Broyles (1921) found much the same results as those of Evans and Long. S. C. Smith (1923) and P. E. Smith (1927) were unable to obtain specific results from feeding of fresh anterior pituitary tissue to either normal or hypophysectomized rats.

Transplantation. Exner (1910) and Schäfer (1909, 1911) made some of the first attempts to transplant pituitary

material in the muscle of an experimental animal but without notable success. Schäfer tried to make a permanent graft of the material into the new tissue but was unable to accomplish this. Both workers noticed an increase in the weight of the experimental animals but no effect on the sex apparatus was found, however this was not definitely looked for. Allen (1916) was able to remove the hypophysis anlage from Rana tadpoles and in so doing was able to keep the tadpoles from undergoing metamorphosis and at the same time inhibited growth and development of the thyroid. By treating such tadpoles with pituitary and thyroid materials he was able to induce metamorphosis in a normal way. P. E. Smith (1916) accomplished the same thing about two months later without knowledge of Allen's work. Since this time P. E. Smith (1926) has developed a method of approach to the pituitary in the rat. He has been successful in its total removal, which resulted in a complete growth stasis and regression of the adrenals, thyroids and sex apparatus. By making transplants of fresh bovine anterior pituitary he was able to restore the growth and sexual regression to a normal state again. P. E. Smith and Engle (1927), working together and separately, have been able to produce precocious sexual maturity in young female rats and mice at about the weaning age by making daily homeo-transplants and also caused a growth in the body and sex apparatus. Super-

ovulation was also induced. Engle (1928) restored regular oestrous and follicular formation in senile female rats by the implantation of fresh anterior pituitary tissue. It was also the work of Engle (1928) which proved that the pituitary glands of rats which have been gonadectomized for at least eight months contain a larger amount of the gonad stimulating principle than the glands from rats which have not had their gonads removed. P. E. Smith and Engle (1927) in making transplants into female rats found that the avian anterior pituitary gave negative results. On the other hand Riddle and Flemion (1928) found that a glycerin extract of bovine pituitary gave positive results when injected intraperitoneally into the ring dove, having an effect on both the male and female.

Extirpation. Palesco (1928) was successful in the complete removal of the hypophysis of the dog and found that the lack of the gland resulted in death without characteristic effects being noticed, and that with only partial removal the dog might live indefinitely without showing any ill effects. Crowe, Cushing and Homans (1910) were able to show that total hypophysectomy in dogs led to death with characteristic symptoms (cachexia hypophyseopriva). They were able to prevent death with implants of pituitary material and also eliminated the symptoms of the disease.

Allen (1916) was successful in the complete removal of the anlage of the hypophysis in the Rana tadpole and P. E. Smith (1926) developed an approach to the pituitary for its removal in the rat and they concluded that hypophysectomy in these two animals was not necessarily fatal. P. E. Smith and Foster (1926) found that metabolism was reduced 35 per cent by complete hypophysectomy and that metabolism was rapidly restored by making daily fresh anterior pituitary transplants. Complete hypophysectomy in the brown leghorn fowl proved fatal in a ten day period as shown by Mitchell (1928), and partial removal did not prove fatal but gave a decided retardation in growth and development of the body and sex apparatus and the thyroid was found reduced in size and filled with colloid to a marked degree.

Injections. Rénon (1908), Franchini (1910) and Iscovesco (1913) were among the first investigators to notice a change in the sex rythym and metabolism of the body by the injections of an extract of the anterior lobe of the pituitary. Klinger (1919), in making injections of extract into guinea pigs was unable to obtain any results either in the body growth or in the sex glands but this was no doubt due to the fact that the injections made were in such small amounts and also that he was not sure of the potency of his extract. By using an extract of fresh anterior pituitary

gland Evans and Long (1921) were able to produce increased weight in the experimental animals and to cause a cessation of sexual development and in some cases oestrous was entirely inhibited. In 1922 they were able to produce giants and to cause a hemming in of the ova in the follicles by the formation of lutein tissue. A like preparation of the posterior pituitary was proved to have no potency. Walker (1925) obtained the same hemming in of the ova in the hen ovary as Evans and Long did in the rat and thus extended the potency of the pituitary extract to birds. Teel (1926a, 1926b) was able to produce placentomata and deciduomata in the virgin rat with the aid of anterior pituitary extract injections through the ability of the extract to form corpora lutea which in turn are necessary for the formation of placentomata and deciduomata. Crafts and Flower (1925) and Evans and Simpson (1926) formed the common opinion that male rats treated with anterior pituitary extract retained their fertility, but later Evans and Simpson (1928) find a loss of sexual interest in male rats treated with anterior pituitary fluid but do not state anything as to the degree of sterility produced. Zondek and Aschheim (1928), in working with the anterior pituitary hormone from the urine of pregnant women, were unable to state with certainty that there was an effect on the male genital system but they state that Biedl (1911) found a hindering effect on the

males and that Fels (no date given) found a decrease in the size of the testis and an increase in the interstitial tissue with the use of anterior pituitary hormone from the urine of pregnant women. Using this hormone in infantile mice they obtained an increase in the size of the testes and in the near organs in the male genital system. They do not state whether or not a histological change was found. Engle (1929) made injections of an extract of urine from pregnant women into male rats and found a marked increase in the interstitial cell mass with no acceleration of spermatogenesis and also found a varying degree of destruction of the seminal epithelium.

METHODS AND MATERIALS

The whole bovine pituitary glands were obtained from the Armour Packing Plant in Kansas City within 24 hours after collection, having been chilled or frozen and packed in ice for the trip. Upon arrival of the glands the anterior lobe was shelled out immediately and an extract made according to the method of Evans (1924) which was modified by Putnam, Teel and Benedict (1928) and again by Johnson and Sayles (1929). The anterior lobes of the glands were weighed and the extract made so that 1 cc. of the extract was equal to 1 gm. of the anterior lobe of the gland.

The method of making the extract follows: The shelled

anterior lobe of the gland was washed in a solution of 8 per cent alcohol. Instruments, dishes and hands were washed in 50 per cent alcohol. The glands were ground in a mortor with sterile sand until they were in a homogeneous paste which was put in a jar and as many cubic centimeters of 0.2 normal sodium hydroxide were added as there were grams of the gland. This was then put in the refrigerator and allowed to stand at about the freezing temperature for 3 to 48 hours. At the end of this time the mixture was strained through a fine cloth to remove the solid particles and the remaining liquid was then neutralized with 0.2 normal acetic acid until the pH value of 7.0-7.2 was reached. The process of neutralizing caused the proteins to be precipitated out and a careful filtering was then necessary, after which the extract was kept in the refrigerator at about the freezing temperature where it would keep for about 30 days without any deterrent action. That the extract retained its potency is shown by the fact that no difference was noted in the growth curves of the animals in the later periods of the use of a batch of extract than from the earlier periods of the same batch of extract.

Animals to be used in the experiment were weaned at the age of twenty days, weighed and each animal given an ear mark which was recorded and the animals of a litter

were kept together and not mixed with those of any other litter. Each litter was given a group number. The animals were paired as nearly even as possible but when a difference occurred the lighter animal was used as the experimental and the heavier one as the control. Injections were begun on the twentieth day of life and the amount given was one-tenth cubic centimeter daily for the first five days, after which time, if the animals of the litter had made a substantial growth, the dose was increased by one-tenth cubic centimeter and thus increases were made every five days until one-half cubic centimeter was reached and over this amount was never given. Weights of all animals were taken at five day intervals.

As nearly as possible all of the animals which were started early enough in the experiment were left to attain the age of 150 days before killing, making a total of 130 days of injection. Other animals were killed at intervals which would make a five day series from 30-150 days of age, the purpose of which is explained in another part of this paper. The testes, most of the prostates, and some of the epididymi were saved from the males and the ovaries and uteri were saved from the females and all tissues saved were fixed in Bouin's fluid, most of them sectioned except the uteri, and stained with Ehrlich's Hematoxylin. Measure-

ments of the ovaries and testes were taken at the time of autopsy as well as certain long bones of the body. Body measurements were taken from the tip of the nose to the base of the tail and from the base of the tail to its tip. The right hind leg was measured from the anterior edge of the acetabulum to the end of the longest toe. The testes and ovaries were measured for the length, width, and thickness and the resulting volumes are therefore only indicative of the volume of the organs measured. The results of these measurements and calculations of such will be found in Tables VI, VII and VIII.

All animals were kept under like conditions and in cages with wire bottoms and sides. Feed cans were cleaned each day and fresh feed given from a source which was free from any insect which might serve as an intermediate host of an internal parasite, and under these conditions, with only two exceptions, all of the animals were kept free from internal parasites and apparently free from external parasites.

RESULTS AND DISCUSSIONS

Growth of Female

Results. The daily injections of the extract of the anterior lobe of the beef pituitary into female albino mice caused a decided increase in the growth of the individual

from the time injections started until at least the 150th day of life, the time when this experiment terminated. The increased growth took place over the entire body as is noticed by the examination of the picture (Fig. 1) of an experimental animal (A) and its control (B), killed at 110 days of age. The experimental animal exceeded the control in total length by 23 mm. and in leg length by 3 mm.

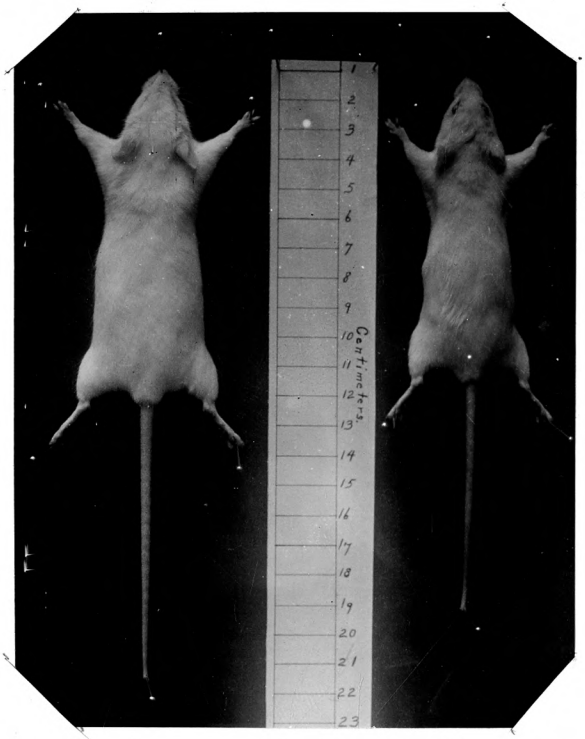

Fig. 1. Two female mice 110 days old, after 90 days of injections. Measurements recorded were taken before the animals were stretched on board for the picture.

A, Experimental (13-2); body length, 111 mm.; tail, 91 mm.; leg, 34 mm.

B, Control (13-5); body length, 102 mm.; tail, 77 mm.; leg, 31 mm.

A comparison of Tables I and II will show that at 70 days the experimentals were 1.42 grams heavier than their controls. When this difference is divided by the probable error the number 2.9 was obtained which indicates that the difference is not significant. However, when all females which had been pregnant up to 70 days were omitted because of their greater weight, the experimental females were 2.16 grams heavier than the controls which statistically treated proved to be significant (Difference ÷ probable error = 4.9). At 150 days the experimentals were 2.4 grams heavier than the controls, a difference which would hardly be significant (D/PE = 3.1), but when animal 2-4, which had been pregnant three times was omitted the difference was 3.30 grams, a significant figure (D/PE = 5.9).

From the weights given it is evident that the experimental females made more striking gains in comparison with the controls before 70 days (2.16 grams) than from 70 to 150 days of age (3.30 - 2.16 = 1.14 grams). A comparison of Tables I and II shows that the average weights of the experimental females rose from below those of the controls between 35 and 45 days of age, becoming distinctly higher at 50 days.

TABLE I. WEIGHTS OF EXPERIMENTAL FEMALES AT FIVE DAY INTERVALS

Animal**	Age in Days																												
	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	
1---1	9.4	10.1	12.4	14.3	14.8	16.3	18.0	20.2	20.6	22.5	21.3	22.1	22.8*	25.9*	33.3*	26.1	26.5	27.1	24.1	26.9	26.5	26.7	27.4	killed					
2---1	6.4	7.6	9.5	13.7	15.9	17.3	19.0	21.3	23.3	23.9	24.4	24.7	25.4	25.2	24.3	26.1	25.7	26.5	26.9	28.2	22.8	21.6	21.2	20.2	19.8	22.9	25.4	killed	
3---1	6.1	8.8	10.6	13.0	13.2	15.9	16.5	15.2	17.3	19.3	19.7	21.2	22.8	23.1	23.1	24.6	22.1	24.7	dead										
3---2	5.3	7.8	9.2	10.3	dead																								
4---1	4.7	6.8	10.0	13.1	16.3	16.7	17.7	20.1	20.3	21.8	23.7	23.7	24.4	25.3	26.4	26.4	23.0	23.2	24.5	23.7	23.7	27.9	26.9	28.0	30.5	27.7	29.1	killed	
4---2	5.7	8.4	11.6	14.6	16.8	17.1	18.4	20.1	21.3	22.1	23.5	23.7	24.8	23.8	25.6	25.9	25.9	25.3	26.7	27.1	26.6	28.5	27.8	28.0	30.0	28.3	28.0	killed	
5---1	6.2	9.0	11.7	13.5	13.7	14.4	17.4	19.2	19.3	19.4	21.0	20.7	20.9	21.3	22.2	22.2	22.2	22.5	21.8	21.8	23.3	24.3	25.0	25.5	25.6	24.9	24.5	killed	
5---2	6.5	9.1	11.3	13.4	13.7	15.0	18.4	18.2	19.7	19.2	21.9	21.0	21.8	22.1	23.1	22.9	22.5	22.7	22.6	22.6	23.0	24.7	25.8	26.4	25.8	26.4	25.0	killed	
5---3	6.4	9.0	11.3	13.7	14.0	15.7	17.8	18.4	18.6	18.9	21.0	20.1	21.2	20.9	22.5	22.6	22.2	23.7	22.8	22.8	23.3	24.7	24.8	25.3	24.1	24.0	24.0	killed	
6---1	4.3	--	7.5	10.3	10.3	12.8	13.4	15.2	16.1	17.2	18.6	18.2	20.6	21.0	dead														
6---2	4.2	5.2	7.4	10.1	10.0	12.2	12.6	13.5	14.8	16.1	16.9	16.4	18.8	19.2	19.5	20.9	21.3	22.1	27.8*	31.1*	26.4*	killed (resorbing litter)							
7---1	8.5	10.4	13.5	17.0	18.2	18.9	20.1	21.4	23.1	22.6	22.7	21.6	23.5	24.4	24.0	24.1	24.6	24.8	26.0	26.4	27.7	25.4	27.3	25.7	26.0	22.2	25.5	killed	
8---1	7.5	10.4	10.7	11.4	13.6	dead																							
8---2	7.2	10.6	10.5	11.0	13.8	dead																							
9---1	6.4	9.0	11.8	14.7	dead																								
10--1	8.7	10.1	13.4	dead																									
12--1	11.5	14.5	16.1	17.9	19.2	21.2	22.3	23.2	23.6	21.4	21.6	22.5	24.5	25.5	26.8	28.4	25.1	26.7	25.8	28.8	28.4	28.5	29.3	28.3	30.2	31.2	29.8	killed	
13--1	5.0	7.5	10.5	12.1	12.5	15.5	18.3	16.9	20.0	20.4	20.0	20.9	21.9	21.7	22.1	22.7	24.9	25.5	25.7	killed									
13--2	8.7	11.5	14.5	16.4	16.9	18.6	21.1	21.1	24.0	25.4	24.5	26.5	26.7	26.9	27.4	27.7	29.8	30.0	30.9	killed									
13--3	5.0	6.8	9.2	10.9	12.5	15.6	19.0	17.8	21.5	22.5	21.5	22.6	23.2	24.1	23.4	23.4	25.2	25.8	26.4	killed									
14--1	9.1	11.8	13.6	15.6	16.8	19.1	23.3	22.1	24.4	25.1	23.9	26.0	24.6	24.8*	27.8*	31.0*	39.0*	31.3*	31.4*	35.8*	42.7*	killed (about 16 days pregnant)							
15--1	7.2	6.2	9.1	12.0	14.3	16.7	17.0	18.8	20.0	20.0	20.0	21.0	20.3	21.6	21.7	23.2	24.1	24.7	killed										
17--3	4.4	6.0	9.8	10.8	11.4	12.7	13.3	14.5	14.8	16.6	16.8	17.7	19.9	18.3	20.5	21.3	killed												
17--4	4.7	6.9	10.6	10.8	11.8	13.3	14.4	15.6	15.5	16.6	16.7	16.7	19.9	escaped from cage															
18--3	8.5	11.8	13.2	16.2	16.9	17.6	19.3	20.5	22.5	22.5	23.2	25.4	25.6	24.6	25.8	killed													
19--2	6.9	8.5	10.9	13.3	15.3	17.1	16.6	19.5	20.4	22.0	22.1	22.7	killed																
20--2	5.5	9.3	10.7	13.3	15.0	15.6	17.9	19.4	21.1	22.5	killed																		
20--3	5.3	8.8	10.4	12.0	13.3	14.0	15.9	17.2	18.1	19.3	killed																		
20--4	5.2	8.8	10.4	12.7	14.4	15.5	17.5	18.4	21.4	23.0	killed																		
21--1	9.0	9.3	10.3	12.8	14.1	13.6	16.5	17.4	killed																				
21--2	9.2	11.3	13.3	16.1	17.3	16.9	18.7	20.5	killed																				
22--1	5.9	8.5	8.5	9.4	10.0	11.0	14.4	killed																					
23--1	7.0	8.6	10.9	12.7	12.5	16.4	killed																						
Average:	6.7	9.0	11.0	12.9	14.3	15.1	17.6	18.7	20.1	20.9	21.2	21.7	22.7*:	23.1*:	24.5*:	24.7*:	25.3*:	25.4	26.0*:	26.8*:	26.8*:	25.8	26.2	25.9	26.5	26.0	26.4		

*Indicates those weights affected by pregnancy.

**The first number indicates the group and the second number the animal of the group. The weights underlined indicate the time when the animal equaled or became heavier than its litter-mate control; those underlined with dashes indicate the lack of a control.

TABLE II. WEIGHTS OF CONTROL FEMALES AT FIVE DAY INTERVALS

Animal**	Age in Days																												
	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		
1---3	9.6	9.8	11.5	12.5	13.6	15.1	17.0	17.6	18.0	19.8	19.5	20.4	21.2	22.7	27.4	23.7	23.7	24.8	26.4	33.1	27.1	24.9	25.0	killed					
2---4	7.2	8.2	9.6	12.6	14.4	15.4	15.6	17.0	18.7	19.1*	20.3	25.4	21.7	20.8	21.5	21.7	22.0	21.8	24.2	26.4	31.3	27.7	26.2	25.9	27.6	31.8	27.7	killed	
3---4	6.1	8.3	10.1	12.3	12.3	15.0	15.3	14.3	17.1	18.5*	19.2	20.8	21.2	21.0	21.5	23.3	21.6	23.9	killed										
3---5	6.0	7.9	8.8	11.3	killed																								
4---3	5.7	7.8	10.8	13.9	15.5	14.9	15.6	17.0	17.6	18.3	19.3	19.5	21.1	21.1	21.5	21.4	22.5	22.6	22.6	23.0	23.5	23.6	24.8	24.3	25.3	23.9	23.3	killed	
5---4	6.6	9.3	12.3	13.6	14.0	14.2	16.0	17.2	19.0	18.5	20.5	20.0	20.8	19.8	20.9	20.7	21.7	21.2	20.8	21.1	21.8	22.4	23.3	22.8	23.4	23.0	23.8	killed	
5---5	6.7	9.1	11.3	12.7	13.1	13.9	15.4	15.1	18.2	17.7	19.5	19.0	19.6	19.5	20.2	20.1	20.6	20.9	18.8	18.8	20.3	21.1	21.3	21.0	21.7	22.3	21.8	killed	
6---4	4.8	5.7	7.4	9.6	9.7	12.0	12.4	13.5	15.9	15.9	16.8	16.5	19.0	18.2	18.4	19.4	19.2	20.1	19.4	20.3	20.0	killed							
6---5	4.7	6.0	8.0	10.1	10.0	12.0	12.6	13.8	16.1	16.4	17.5	19.6	22.9	19.4	killed														
7---2	8.5	10.1	12.0	13.8	15.6	16.3	17.6	18.4	18.6	19.7	21.0	20.1	22.0	22.1	21.1	22.6	22.6	22.9	22.4	22.7	23.9	22.5	23.2	22.6	23.3	21.2	23.5	killed	
8---3	7.7	9.9	10.0	11.4	14.0	killed																							
9---3	7.4	8.9	12.2	15.3	killed																								
10--2	10.5	11.7	14.6	killed																									
12--3	12.3	14.6	15.5	16.5	18.0	20.1	20.6	21.4	27.1	24.0	24.1	24.5	23.7	23.7	23.2	24.8	22.1	22.4	22.1	24.7	25.2	24.8	25.5	24.8	26.0	32.0	dead after abortion		
13--5	9.0	12.0	14.6	16.0	16.4	16.1	19.2	20.2	28.0	22.6	23.0	23.6	24.3	24.5	22.2	23.4	23.9	24.6	25.4	killed									
14--2	9.5	11.3	13.4	14.9	15.2	16.0	18.3	18.1	20.0	20.0	20.2	20.3	20.3	20.9	22.1	22.7	25.4	29.7	34.7	28.7	28.0	killed							
15--2	7.3	6.2	8.6	10.3	12.4	14.8	14.7	16.5	17.6	18.1	18.6	19.8	18.3	20.2	20.0	21.3	20.7	20.4	killed										
17--6	5.4	7.1	10.0	11.4	12.3	13.4	14.0	15.3	15.4	17.0	17.2	17.5	21.0	21.1	19.6	21.7	killed (heavy adhesions)												
18--6	8.9	10.2	12.0	14.4	15.3	16.5	17.0	17.3	19.6	22.2	19.8	20.0	21.2	20.6	20.6	killed													
19--4	7.5	9.0	10.9	12.7	13.6	14.9	15.0	16.2	18.0	escaped from cage																			
20--7	6.0	9.5	10.9	12.6	13.8	14.8	16.4	17.8	18.5	20.7	killed																		
21--4	9.5	11.1	12.2	14.6	15.7	16.4	17.7	19.4	killed																				
22--3	6.7	9.0	9.5	10.6	10.8	13.5	15.7	killed																					
23--3	7.7	9.8	12.0	13.1	13.0	16.3	killed																						
Average:	7.6	9.3	11.2	12.9	13.8	15.1	16.1	17.0	19.0	19.3	19.8	20.5	21.2	21.0	21.4	22.1	22.2	22.9	23.7	24.3	24.6	23.9	24.2	23.6	24.6	25.7	24.0		

*Those weights affected by pregnancy.

**The first number indicates the group and the second number the animal of the group.

TABLE III. GROWTH OF FEMALES DURING PREGNANCY COMPARED WITH THE AVERAGE GAIN OF NON-PREGNANT FEMALES

	PREGNANT INDIVIDUAL				NON-PREGNANT GROUP
		Weight in Grams			
Animal*	Age	Before Pregnancy	After Pregnancy	25 Da. Gain	Group gain in 25 days
		Saline Injected Females			
1---3	75	20.4	23.7**	3.3	1.8
1---3	100	23.7	24.9**	1.2	2.0
2---4	60	18.7	20.8	2.1'	3.1
2---4	105	21.8	26.2	4.4	2.1
2---4	130	26.2	27.7	1.5"	-.2"
12--3	45	20.1	24.1	4.0'	4.6
13--5	45	16.1	23.0	6.9	4.6
14--2	90	22.1	28.7	6.6	1.8
Average:				3.8	2.5
		Anterior Pituitary Injected Females			
1---1	75	22.1	26.5	4.4	2.6
14--1	80	24.6	31.3	6.7	2.7
Average:				5.6	2.7

*The first number indicates the group and the second number the animal in the group.

'The gain of these females in growth during pregnancy is less than the average gain of the group during a corresponding time, which may be accounted for by the fact that the pregnancy occurred during early life when the growth of the group was going on at its greatest rate.

"Taken for a period of 20 days on account of a previous pregnancy and an early autopsy.

**This weight was taken 6 days after parturition. Weight was 27.1 gm. 1 day after parturition.

One of the striking results of the experiment was the greater permanent growth made by females during pregnancy than during a like period of non-pregnancy as is shown in Table III. This growth was greater in the females which received the anterior pituitary injections than in those which did not.

Discussion. While the extract produced a significant increase in growth, the fact that no striking giantism was produced such as reported in rats by Evans (1924) may be due partly to the fact that the experiment terminated at 150 days and partly to the probability that his extract was more potent for the production of growth because his extract was prepared by centrifuging the crushed glands. The growth was distributed over the entire body (Tables VI and VII). The results obtained are similar to those reported by Johnson and Sayles (1929) on the albino rat.

There are two possible explanations for the increased growth during pregnancy. One of these is advanced by Slonaker (1927) who suggests that increase in growth may be caused by the decrease in the amount of follicular hormone present in the ovary at the time of pregnancy, which, when present, may be an inhibitor of growth. The other theory is advanced by Zondek and Aschheim (1928) and Engle (1929) that during pregnancy there is an increased amount of the

gonad and growth stimulating principles released from the anterior pituitary of the individual and that the pituitary gland is larger during pregnancy than during a non-pregnant period. The increase in growth made by the experimental female over the growth of the control female while both are pregnant, may be accounted for by the growth stimulating principle which she received from the daily injections of the potent extract. Since it is known that the secretion of the anterior pituitary causes growth, and since the inhibitory function of the follicular hormone to growth has not been established, it seems most likely that the increased secretion of the anterior lobe of the pituitary during pregnancy is the cause of the increase in growth during that period of time.

Growth of Male

Results. The male albino mice were stimulated to increased growth by the daily injection of anterior pituitary extract (Tables IV and V). Treated males became heavier than their controls at the age of 38 days although they entered the experiment 0.6 grams lighter. At the age of 70 days the experimental males averaged 1.42 grams heavier than the controls. Statistical treatment of this (D/PE = 2.4) gives the figure 2.4 which bears no significance. By the

time the experimentals had reached 90 days of age they were 2.3 grams heavier than their controls. With the same treatment of this the figure 3.17 is obtained, the significance of which is doubtful. It does, however, show a decided increase in growth in the experimental males from the age of 70 to 90 days. The increase in male mice was not as great as the corresponding increase in females treated with the extract. There appears to be a possibility that the males may make a greater growth late in life to surpass the early growth made by the females. The number of males left at the end of the experiment was too small to demonstrate this period of catching up with the females in increased growth, but an examination of the tables indicates a steadier and a greater growth at the age of 150 days than among the females. The growth in the male takes place over the entire body and is not localized in any particular organs or systems of the body (Tables VI and VII).

Discussion. The growth of male albino mice which I have been able to produce is confirmatory to the work of Johnson and Sayles (1929). It also confirms the work of Evans (1924) and others as to the potency of anterior pituitary extract in causing growth. Although the work of Johnson and Sayles (1929) and the present work has not proved the significance of the growth stimulating principle in male

TABLE VI. RELATIVE LENGTH OF BODY, TAIL, AND RIGHT HIND LEG OF FEMALES

EXPERIMENTAL FEMALES					CONTROL FEMALES				
Animal*	Body	Tail	Leg	Age	Animal*	Body	Tail	Leg	Age
1---1	102.0	86.0	35.0	130	1---3	99.0	85.0	31.0	130
2---1	118.0	85.0	33.0	150	2---4	115.0	89.0	32.0	150
3---1	100.0	86.0	34.0	108	3---4	99.0	83.0	35.0	108
4-1&2'	106.0	89.0	31.0	150	4---3	98.0	85.0	31.0	150
5-1,2,3"	101.0	86.7	33.0	150	5-4&5"	95.5	86.0	32.5	150
6---1	96.0	83.0	30.0	87	6---5	93.0	81.0	31.0	87
6---2	103.0	81.0	31.0	120	6---4	97.0	82.0	31.0	120
7---1	105.0	93.0	36.0	150	7---2	100.0	89.0	35.0	150
8-1&2'	79.5	74.5	29.5	41	8---3	81.0	71.0	30.0	41
9---1	80.0	59.0	27.0	36	9---3	83.0	'''	30.0	36
10--1	81.0	69.0	31.0	32	10--2	82.0	71.5	33.0	32
12--1	105.0	82.0	34.0	150	12--3	97.0	93.0	33.0	149
13-1,2,3'	103.7	82.0	32.3	110	13--5	102.0	77.0	31.0	110
14--1	117.0	90.0	37.0	120	14--2	102.0	86.0	33.5	120
15--1	101.0	'''	31.5	105	15--2	94.0	69.0	31.0	105
17--3	94.0	67.0	32.0	95	17--6	89.0	65.0	28.0	95
18--3	102.0	84.0	32.0	90	18--6	91.0	81.0	30.5	90
20-2,3,4'	92.7	76.7	31.3	65	20--7	91.0	81.0	31.5	65
21-1&2'	92.0	75.0	30.8	55	21--4	90.0	74.0	29.0	55
22--1	79.0	62.0	29.0	50	22--3	80.0	64.0	31.0	50
23--1	84.0	62.0	30.0	45	23--3	83.0	61.0	31.0	45
Average:	97.23	78.65	31.92	97.1		93.40	78.68	31.48	97.1

*The first number indicates the group and the second number the animal in the group.

'Averages of two or more individuals which had but one control.

"Three experimental animals averaged against two controls.

'''Tails had been injured so that measurements could not be taken. Each experimental animal has a litter-mate control in the opposite position of table.

TABLE VII. RELATIVE LENGTH OF BODY, TAIL, AND RIGHT HIND LEG OF MALES

EXPERIMENTAL MALES					CONTROL MALES				
Animal*	Body	Tail	Leg	Age	Animal*	Body	Tail	Leg	Age
2---3	115.0	86.0	30.0	150	2---6	112.0	92.0	31.0	150
3---3	93.0	82.0	33.0	80	3---6	89.0	83.0	32.0	80
6---3	96.0	82.0	33.0	150	6---6	94.0	85.0	32.0	150
11--1	97.0	87.0	33.0	72	11--2	93.0	87.0	34.0	72
13--4	92.0	84.0	31.0	62	13--7	93.0	80.0	31.0	62
16--2	102.0	87.0	34.0	100	16--7	87.0	78.0	31.7	100
16--3	98.0	84.0	34.0	105	16--9	95.0	86.0	32.5	105
16--4	89.0	82.0	31.0	44	16--6	80.0	77.0	28.0	44
16--5	103.0	89.0	33.0	120	16--8	97.0	87.0	33.0	120
18--1	101.0	81.0	33.5	100	18--4	95.0	82.0	31.5	100
18--2	101.0	84.0	32.5	90	18--5	96.0	80.0	31.5	90
20--1	93.0	76.0	33.0	60	20--5	89.0	73.0	31.0	60
21--3	90.0	75.0	31.0	55	21--5	91.0	72.0	29.0	55
22--2	83.0	66.0	29.0	50	22--4	77.0	65.0	30.0	50
23--2	83.0	61.0	30.0	45	23--4	81.0	57.0	28.0	45
24--1	84.0	74.0	31.0	40	24--2	83.0	73.0	29.0	40
25-1&2'	73.5	62.0	30.0	35	25--3	80.0	70.0	32.0	35
26--2	77.0	69.0	29.0	30	26--3	76.0	69.0	28.0	30
Average:	92.81	78.39	31.72	77.11		89.33	77.56	30.84	77.11

*The first number indicates the group and the second number the animal in the group.

'Two experimental animals averaged against one control. Each experimental animal has a litter-mate control in the opposite position of this table.

rats and mice it shows that there is a decided gain in these animals. It is possible that further work will prove the difference between the growth of the treated male and the untreated male will be significant when treated biometrically.

Effect on Female Genital System

Results. The ovaries are the only organs of the female genital system which were studied. In all females which had received injections for 50-130 days the ovary was completely filled with lutein bodies, and the size of the ovary varied much as the age of the individual, because of the increasing number of lutein bodies and the more or less persistence of the old lutein bodies. The ovary of the treated female of 150 days of age would have a diameter approximately three times that of its normal control. Between the ages of 20 and 65 days the lutein bodies were in the forming process until at the age of 65 days they were well formed, the ova in most of them were entirely gone, and they were nearing the size of the larger bodies in the ovary of the female of 150 days of age. Very few ova were released from the ovaries of the treated females as determined by microscopical examination and also by the number of treated females which became pregnant during the time of the experiment. The ovum was found to be hemmed in the follicle by lutein cells where

it underwent atresia or granular disintegration and absorption and disappeared. Evans (1924) and Johnson and Sayles (1929) found the same things in the rat ovary; and Walker (1925), in the ovary of the hen. The female which had been treated as short a period as ten days shows the starting of the formation of lutein bodies in the ovary with some of the ova entirely surrounded with lutein cells.

Four pregnancies were produced in the experimental females during the experiment with a total of three mice born and eight embryos taken in the uteri at the time of autopsy, four of which were being resorbed in the uteri of one of the females and the other four apparently normal in the uteri of another female. In the control females there were nine normal pregnancies and two abortions.

Discussion. The formation of lutein bodies in the ovary has been noted by many workers as a result of anterior pituitary extract injections, and this work extends the potency of a lutein forming principle in the anterior pituitary to the albino female mouse. The process of lutein formation is a gradual one and starts taking place as early as 10 days after the starting of injections which places the animal at the age of 30 days. At the age of 30 days most of the large follicles have a strong wall of lutein tissue formed around the inner part of stratum cells to such an extent that the rupture of the follicle would be dif-

ficult, and at the age of 45 days the process has extended greatly so that it is rare to see a large follicle which has escaped the formation of lutein cells. The age at which the average female reaches maturity is about 45 days. If the female has received pituitary injections from the age of 20 days to the age of 45 days the follicles in her ovaries will be greatly hindered from rupturing by the hemming in wall of lutein cells which has formed during the period of injections, and in this manner she may be considered practically a sterile female. With the increased number of injections and an increase in age it is indeed a rare thing to find a ripe follicle in the ovary which is not filled with lutein cells and which is apparently normal and ready to rupture from the ovary. That normal follicles do occur and that fertilization takes place is proved by the fact of pregnancy among the experimental females of the group. This is also proved by the work of Johnson and Sayles (1929).

Effect on Male Genital System

Results. The size of the testes was not greatly affected by the injection of extract of anterior pituitary into male albino mice as will be noticed by the examination of Table VIII. In some instances the experimental testis

TABLE VIII. RELATIVE TESTES VOLUMES IN CUBIC MILLIMETERS

EXPERIMENTAL ANIMALS				CONTROL ANIMALS			
Animal*	Right Testis	Left Testis	Age	Animal	Right Testis	Left Testis	Age
2---2	122.50	122.50	90	2---5	126.00	84.00	90
2---3	110.25	122.50	150	2---6	200.00	160.00	150
3---3	43.75	30.00	80	3---6	133.00	141.26	80
6---3	84.00	91.00	150	6---6	134.55	117.00	150
11--1	135.00	121.50	72	11--2	168.75	168.75	72
13--4	70.00	70.00	62	13--7	168.75	168.75	62
16--2	169.20	154.60	100	16--7	109.20	108.00	100
16--3	150.75	120.60	105	16--9	157.50	145.80	105
16--4	77.00	77.00	44	16--6	96.00	96.00	44
16--5	176.25	147.00	120	16--8	160.95	169.20	120
18--1	141.75	126.00	100	18--4	148.00	162.90	100
18--2	175.20	135.00	90	18--5	168.80	152.30	90
20--1	109.37	109.37	60	20--5	131.63	122.85	60
21--3	176.25	168.75	55	21--5	157.50	148.05	55
22--2	96.00	95.60	50	22--4	84.00	67.38	50
23--2	110.00	77.00	45	23--4	96.00	77.00	45
24--1	104.00	91.00	40	24--2	104.00	117.00	40
25-1&2'	92.50	84.10	35	25--3	126.00	57.75	35
26--2	75.30	67.38	30	26--3	57.75	61.25	30
Average:	116.793	105.837	77.8 days		133.073	122.381	77.8 days

*The first number indicates the group and the second number the animal in the group.

'An average for 1 and 2 is given as there was only one control for these two experimental animals. Each experimental animal has a control in the corresponding opposite position; of same litter; of same autopsy date.

was larger than the control testis, but the average taken for the entire group shows that there is a slight retardation in the size of the testis of the treated animal. There is a high degree of sterility produced in the male mice by the injections as shown by the fact that only one fertilization was produced in 222 days compared with the normal of one fertilization in every 46.9 days (Table IX). During the course of the experiment there was only one fertilization accomplished by the treated males and in that single case five embryos were aborted at half term, all of which appeared normal upon macroscopical examination.

Upon removal of the testes of those animals which had been injected for about 100 days a macroscopical examination brought to notice a rather hard calcareous deposit in the walls of the testes. By probing with needles this shell or calcareous appearing deposit resembled the very thin shell often found on an abnormally thin shelled hen's egg. As it was apparently laid down in the membrane it was impossible to pick off pieces of the shell as from a hen's egg. No abnormalities of any of the other parts of the male genital system were noticed at the time of autopsy.

In examining the sections of those testes which had the calcareous-like deposit in the membranes at the time of autopsy it was noticed that the membranes had areas which had different staining properties and which occupied only a

portion of the membranes, starting in the inner layer and working outward. In the most advanced stages of such deposits all of the nuclei seemed to have disappeared, leaving only the fibrous lavender stained content inside the outer cellular sheath.

Further examination of the testes revealed that a change had taken place in the testes of those animals which had received injections for about 80-130 days. Before the age of 100 days not much change takes place in the histology of the testis although two individuals exhibited abnormal testes as early as the age of 62 and 80 days, respectively. There is a probable physiological change which takes place before the age of 62 or 80 days as is shown by the fact that treated males will breed but very little at any age (Table IX), although the normal male is fertile as early as 45 days of age. Among those changes which take place in the testis the most prominent is the increased amoung of interstitial tissue in the testes of those animals which have been injected from 42-130 days. The variation is great in different individuals and does not seem to correlate with the age of the individual or the dosage given although the doses used in this experiment did not vary after the animal had attained the age which would allow 0.5 cc. to be given, that age being about 40-45 days.

TABLE IX. PREGNANCY OCCURRING IN CONTROL FEMALES MATED WITH NORMAL AND WITH ANTERIOR PITUITARY TREATED MALES

WITH NORMAL MALES				WITH ANTERIOR PITUITARY TREATED MALES		
Saline* injected females	Age in days when with normal males	Number of days	Times pregnant	Age in days when with A.P. injected males	Number of days	Times pregnant with A. P. injected males
1---1	45---115	70	2	----	--	---
2---4	45---150	105	3	----	--	---
3---4	45--- 80	35	0	----	--	---
6---4	45--- 92	47	0	92----120	28	0
6---5	45--- 92	47	0	----	--	---
12--3	45--- 62	17	1	62----149	87	1**
13--5	45--- 57	12	1	57----110	53	0
14--2	45---120	75	1	----	--	---
17--6	45--- 68	23	0	68---- 95	27	0
18--6	45--- 63	18	1***	63---- 90	27	0
20--7	45--- 65	20	1	----	--	---
Average number of days for each pregnancy		46.9			222	

*The first number indicates the group and the second number the animal in the group.

**Five embryos aborted at half term.

***One embryo aborted at about sixteen days.

In accordance with the increase in the amount of interstitial tissue in some of the testes there is often a reduction in the number of seminiferous tubules to half or less than half the number found in the normal control testis. In one testis of one individual (Group 3, animal 3) injected 60 days, the number of tubules was greatly reduced and those tubules which were present did not have any sign of spermatogenesis but were filled with what appeared to be a hard colloid. Between the tubules the testis was filled with tissue which was not interstitial in nature, but rather fibrous connective tissue. In the section of some of the testes there are some tubules which appear to have a great mixture of cells and stages of spermatogenesis and which do not have a distinct separation from the interstitial tissue surrounding them. In no instance were the tubules of a whole testis found to have this relationship with the surrounding tissue or the unbalance of spermatogenesis. In some of the males which had received injections of the pituitary extract for over 100 days no change was found in the testes, and in all cases when the injections had not extended to at least 42 days (age of 62 days) the testes appeared perfectly normal with normal spermatogenesis. Spermatogenesis was not noticeably accelerated in the younger and immature animals.

The prostate glands did not exhibit as much change as the testes, and the only change which did occur in the prostates was a variation in the amount of colloid formed. There was no definite correlation with age and the amount of colloid formed, but those which showed a colloid formation were among the older animals. Colloid was found in the control animals, but not quite so extended or frequent as in the treated animals. There was also noted among the treated animals an apparent breaking down in the cells which lined the lumen of the tubules. This was found in the control animals but not to such a great extent.

Discussion. Engle (1929) found that the injections of the anterior pituitary hormone from the urine of pregnant women caused an acceleration in the growth of the testis of the immature rat. He also found a decided increase in the amount of interstitial tissue in the testes of treated animals and varying degrees in the destruction of the seminal epithelium besides a marked hypertrophy in the rest of the sex apparatus of the male. In my work I did not find a marked hypertrophy in the sex apparatus of the male mouse nor did I find an acceleration in the growth of the testis of the immature animal. I do agree with Engle in the finding of a marked increase in the interstitial tissue of those animals which had received injections for a considerable time, and in the finding of varying amounts of

destruction of the seminal epithelium. Engle does not mention sterility in rats as a result of such treatment. Some hypertrophy was noted in a few of the prostate glands of experimental males although no measurements were taken at time of autopsy to determine such a difference.

Johnson and Sayles (1929) found some but not complete sterility in male rats after extensive treatment with anterior pituitary extract. Evans and Simpson (1928) found a lack of sex interest in rats when treated with anterior pituitary extract, although they did not make a correlation between the length of treatment, size of dose and degree of loss of such sex interest. Zondek and Aschheim (1928) found a change in the testes of the male treated with the anterior pituitary hormone prepared from the urine of pregnant women but they did not feel that they had enough evidence to publish their findings.

From the results which I have found and from the results of other workers it appears there must be several factors in the anterior lobe of the pituitary. P. E. Smith (1927), P. E. Smith and Engle (1927), Riddle and Flemion (1928) and others have established the presence of a follicle forming principle in the anterior pituitary, and Evans (1924), Walker (1925), Johnson and Sayles (1929) and others (including this experiment) have established the

presence of a lutein stimulating principle in the anterior pituitary. The apparently abnormal conditions which I have obtained in the male genital system and with some corresponding results found in rats by Engle (1929) suggest that the principles of the anterior pituitary which stimulate the female genital system may inhibit or pervert the normal function and development of the male genital system. It appears that this adverse effect may be manifest in the early life of the male only through the lack of proper function of the genital system but in later life not only through the lack of function but also through the rise of abnormalities in the testes and prostates.

The lack of functioning in the experimental males is evident since only one fertilization was produced by one of five experimental males in a total period of 222 days spent with normal females. The male concerned had received anterior pituitary injections for about 115 days when the fertilization took place. The resulting embryos of this copulation were aborted at half term (5 embryos) which may or may not be the result of fertilization by an experimental male. The embryos appeared normal to a macroscopical examination. The testes of the male producing the fertilization had some increase in the interstitial tissue over the normal control, and in only an occasional tubule did spermatogenesis appear abnormal. Sperms were found free in the

lumen of some of the tubules as in the lumen of tubules of other experimental males. The testes were enclosed in extra thick membranes and were hard at autopsy.

Sterility among the treated males may also have been produced by a physiological action of the principles of the anterior pituitary on the spermatozoa which rendered them incapable of entering the ova even after they had been successfully placed in the vagina of the female, or incapability of producing normal segmentation if they were able to gain entrance to the egg. There may also be other ways in which the acting principles of the anterior pituitary affect the male genital system which this and other experiments have not brought to light. It is certain, however, that these principles have an effect on the male genital system but it is not proved in what manner or through what avenues the principles act to cause an effect.

CONCLUSIONS

The following conclusions may be drawn from these experiments:

1. That the extract of the anterior pituitary injected into albino mice for a period of 130 days (from 20-150 days of life) caused an increase in growth which was correspondingly greater in the female than in the males,

and that the increased growth which took place was not localized in any organ or system but was general over the entire body.

2. That females grew more while pregnant than during a corresponding period of non-pregnancy and that this growth was possibly brought about by increased function of the anterior pituitary during pregnancy, or less probably by the decrease in the follicular hormone during pregnancy, or by a combination of these two methods.

3. That there was a great increase in the size of the treated ovary through the formation and persistence of lutein bodies and that very few follicles reached maturity due to the hemming in of the ovum and the filling of the follicle by lutein cells, causing the ovum to degenerate in the follicle, leading to a high degree of sterility in female mice.

4. That there was an effect on the male genital system manifested (a) by different degrees of excess formation of interstitial tissue, (b) by a hardening of the wall of the testis in those animals which have received the injections for about 60 days or over, (c) by varying degrees in the destruction of the seminal epithelium, (d) by a physiological effect which cannot be seen with the aid of a microscope, (e) by an indication of a surplus of colloid formation in

the prostate gland, (f) and by the fact that these conditions are associated with an exceedingly high degree of sterility in male mice.

ACKNOWLEDGMENT

I wish to express my appreciation to Dr. George E. Johnson for his unceasing interest and aid in the experimental work and writing of this paper and for the suggesting of the original problem.

LITERATURE CITED

Aldrich, T. B.
1912. Feeding Young Pups the Anterior Lobe of the Pituitary Gland. Amer. Jour. Physiol., 30:352.

Allen, B. M.
1916. Extirpation Experiments in Rana pipiens Larvae. Science, N.S., 44:755-757.

Crafts, J. G., and Flower, F.
1925. Preservation of Fertility in the Male Rat after Extensive Treatment with Anterior Hypophyseal Fluid. Anat. Rec., 29:381.

Crowe, S. J., Cushing, Harvey; Homans, John
1910. Experimental Hypophysectomy. Johns Hopkins Hosp. Bull., 21:127-169.

Engle, E. T.
1928a. The Role of the Anterior Pituitary in Compensatory Ovarian Hypertrophy. Anat. Rec., 37:275-287.

1928b. The Effect of Daily Transplants of the Anterior Pituitary from Gonadectomized Rats on Immature Test Animals. Amer. Jour. Physiol., 88:101-106.

1929. The Response of the Male Genital System to Treatment with Urine from Pregnant Women and from Men. Anat. Rec. (In Press)

Evans, H. M.
1924. The Function of the Anterior Hypophysis. The J. B. Lippincott Co., Philadelphia.

Evans, H. M. and Long, J. A.
1921a. Effects of Feeding Anterior Lobe of Hypophysis on the Oestrous Cycle of the Rat. Anat. Rec., 21:62.

1921b. The Effect of Anterior Lobe Administered Intraperitoneally Upon the Growth and Maturity and the Oestrous Cycles of the Rat. Anat. Rec., 21:62.

Evans, H. M. and Long, J. A.
1922. Characteristic Effect upon Growth, Oestrous and Ovulation Induced by the Intraperitoneal Administration of Fresh Anterior Hypophysis Substance. Proc. Nat'l Acad. Sci. Wash., 8:38-39.

Evans, H. M. and Simpson, M. E.
1926. Effects of the Anterior Hypophyseal Extracts on the Male. Anat. Rec., 25:36-37.

1928. Antagonism of the Growth and Sex Hormones of the Anterior Pituitary. Jour. Am. Med. Assoc., 91, November 3, 1928.

Exner, A.
1910. Über Hypophysentransplantationen und die Wirkung dieser Experimentellen Hypersekretion. Deutsch. Zeitschrift f. Chir., 107:172-182.

Franchini, G.
1910. Die Funktion der Hypophyse und die Wirkungen der Injektion ihres Extraktes bei Tieren. Berlin klin. Wchnschr., 47:613-617.

Hoskins and Hoskins
1920. Inter-relation of Thyroid and Hypophysis in the Growth and Development of the Frog Larvae. Endocrinology, 4:1-32.

Iscovesco, H.
1913. La Physiologie du lobe Anterieur de l'hypophyse. Le Lipoide du Lobe Anterior. (Paris), C. R. Soc. Biol., 75:450-451.

Johnson, G. E. and Sayles, E. D.
1929. The Effects of Daily Injections of Bovine Anterior Pituitary Extract Upon the Developing Albino Rat. Physiol. Zool., 2:285-301.

Klinger, R.
1919. Versuche ueber den Einfluss der Hypophyse sur das Wachstum. Pflüger's Arch., 177:232-238.

Lewis, D. D., and Miller, J. L.
1913. The Relation of the Hypophysis to Growth and the Effect of Feeding Anterior and Posterior Lobe. Arch. Int. Med., 12:137.

Marinus, C. J.
1919. Effect of Feeding Pars Tuberalis and Pars Anterior Proprior of Bovine Pituitary Glands upon the Early Development of the White Rat. Amer. Jour. Physiol., 49:238-247.

Mitchell, J. B.
1928. Hypophysectomy in the Brown Leghorn Fowl. Anat. Rec., 41. (Abstract)

Paulesco, N. C.
1908. L'Hypophyse du Cerveau. Vigot Freres, Eds. Paris.

Putnam, T., Teel, H. M. and Benedict, E. B.
1928. The Preparation of a Sterile Active Extract of the Anterior Pituitary with Some Notes on Its Effects. Amer. Jour. Physiol., 84:157-164.

Renon et Delille
1908. Effects des extracts d'hypophyse, thyroids, suprarenal, ovaris, en inj. intraperitoneals chez de Lapin (Simples et combinees). (Paris), C. R. Soc. Biol., 65:494-501.

Riddle, Oscar and Flemion, Florence
1928. Studies on the Physiology of Reproduction in Birds. XXVI. The Role of the Anterior Pituitary in Hastening Sexual Maturity in Ring Doves. Amer. Jour. Physiol., 87:110-123.

Schafer, E. A.
1909. Croonian Lecture. June 10. Proc. Roy. Soc. Ser. B., 81:442-467. (Same as below)

1911. Die Functionen des Gehirnanhanges (Hypophysi-Cerebri). Berner Universitatschriften. H. 3.

Sisson, W. R., and Broyles, E. N.
1921. The Influence of the Anterior Lobe of the Hypophysis upon the Development of the Albino Rat. Johns Hopkins Hosp. Bull., 32:22-30.

Smith, C. S.
1923. The Alleged Effects of Body Growth and Gonad Development of Feeding Pituitary Gland Substance to Normal Rats. Amer. Jour. Physiol., 65:277-281.

Smith, P. E.
1916. Experimental Ablation of the Hypophysis in the Frog Embryo. Science, N. S., 49:280-282.

1926. Ablation and Transplantation of the Hypophysis in the White Rat. Anat. Rec., 32:221.

1927a. The Experimental Feeding of Fresh Anterior Pituitary Substance to the Hypophysectomized Rats. Amer. Jour. Physiol., 81:20-26.

1927b. The Induction of Precocious Sexual Maturity by Pituitary Homeotransplants. Amer. Jour. Physiol., 80:114-125.

Smith, P. E. and Engle, E. T.
1927a. The Induction of Precocious Sexual Maturity in the Mouse by Daily Homo- and Hetero-transplants of Anterior Pituitary Substance. Proc. Soc. Expt. Biol and Med., 24:561-562.

1927b. The Role of the Anterior Pituitary in the Development and Regulation of the Genital System. Amer. Jour. Anat., 40:160-215.

Smith, P. E. and Foster, G. L.
1926. The Effect of Hypophysectomy on Metabolism in Rats. Jour. Amer. Med. Assoc., 87:2151-2153.

Teel, H. M.
1926a. The Effects of Injecting Anterior Hypophyseal Fluid on the Production of Placentomata in Rats. Amer. Jour. Physiol., 79:184.

1926b. Invariable production of Deciduomata in the Virgin Rat with Anterior Hypophyseal Fluid. Anat. Rec., 32:243.

Uhlenhuth, E.
1919. Experimental Production of Giantism by Feeding Anterior Lobe of the Hypophysis. Jour. Gen. Physiol., 3:317-347.

Walker, A. T.
1925. An Inhibition in Ovulation in the Fowl by the Intraperitoneal Administration of Fresh Anterior Hypophyseal Substance. Amer. Jour. Physiol., 74:249-256.

Zondek, B. and Aschheim, S.
1928. Das Hormone des Hypophysenvorderlappens. Klin. Wchnschr., 7:831-835.