THE RELATION OF THE HYPOPHYSIS TO THE SEX GLANDS

BY

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INTRODUCTION.

A casual reading of the literature that follows shows the wide divergence of results on the relation of the hypophysis to the sex glands. The results of work of Herbert M. Evans, on the effects produced by the injection of extracts of the anterior lobe of the hypophysis on the oestrus cycle and on ovulation by the production of atretic changes in the ovary along with stimulation of the formation of corpus lutea, were so unexpected that it was thought advisable to repeat the work with pituitary extracts upon which nitrogen analyses had been made. It was also thought that another series of animals should be injected with some other glandular extract in order to determine whether or not the results of Evans were specific for the pituitary gland, or due to some protein toxic effect.

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Introduction.

Part 2.

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1. Effects of pituitary injection on ovary and

The Pituitary body (Hypophysis cerebri) is a small organ located in the sella turcica of the brain. It is divided into a posterior part of nervous origin, and an anterior part consisting chiefly of glandular epithelium. Both lobes are contained in a common fibrous sheath.

The anterior and posterior lobes, though closely associated, seem to have entirely different functions, as might be expected from their different embryonic origin. Since we are not concerned with posterior lobe, no further mention will be made of it.

The discovery, that in the human subject, enlargement of the anterior lobe is associated with increase of growth, expecially of the skeleton, led to attempts to determine experimentally, whether such an effect would be caused by an increase of anterior lobe substance in animals by implantation, or administration by mouth, or injected paraenterally.

Scientific interest was not aroused in the pituitary body until Marie (1886) called attention to the relation of diseases of the pituitary body and acromeglia. Marie thought acromegalia was due to a hypofunction of the gland. So Horsley in the same year endeavored to produce the disease by removal of the gland, but it was not until Paulesco (1907) perfected the operative technique, were any definite results obtained.

Paulesco was the first to state definitely that complete removal is in every case fatal. Most of the hypophysectomized dogs died within two to three days. Harvey Cushing and L. R. Lewis (1909) restricting most of their work to dogs, confirmed the results of Paulesco. Symptoms of complete removal are also described by Cushing as follows: "On the day after the operation the animal usually appears normal with fair appetite. Gradually it becomes lethargic and refuses food. Later the respiration becomes slowed, the pulse both slow and feeble, the musculature limp. Finally after about forty eight hours the animal becomes comatose and dies in this condition." This condition has become known as cachexia hypophysepriva. Blair Bell (1917) obtained results somewhat similar to those of Cushing; but failed to get the specific cachexia. All of Bell's dogs died within thirty six hours following removal of the entire gland. Removal of the anterior lobe alone resulted in death within a few hours. Partial removal of the anterior lobe produced no observable increase in weight. In three out of five cases the uterus and ovaries were distinctly atrophied, atrophy occuring first in the muscular coat, followed by atrophy in the endometrium. The ovary was shrunken in size, the Graafian follicles degenerated, while the ova and epithelial contents (granulosa) tended to dis-

appear. Similiar results have been obtained by Bidel, Houssay and Dott, while Dandy and Reichert (1925) using the temporal approach failed to get any symptoms whatever from the removal of the hypophysis. The fact that the entire gland was removed was ascertained at autopsy by serial sections through that region.

Many feeding experiments of pituitary glands have been performed from 1905 on with widely different results. Johnson and Thompson (1905) noticed a dimunition in weight of dogs fed of sheeps pituitary, as did Aldrich (1912). Their results, however, were not confirmed by Oswald (1902) who failed to get a loss of weight.

Schafer (1912) in a series of young rats fed on anterior lobe substance found very little difference between the pituitary fed, and the control animal during the first six weeks, the average weight being forty four grams. At the end of three months, however, a distinct difference was noticeable, the average weight of the pituitary-fed animals being one hundred fifty grams and the controls being one hundred thirty one grams.

E. Goetch (1916) by feeding powdered extract to rats three and one half to four weeks old reported stimulating effects in forty two days, both in growth and sexual development. C. J. Marinus (1919) fed one-sixth lobe of hypophysis to rats two to three weeks old. After twelve

weeks of feeding all the famales had give birth to at least one litter of young, or were in some stage of pregnancy. In the female the reproductive organs were well developed, and in the males the testes weighed nearly twice as much as the controls of the same age. Sisson and Broyles (1921) in a series of sixty eight rats from three to ten weeks old found no change in normal development as a result of feeding the dessicated powder of the anterior lobe of hypophysis of calves. The ovaries showed evidence of active ovulation; but no corpora lutea. The uterine mucosa was flat, showing no evidence of hyperplasia. In two series of carefully controlled experiments on albino rats C. S. Smith (1923) reported no definite effects produced by feeding anterior lobe of the hypophysis. N. M. Dott (1923), however, reports unmistakeable evidence of acceleration of growth, expecially of osseous development of both kittens and puppies as a result of feeding anterior lobe substance, however, relatively large amounts were required to obtain these results. P. E. Smith (1927) reports results similiar to these of C. S. Smith.

By extracting the anterior lobe of the hypophysis with alcohol-ether T. B. Robertson (1916) obtained an extract he named tethelin. He claimed this extract contains the growth promoting autacoid of the anterior lobe.

By a statistical method using a large number of animals he attempted to show its growth accelerating effect on white mice, however, there was only a ten per cent difference between the treated and the control animals. Drummond and Cannon (1921) failed to get similiar results, they state tetelin is not a pure compound; but a very impure mixture of lipoids and that it was impossible to obtain a pure product by the method used. Futher they state that Robertson was biased in his selection for control mice for comparison.

H. M. Evans (1927) by injecting an alkaline extract of the anterior lobe of the hypophysis into white female rats intra-peritoneally, beginning on the fourteenth to the twenty first day of life, was able to produce a true overgrowth of both skeleton and visera with the exception of the reproductive system(uterus and oviduct) which remained infantile. In a series of thirty eight normal rats ovulation began on about the fifteith day of life, ovulation continuing every four to six days. The hypophysis fed rats usually ovulated only four or six times; or not at all. But some ovulated seven or eight times, with greater periods between cycles (four to twenty nine days). The age in days of the initial ovulation was much greater in the hypophysis injected rats. Some of them did not

ovulate until the one hundredth day of life. A few ovulated on about the fiftieth day of life. The delay of ovulation was presumably due to some toxic action of hypophysis extract on the ovary. Instead of follicular healing through death of the granulosa and hypertrophy of the thecal cells, the granulosa has taken on growth changes and corpora lutea formed imprisoning the normal ova. Growth and maturity of the ova was also prevented. There seemed to be a specific stimulation of luteous tissue. Neither growth nor ovarian effects were obtained if the proteins of this extract had been coagulated by heat or alcohol and the coagulum injected. If the concentration of the alcohol added never rose above fifty per cent and the precipitated proteins were removed, the fluid gave typical ovulation effects; but no growth stimulation.

A. J. Walker (1925) found the injection of an extract of the anterior lobe of the hypophysis in the white Leghorn hen produced degenerative changes in the ovary. The ovaries of the injected fowls were smaller and more nodular than the normal. There was a liquefaction and an invasion of the yolk in all that had attained a diameter of seven millimeters. This degeneration prevented the formation of yolk of larger size. These degenerative changes were not due to handling as

controls were injected with Locke's solution and not due to protein absorption as muscle extract was also used. The inhibition was not due to a systemic toxic effect as the fowls remained in excellent health and gained in weight. Pearl, R. and Surface, F. M. (1915) injected into the abdominal cavity of hens in a resting condition one to two grams of the dried extract of the anterior lobe of the hypophysis and found these injections did not stimulate ovulation.

The grafting of the anterior lobe of the hypophysis has met with many difficulties and rarely if ever been surmounted. The most successful up to the time of the daily homeo- and hetero transplants of P. E. Smith (1927) were those of Crowe, Cushing, Homans (1909) who in seven cases out of nine succeeded in prolonging the life of their hypophysectomized dogs (which usually died within forty eight hours without treatment) from ten to twenty-six days. Some of the animals were eventually sacrificed to examine the condition of the transplant, which was found to show active anterior cells. The next report of successful transplants was that of P. E. Smith (1927) who by injecting homeotransplants intramuscularly in female white rats beginning on the fourteenth day of life was able to obtain sexual maturity as early as the weaning date. Four to nine transplants

invariably caused complete establishment of the vaginal canal, a uterine hyperaemia, oestrus, formation of large follicles, and corpera lutea in the ovary. Autopsy immediately after opening of the vaginal canal showed large follicles, but no corpera lutea, while delaying the autopsy for a few hours invariably showed the presence of corpora lutea. General body development did not take place. Sexual maturity was brought about even sooner in the mouse by both hetero- and homo- transplants (Smith and Engle 1927). Similiar results have been reported with mice by Zondek and Asheim (1927). They state the anterior lobe contains a homone whose specific function is to start folliculation in the ovary. This process once started leads in the normal manner to ovulation, corpus lutea formation, and generation of the ovarian hormone.

THE OVARY.

That the ovary is concerned with sexual characteristics has been known for a long time. Knauer (1900) was probably the first to produce definite experimental evidence that the ovary was concerned with the phenomena of oestrus and that the results of spaying an animal could partly be overcome by an ovarian graft. Atrophy of the uterus and tubes following castration, however, has been known for a long time.

A striking point in the early experimental work on the ovary was that most of the feeding and injections of the glands was done on humans and with the exception of Knaeur very little animal experimental work was preformed. Landau (1896) at about the same time as Knaeur began to give dried ovaries by mouth for relieving symptoms of the climateric, and for those of double oophorectomy in younger women. These results were repeated by Maenzer (1903) with good results.

The ovary contains the Graafian follicles with their ova, follicular epithelium, and liquor folliculi and the corpora luteum. These are embedded in a highly vascular stroma formed of a peculiar connective tissue, firm in texture and containing numerous spindle shaped cells. In most animals there are present in the stroma, cells of a different appearence from the ordinary stroma cells. These have been named the interstitial cells and like the interstitial cells of the testes they are regarded as a source of internal secretion that regulates the development of the secondary sexual characteristics. The cells of the Graafian follicles, liquor folliculi, and the corpora lutea also play an important part in the endocrine function of the organ.

The Graafian follicles are derived from the epithelium of the germinal ridge; this epithelium grows

into the substance of the ovary in the form of cords that that presently break up into cell nests, each of which contains an ovum differentiated from the germinal epithelium. The surrounding cells proliferate and form two layers that soon become multiple. The stroma of the ovary is differentiated around each follicle into two layers, termed the external and internal thecae. Those interna is very vascular and contains besides the ordinary stroma cells of connective tissue origin, large epithelium like cells, known as thecae cells.

With the approach of sexual maturity the Graafian follicles enlarge partly by multiplication of the epithelial cells and partly by formation of liquor folliculi within them. With rupture of the follicle the ova is swept out with the liquor folliculi leaving behind in the remaining epithelial cells, which form a layer several cells thick lining the follicular wall and termed the stratum granulosa. After rupture of the follicle very remarkable changes take place within it leading to the formation of the corpus lutea.

There are two schools of thought as to the origin of the cells of the corpus luteum. It is said Von Baer first stated the corpus luteum is derived from the theca interna of the Graafian follicle and that Bischoff (1842) first discarded this view in favor of the menbrana granu-

losa as the site of origin. The first investigation was done before the days of Schleiden and Shwann's enunciation of the cell theory and the other later when histology was studied by means of needles and pincetts rather than sections.

The studies of His (1865) led to the complete formulation of the view that corpus luteum is formed from the theca interna of the Graafian follicle. The chief arguments in favor of this view are that: first, the membrana granulosa of the large follicles is often degenerated and believed to be cast off at the time of rupture: second, as the Graafian follicle ripens, the cells of the theca interna show marked changes- they swell in volume, become rounded and acquire yellowish granules: and third, such follicles that do not rupture loose their granulosa by degeneration, become obliterated by proliferation of the theca interna, and in this process of atresia attain a resemblance to the corpus luteum, a fact that should be considered in studying atretic follicles.

None of the contributions disagreeing with this view in favor of the granulosa origin of the lutean cells were of all convincing until the appearence in 1895 and 1896 of Sorbota's first researches. He stated the problem should be studied from a series of specimens gathered at known periods after rupture of the follicle in order to avoid confusion with atresia or other irrevelant

processes.

According to the second theory the menbrana granulosa becomes greatly thickened by enlargement of the cells (with or with their multiplication) and deposition within them of a yellowish lipodial material. They now resemble the epithelium of a secreting gland and collectively form the spheroidal or oval mass known as the corpus luteum. Very soon the mass becomes vascularized by the ingrowth from the theca interna of stroma tissue containing blood vessels; these ingrowths are accompained by the large theca cells already mentioned as derived from the interstitial cells. Utimately they are transformed in the lipoid containing cells and become indistinguishable from the original cells of the corpus luteum which are derived from the membrana granulosa. The strands of luteal cells and blood vessels converge toward a scar like hilum which is formed at the surface of the ovary at the place where the ovum was extruded.

If the ovum remains unfertilized the corpus ceases relatively early any futher development and begins to undergo retrogressive changes and is termed a corpus luteum spurium. If the ovum becomes fertilized and fixed in the uterus the corpus luteum undergoes further development appearing as a globular mass yellow in color with strands of cells alternating with cap-

capillaries and is termed a corpus luteum vera.

OESTRUS.

It has long been known that sexual reproduction takes place when the female is in a physiological condition known as oestrus. While in some animals oestrus is evident either by peculiarities of behavior or other easily recognized signs, in others this is not true. To the latter belong such animals as the rat. This animal is scientific importance on account of its avalibility for scientific work.

The idea of the histological examination of the vaginal smears to determine the changes going on in the cellular content of the vagina of the guinea pig during the various phases of the sexual cycle belongs to Stockard and Papanicolaou (1917). These men discovered that definite, characteristic changes occur during each phase of the oestrual cycle and ovulation as well. Ovulation, of course, occurs spontaneously in all animals except in the cat and rabbit in which species copulation is necessary to bring about a rupture of the Graafian follicle.

Upon the basis of Stockard and Papanicolaou's work on the cellular changes in the vaginal content of the guinea pig during oestrus Long and Evans (1922) observed the oestral changes in the vagina of the rat. The found characteristic changes both in the cell of

the vagina as well as gross changes in the vaginal lips and mucosa.

During the interval or dioestrus pause, which constitutes about half of the entire cycle, and during which the mucosa is clear, moist, glistening, somewhat translucent, and pink, there may be withdrawn from the vaginal lumena variable quantity of thin, stringy mucous in which are entangled leucocytes and small irregularily shaped free epithelial cells. The leucocytes are usually fairly abundant and are the characteristic small polymorphouclear elements, which often have an annular mucleus. The epithelial cells are always single, never in groups.

The oestrual cycle is inagurated by the occurence of a distinct stage, (designated as stage one) and characterized by a distinct histological picture of the vaginal content, and often be detected macroscopically by the dry, opaque appearance of the surface of the vaginal mucosa, as seen by speculum. To the naked eye, however, these appearence are overlooked, but the onset of this stage is readily confirmed by a microscopic examination of a sample of the vaginal contents. A narrow speculum may be applied to a small drop of physiological saline after its withdrawl from the vagina, in the translucent jelly like mass withdrawn from the vagina leucocy-

tes have almost disappeared, but there are found, in great, numbers, small, round, nucleated epithelial cells, of uniform appearence and size. The microscopic picture is characteristic, for these cellular elements occur at no other time in the oestrus cycle. During stage one, females will usually not accept copulation, hence it is proper to recognize this as the stage normally preparatory for oestrus, called by some pro-oestrus. In some cases, however, at about the middle of the stage, and in still more cases toward the end of the stage animals will mate, but do not show oestrus excitement in the usual manner.

In stage two the macroscopic changes noted as characterizing stage one, notably the beginning swelling of the vaginal lips and the dry mucosa, are now more marked. Equally definite changes take place in the microscopic appearance of the vaginal smear. The small, nucleated, granular epithelial cells characteristic of the first stage, have been suddenly replaced by large, thin, transparent, non-nucleated scale-like elements the cornified cells. For this reason stage two may be designated as the cornified cell stage. The sample, while still scanty in amount, is opaque, whitish, and granular. There is still a singular absence of leucocytes, whose sudden disappearence was so marked a characteristic of stage one.

It is during this stage that females usually show unmistakeable signs of heat. If placed in a mating cage and not approached by the male she will manifest oestrus excitement i.e. quick darting movements, with back arched and occasional quiverings of the body. This behavior is never encountered at any other time than oestrus.

Stage three cannot be sharply separated from stage two. The histological picture in this stage is similiar to stage two, but is mainly or practically an exaggeration of the characteristics of the preceding stage. The accumulation of cornified, non-nucleated epithelial plates within the lumen of the vagina now proceeds so rapidly that easily visible masses of whitish, granular or pasty substance are seen deep in the vagina. These masses of cellular elements may, at first, be confused with the plug left in the vagina by the male; but they may be readily differentiated by microscopic examination. Besides this accumulation of cheesy substances stage three is also characterized by the fact that animals in this stage will usually no longer accept coitus.

Stage four, the metoestrum, is inagurated by the appearance in the vaginal smear of leucocytes among the cornified cells and ends with the disappearence of the latter. The leucocytes cause a softening of the granular masses seen in stage three and converts them into a sub-

stance which has a thin cheesy, creamy consistency. Before the cornified cells completely vanish from the smear, epithelial cells appear. So during a short interval all three types of cells are present. This ushers in the beginning of the dioestrus pause, which may be recognized by complete disappearence of cornified epithelial elements and leucocytes.

Long and Evans on using over three hundred females from four to six months of age and isolated from males on the weaning date, found the oestrual cycle to be from four to six days. Under nutrition has a marked tendency to prolong the oestrual cycle. So also does disturbance in feeding hours. Impairment of respiration, which is invariably produced when too many animals are allowed to lie together, has also been found to prolong the cycle.

TESTES

The essential sexual gland of the male is the testis. It is a gland with a double function. It produces sperm cells (spermatozoa), hence it is a cytogenic organ. It also produces internal secretion, hence it is an endocrine gland. The testis is made up of a collection of convuluted tubules; the seminal tubules, which are contained in a number of compartments separated by fibrous septa. The tubules present few or no branches, each one being about

five hundred millimeters long. The testis is formed in the peritoneal cavity from germinal epithelium. In most animals it leaves the abdominal cavity early in life, and comes to lie in a pouch of skin, the scrotum. In the rat, however, the inguinal canal remains patent so that the testis can be drawn into the abdominal cavity. Several seminal tubules unite to form a straight tubule, which leads by a series of communicating spaces, the rete testes, into the vasa efferenta. These vasa join to form the duct of the epididymis coiled into a mass lying at the back of the testis.

In the immature testis i.e. before sexual maturity, the seminal tubules are filled with cells having large nuclei. Some of these are the spermatogonia, the mother cells of the spermatozooa, while others form the cells of Sertoli, whose function it is to act as nurse cells to the developing spermatozoa. The actual formation of spermatozoa begins at sexual maturity, when they in turn undergo heterotype mitosis to form the spermatids.

The walls of the seminiferous tubules consist of fibroelastic tissue. They are lined with several layered epithelium, the cells representing the several stages in spermatogensis. In addition to the germ, or sex cells, the epithelium contains the sustentacular cells (Sertoli cells), to which the spermatids become attached during the process of metamorphsis into ripe spermatozoa. It

seems probable that the spermatids draw nourishment from the sustentacular cells of the work of metamorphsis, hence also known as trophocytes.

Throughout the inter-tubular connective tissue stroma are peculiar large, polygonal cells; the interstitial cells of Leydig. Each of these cells contains a large spherical nucleous, well marked nucleolus, and often a double centrosome. Mitosis is rarely observed. The cells tend to accumulate in masses in the angular spaces between three or four seminiferous tubules, with surrounding blood vessels and large lymph spaces, separating them from the tubules. The cell masses are not very vascular, indeed they are less so than most other endocrine organs. Various functions have been ascribed to the interstitial cells, especially those apparently dependant upon the internal secretion of the testis--prominently origin and maintenance of secondary characteristics and basis of sex instinct. Other cells of the testis might serve these functions, namely, the sustentacular cells or the sex-cells. However, observations on mules and cryptorchid horses, and the findings of Whitehead (1908) of a third abdominal testicle in a horse where only interstitial cells in great abundance persisted would seem to indicate these functions belong to the interstitial cells.

Methods

Preparation of Liver and Pituitary Extract

Method of H. M. Evans. (1923)

The hypophysis lobes were weighed in sterile dishes and put into forty per cent alcohol, agitated for ten minutes with a glass stiring rod, so that every portion of the gland was washed, then transferred into two changes of normal saline, drained, and again transferring into a sterile mortar where they were ground with force and speed for thirty minutes with sterile sand. The homogeneous paste was now diluted with saturated sodium bicarbonate solution in the porportion of one third of the weight of the gland, and the whole poured into large sterile contrifuge tubes and whirled for half an hour at three thousand revolutions per minute. The layers of sand, cell fragments, and pink fluid permitted the later to be decanted. The supernatant liquid was injected without filtering, in order to risk no possible absorption of the hormone during the filtration process. Each cubic centimeter of the anterior lobe extract represented slightly over two grams of the beef anterior glandular substance.

The preparation of the liver extract followed very closely the above method.

Six hundred twenty grams of fresh hog's liver was selected and washed in forty per cent ethyl alcohol, drained, washed in sterile saline and then ground in a meat

grinder, it was then transferred to a sterile ball mill and turned for one hour. The homogeneous paste was now diluted with two hundred cubic centimeters of a saturated solution of sodium bicarbonate and an equal amount of sterile saline. The whole amount was poured into sterile centrifuge tubes and whirled for half an hour at three thousand revolutions per minute. The dark red supernant liquid was placed in sterile test tubes and plugged with sterile cotton. One tenth of one per cent tri-cresol was added as a preservative and they were kept in the ice box.

The nitrogen analysis made on both the pituitary and the liver extract was done by the Koch and McMeekin method (1924).

VAGINAL SMEARS.

The most satisfactory method for obtaining the vaginal contents for smears was suggested to us by R. T. Frank (1). The smear can be made alone by holding the rat in the left hand head downward, one hind foot being held aside by the thumb and the other by the first finger. A moistened tooth pick is then inserted into the vaginal canal with the right hand care being taken not to scrape off any of the vaginal wall. The tooth pick is then withdrawn and stirred in a small drop of saline or water on a

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(1) Personal Communication.

clean glass slide. The slides are allowed to dry in air and are fixed by passing through a flame several times. They are then stained for thirty seconds with a one per cent aqueous solution of thionin and examined microscopically. The low power is usually sufficient to differentiate the stages, the characteristics of which have previously been mentioned.

After the animals were killed by ether anathethesia, the tissues were immediately removed and placed in Bouin's fluid for from six to twelve hours. They were then washed several times in fifty per cent alcohol, transferred successively into seventy, eighty, ninety five per cent and absolute alcohol, from which they were placed in cedar oil and allowed to clear. Next, they were infiltrated by paraffin by placing them in a beaker and pouring melted paraffin on them. Here they were allowed to remain for six hours care being exercised to keep the temperature of the oven as nearly constant as possible.

Sufficient melted paraffin to cover the bottom of a paper box was poured in, then, with warm forceps the tissues were removed to the box after which it was filled with paraffin. As soon as the paraffin had cooled sufficiently for the surface to become opaque, it was cooled rapidly by plunging it into cold water. With a sharp

knife the paraffin block was trimmed until it was perfectly rectangular and just a little larger than the tissue. The paraffin block was made fast to the carrying block and the sections cut serially at ten microns. A small drop of albumen fixative was placed on the slide after which it was flooded with water. Then the sections were placed on the slide and warmed to facilitate straightening of the sections. The excess water was drained off and the slide allowed to dry. The paraffin was removed from the sections by placing in xylol for ten to fifteen minutes. Next the xylol was removed by placing the slides in absolute alcohol for one minute. The slides were then passed through the alcohols (95-80-70-and 50 per cent) and stained with Delafield's hematoxylin for ten minutes, washed in water and stained for thirty seconds with eosin. They then were run back through the alcohols, cleared in cedar oil, the cedar oil dissolved in xylol and the sections mounted with balsam.

Rat 1.

Injected with pituitary extract. Sex: female.

Litter Al.

Date of birth 10/11/27. Weaned 11/11/27.

12/8/27. Initial injection of pituitary extract 1/4 C. C.

12/8/27. Weight 105 grams.

1/5/27. Animal died of peritonitis. Age three months.

Rat 4.

Control. Sex: male. Litter Al

Date of birth. 10/13/27. Weaned 11/11/27.

12/5/27. Weight 83 grams.

1/5/27. Weight 169 grams.

2/2/28. Weight 213 grams.

2/28/28. Weight 248 grams.

3/20/28. Animal killed. Weight 253 grams.

General condition: excellent.

Weight of testes 2.6 grams. Weight of kidneys 1.53 grams.

Weight of liver 7.3 grams.

Histological findings:

Tubules of the testes were shrunken probably due either to the fixing agent or to the paraffin oven. The section gave every appearence of a normal testis with normal interstitial tissue and cellular cytology.

Rat 5

Control. Sex: male Litter A1

Date of birth. 10/13/27 Weaned 11/11/27

12/5/27 Weight 96 grams.

1/5/28 Weight 169 grams.

2/28/28 Weight 248 grams.

3/20/28 Animal killed with ether anethesia. Weight 262 grams. Total body length 38 cms. Body length cms. Weight of testes 2.85 grams. Weight of kidneys 1.95 grams. General condition: excellent.

Histological findings:

The tubules were filled with spermatozoa and the mitotic figures indicated actove development of the spermatogonia. The interstitial was normal with no evidence of degeneration. There was no disarrangement of the tubules within the capscule.

Rat 6

Received liver extract. Sex: male Litter B1

Born 10/11/27 Weaned 11/11/27

12/2/27. Weight 72 grams.

12/24/27. Received initial injection.

12/14/27. Injections increased to 1/2 C. C.

1/5/28. Weight 164 grams.

1/24/28. Injections increased to 3/4 C. C.

2/2/28. Weight 214 grams.

2/6/28. Injections increased to 1 C. C.

2/13/28. Injections increased to 1 1/2 C. C. and this amount continued every other day through out the course of the experiment.

2/28/28. Weight 239 grams.

3/20/28. Weight 262 grams.

Total body length 40 cms. Body length 21 cms.

General condition: excellent.

Animal was killed with ether anesthesia.

Macroscopic findings at autopsy: normal.

Weight of testes: 2.4 grams. Weight of kidneys: 1.65 gr.

Weight of liver 8.15 grams.

Histological findings of the testes:

The tubules have shrunken greatly so that the interstitial tissue is free. Active spermatogensis is evident by the large number of spermatozoa and the mitotic

figures in the lower stages of their development. Practically a normal testis with no evidence of degeneration.

Rat 7

Received liver extract. Sex: female. Litter B1

Date of birth 10/13/27. Weaned 11/11/27

12/2/27 Weight 75 grams.

12/24/27 Initial injection of 1/4 C. C. Liver extract

12/14/27 Injections increased to 1/2 C. C.

12/20/27 Oestrus smears were begun.

1/5/28 Weight 138 grams.

1/24/28 Injections increased to 3/4 C. C.

2/13/28 Injection increased to 1 1/2 C. C. and this amount continued during the course of the experiment.

2/28/28 Weight 239 grams.

3/19/28 Animal died of peritonitis.

Total body length 35 cms. Body length 17 cms.

Weight 154 grams.

Findings at autopsy:

Abdomen markedly distended. Adhesions in the lower part of the abdominal cavity not only to the abdominal wall but also the intestines themselves. The peritoneum was greatly inflammed, and on the surface of which a purulent exudate was present. The intestines were congested giving the indications of stricture.

The oestrus cycle was normal until the last nine days of life. No tissues were removed for histological examination.

Rat 8

Received liver extract. Sex: male Litter B1

Date of birth 10/13/27. Weaned 11/11/27

12/2/27. Initial injection of 1/4 C. C.

12/5/27. Weight 70 grams.

12/14/27. Injections increased to 1/2 C. C.

1/5/28. Weight 140 grams.

1/24/28. Injections increased to 3/4 C. C.

2/6/28. Injections increased to one C. C.

2/13/28. Weight 241 grams.

2/13/28. Injections increased to 1 1/2 C. C. and this amount was continued throughout the course of the experiment.

2/28/28 Weight 208 grams.

3/12/28 Weight 241 grams.

3/12/28 Animal died of intestinal obstruction.

Findings at autopsy:

Adhesions in lower left abdominal quadrant marked intravascular clotting. Intestines engorged with blood. Emphysema on left side with slight heptization on right.

Weight of testes 2.3 grams.

Histological findings of the testes.

Active spermatogensis and mitosis present. No interstitial tissue present probably due to sectoning. Normal sustentacular cells, and in short, gave the appearence of a perfectly normal organ.

Rat 9

Received liver extract. Sex: male. Litter B1

Born 10/11/27 Weaned 11/11/27

12/2/27 Received initial injection of 1/4 C. C. of liver extract.

12/2 /27 Weight 80 grams.

12/14/27 Injections increased to 1/2 C. C.

1/5/28 Weight 150 grams.

1/24/28 Injections increased to 3/4 C. C.

2/2/28 Weight 204 grams.

2/6/28 Injections increased to 1 C. C.

2/13/28 Injections increased to 1 1/2 C. C. and this amount continued every other day through out the course of the experiment.

2/28/28 Weight 231 grams.

3/20/28 Weight 255 grams.

Total lingth of body: 40 cms. Body length: 21 cms.

General condition: excellent.

Animal was killed by ether.

Macroscopic findings at autopsy: normal.

Weight of testes: 2.63 grams. Weight of kidneys 1.93 gr. Weight of liver 10.05 grams.

Histological findings:

The testes showed active spermatogensis as evidenced by all stages in their metamorphysis. The tubules were shrunken somewhat probably due to the heat of the paraffin oven. The interstitial tissue was scarce in amount but no degenerative changes were present. The tubules were teeming with spermatozooa.

Rat 15

Received liver extract. Sex: female Litter C1

Date of birth 10/13/27 Weaned 11/26/27

12/2/27 Weight 57 grams.

12/2/27 Received initial injection of 1/4 C. C. of liver extract.

12/14/27 Injections increased to 1/2 C. C.

1/5/28 Weight 131 grams.

12/16/27 Vaginal smears were begun.

1/24/28 Injections were increased to 3/4 C. C.

2/6/28 Injections were increased to 1 C. C.

2/9/28 Weight 158 grams.

2/18/28 Injections increased to 1 1/2 C. C. and this amount continued throughout the course of the experiment.

3/19/28 Animal killed. General health: excellent.

Total body length 36 cms. Body length 17 cms. Weight 195 grams.

Weight of uterus .47 grams. Ovaries normal in appearance. Weight of kidney 1.5 grams. Weight of liver 6.65 grams.

Histological findings:

Numerous large corpera lutea present almost replacing the ovarian stroma. Some of the sections show congestion with an apparent diminution in the number of follicles.

Oestrus cycle: Regular, with four and a half to five day intervals.

Rat 16.

Received liver extract. Sex: female Litter Cl.

Date of birth 10/29/27. Weaned 11/26/27.

12/2/27 Received initial injection of 1/4 C. C. of liver extract.

12/2/27 Weight 57 grams.

12/9/27 Injections increased to 1/2 C. C.

1/5/28 Weight 124 grams.

12/16/28 Vaginal smears begun.

2/6/28 Injections increased to 3/4 C. C.

2/9/28 Weight 152 grams.

2/9/28 Injections increased to 1 C. C.

2/18/28 Injections increased to 1 1/2 C. C. and this amount injected every other day during the

course of the experiment.

3/19/28 Animal killed.

Total body length 35 cms. Body length 18 cms.

Weight 165 grams.

General health: excellent. Coat smooth and glossy.

Gross findings at autopsy:

Ovaries slightly reddened.

Weight of uterus .25 grams. Weight of liver 9.2 grams.

Weight of kidneys 2.15 grams.

Histological findings:

The ovaries were markedly congested and the blood vessels seemed dilated with extravasations into the tissue spaces especially into the corpus lutea; the cells of which seem very much smaller than normal and are not arranged in the typical manner, but vary in size. There also seem to be an abnormal proliferation of the germinal epithelium.

Oestrus cycle was regular occuring about every five days.

Rat 22.

Liver injected. Sex: female Litter E1

Date of birth 10/30/27 Weaned 11/26/27

12/2/27 Weight 53 grams.

12/2/27 Received initial injection of 1/4 C. C. of liver extract.

12/14/28 Injections increased to 1/2 C. C.

1/5/28 Weight 80 grams.

12/26/27 Vaginal smears begun.

1/24/28 Injections increased to 3/4 C. C.

2/9/28 Weight 118 grams.

2/6/28 Injections increased to 1 C. C.

3/19/28 Animal killed. Weight 129 grams.

Gross findings at autopsy:

Evidence externally of a large amount of the material being walled off which on opening the abdominal wall was found walled off by the omentum and the peritoneum. Animal apparently in good health.

Weight of uterus .3 grams. Weight of kidneys 1.44 gr. Weight of liver 6.17. Ovaries normal.

Histological findings:

Numerous follicles were present in the ovary in all stages of development. Atresia present in quite a few as evidenced by the loose cells of the granulosa free in the follicular fluid. There also seem to be some pressure on the ova that they have assumed an elongated form. Stroma though very vascular; there is no evidence of extravasation into the tissue spaces of congestion.

The oestrus cycle was regular occuring about every five days.

Rat 26.

Pituitary injected. Sex: male. Litter B1

Date of birth 11/11/27 Weaned 12/5/27

12/7/27 Weight 35 grams.

12/10/27 Initial injection of 1/4 C. C. of pituitary extract.

1/5/28 Injections increased to 1/2 C. C.

1/24/28 Injections increased to 3/4 C. C.

2/2/28 Weight 179 grams.

2/6/28 Injections increased to 1 C. C.

2/13/28 Injections increased to 1 1/2 C. C. and this amount continued throughout the course of the experiment.

2/28/28 Weight 229 grams.

3/12/28 Weight 233 grams.

3/21/28 Animal killed. Weight 246 grams.

Total body length 38 cms. Body length 18 cms.

General condition: Excellent.

Weight of testes 2.15 grams. Weight of liver 8.01 grams.

Weight of kidneys 1.53 grams.

Gross findings at autopsy:

Hard indurated area resembling a scab in the lower right abdominal wall.

Histological findings:

Practically normal number of spermatozoa present. There is an increase in the mitotic figures of the lowest stages of spermatogensis, the intermediate stages of which seem to be also affected somewhat. There is some evidence of degeneration in these stages but the section certainly does not show sterility. The interstitial tissue appears normal but the tubules appear shrunken. There is no disarrangement of the cells but merely a diminution in the sixe. The sustentacular cells appear normal.

Rat 27

Pituitary injection. Sex: male. Litter F1

Date of birth 11/11/27 Weaned 12/5/27

12/7/27 Weight 34 grams.

12/10/27 Received initial injection of 1/4 C. C. of pituitary extract.

1/5/27 Weight 95 grams.

1/5/27 Injections increased to 1/2 C. C.

1/24/28 Injections increased to 3/4 C. C.

2/2/28 Weight 172 grams.

2/6/28 Injections increased to 1 C. C.

2/13/28 Injections increased to 1 1/2 C. C. and this amount continued through out the course of the experiment.

2/28/28 Weight 214 grams.

3/12/28 Weight 230 grams.

3/21/28 Animal killed. Weight 239 grams.
Total body length 39 cms. Tail length 21 cms.
Gross findings at autopsy: Normal.
Weight of testes 2.3 grams. Weight of liver 8.51 grams.
Weight of kidneys 1.55 grams.

Indurated area in the lower left abdominal wall. Large adhesions at the ileo-caecal juncture showing evidences of past peritonitis. Rat apparently in perfect health.

Histological findings:

Intestitial tissue normal. Mitotic figures present in the spermatogonia showing active spermatogensis but there is evidence of beginning degeneration in some of the tubules although large numbers of sperm are present in others. The sustentacular cells are normal.

Rat 28.

Pituitary injected. Sex: male. Litter Fl.
Date of birth 11/11/27 Weaned 12/5/27

12/7/27 Weight 33 grams.

12/10/27 Received initial injection of 1/4 C. C. of pituitary extract.

1/5/28 Weight 93 grams.

1/5/28 Injections increased to 1/2 C. C.

1/24/28 Injections increased to 3/4 C. C.

2/2/28 Weight 169 grams.

2/6/28 Injections increased to 1 C. C.

2/13/28 Injections increased to 1 1/2 C. C. and this amount given every other day throughout the course of the experiment.

2/28/28 Weight 198 grams.

3/12/28 Animal died. Weight 200 grams.

Gross findings at autopsy:

Diarrhea present. Abdomen distended which on opening showed a large amount of yellowish exudate and also serous fluid. The entire gastro intestinal tract was held together by yellowish fibrous material. The right testicle was rather small.

Weight of testes: 1.05 grams.

Histological findings:

The tubules are normal in size but there is quite a bit of hemmoragic congestion both within the tubules and in the interstitial spaces. There is an apparent decrease in the number of spermatozoa, the intermediate stages in the metamorphsis seem particularly affected. There are however numerous sperm throughout the section.

Rat 29

Pituitary injected. Sex: male Litter Fl

Date of birth 11/11/27 Weaned 12/5/27

12/7/27 Weight 30 grams.

12/10/27 Received initial injection of 1/4 C. C. of pituitary extract.

1/5/27 Injections increased to 1/2 C. C.

1/5/27 Weight 93 grams.

1/24/27 Injections increased to 3/4 C. C.

2/28/28 Weight 169 grams.

2/6/28 Injections increased to 1 C. C.

2/13/28 Injections increased to 1 1/2 C. C. and this amount given every other day during the course of the experiment.

2/28/28 Weight 204 grams.

3/12/28 Weight 203 grams.

3/21/28 Animal killed. Weight 222 grams.

Total body length 36 cms. Body length 17 cms.

Gross findings at autopsy: Normal

Weight of testes 2.0 grams. Weight of liver 7.12 grams.

Weight of kidneys 1.22 grams.

Histological findings:

The spermatozoa are decreased in number and in certain of the tubules degeneration is evident in its early stage. The sustentacular cells have disappeared leaving the undifferentiated germ cells along the basement membrane. These changes are more evident than in

the other sections studied.

Rat 32.

Received pituitary extract. Sex: female Litter G1

Date of birth 11/11/27 Weaned 12/15/27.

12/7/27 Weight 34 grams.

12/8/27 Received 1/4 C. C. pituitary extract. Initial injection.

12/28/27 Vagina opened.

1/5/28 Weight 93 grams.

1/5/28 Injections increased to 1/2 C. C.

1/24/28 Injections increased to 3/4 C. C.

2/9/28 Weight 156 grams.

2/12/28 Injections increased to 1 C. C.

2/15/28 Injections increased to 1 1/2 C. C. and this amount continued throughout the course of the experiment.

3/2/28 Animal killed. Weight 190 grams.

Total body length 34 cms. Tail length 18 cms.

General condition: Excellent.

Weight of kidneys 1.15 grams. Weight of liver 7.5 grams.

Weight of uterus .3 grams. Ovaries normal.

Histological findings:

Marked increase in luteous tissue with very few follicles present. Those that are present however,

appear normal. Since in several cases the ova were imprisoned, it seems that the corpora lutea were formed without rupture of the follicles. There were an abnormally large number of corpora lutea and degenerated follicles present in this ovary. In the unruptured follicles the outer covering appears normal; but there is some hyperplasis of the granulosa. There is a marked increased in luteous tissue that is not normal in histological structure for the cells are arranged in a very disorderly manner. The ovarian stroma has been almost entirely replaced by luteous tissue.

The oestrual cycles were run from about ninety days. During the first forty days the cycles were regular, oestrus occuring about every five days. From the forty-fifth to the sixtieth day, however the cycle occurred at irregular intervals the length of dioestrum gradually increasing until about the seventieth day when oestrus ceased entirely.

Rat 40.

Date of birth 12/15/27 Weaned 1/20/28.

1/20/28 Weight 32 grams.

1/22/28 Received initial injection of 1/4 C. C. of liver extract.

2/2/28 Weight 70 grams.

2/7/28 Injections increased to 1/2 C. C.

2/13/28 Injections increased to 3/4 C. C.

2/15/28 Injections increased to 1 C. C.

2/15/28 Vagina opened.

2/9/28 Weight 95 grams.

3/4/28 Injections increased to 1 1/2 C. C. and this amount continued throughout the course of the experiment.

4/11/28 Weight 172 grams. Animal killed.
Total body length 35 cms. Body length 18 cms.
Uterus .5 grams. Kidneys 1.3 grams.
Liver 7.1 grams. Ovaries normal.
Gross findings at autopsy: normal.
Histological findings:

There are numerous large follicles with intact normal ova. Many small follicles are also present that do not show any evidence of degeneration. The corpus lutea and ovarian stroma are normal except for some congestion of the blood vessels that is an acute condition as the degenerative changes accompanying passive congestion are absent. The ovary is normal.

Rat 23.

Control Sex: female Litter E1.

Date of birth 10/30/27 Weaned 11/26/27.

12/8/27 Weight 63 grams.

1/5/28 Weight 80 grams.

2/2/28 Weight 153 grams.

2/28/28 Weight 181 grams.

4/3/28 Weight 198 grams. Animal killed.
Total body length 37 cms. Body length 19 cms.
Weight of uterus .65 grams. Weight of liver 5.3 grams.
Weight of kidneys 1.3 grams.
Histological findings:
Normal in every respect.
Oestrus cycle:
Regular four and half day rhythm.

Rat 10.

Control Sex: female Litter D1
Date of birth 10/13/27 Weaned 11/11/27
12/8/27 Weight 69 grams.
1/5/28 Weight 110 grams.
2/2/28 Weight 140 grams.
2/28/28 Weight 163 grams.
4/3/27 Weight 170 grams. Animal killed.
Total body length 35 cms. Body length 18 cms.
Weight of uterus .37 grams. Weight of liver 5.3 grams.
Weight of kidneys 1.3 grams.
Histological findings:
Blood has extravasated into the ovarian stroma and into the corpora lutea but there is no evidence of passive congestion i.e. degeneration and

necrosis. The follicles are normal in both number and structure.

Rat 25.

Control Sex: female Litter El.

Date of birth 10/30/27. Weaned 11/26/27.

12/8/27 Weight 63 grams.

1/5/28 Weight 152 grams.

2/2/28 Weight 140 grams.

2/28/28 Weight 155 grams.

4/3/28 Weight 168 grams. Animal killed.

Total body length 35 cms. Body length 18 cms.

Weight of uterus 4.5 grams. Weight of liver 5.3 grams.

Weight of kidneys 1.3 grams.

Histological findings:

The ovaries were very congested blood having estravasated both into the ovarian stroma and into the corpora lutea. The blood vessels also appear dilated. The follicles and the corpora lutea however are normal both in histological structure and number.

Rat 14.

Normal. Sex: female Litter Cl.

Date of birth 10/29/27. Weaned 11/26/27.

12/9/27 Weight 48 grams.

1/5/28 Weight 100 grams.

2/2/28 Weight 130 grams.

2/28/28 Weight 161 grams.

4/3/28 Weight 173 grams. Animal killed.

Total body length 34 cms. Body length 17 cms.

Weight of uterus .3 grams. Kidneys 1.3 grams.

Weight of liver 5.2 grams.

Histological findings:

Normal in every respect, The oestrus cycle: Regular five day cycle.

The Nitrogen analysis made by the Koch and McMeekin method (1924) gave the following results:

Liver extract La	543.5 mgm.	per 100 C. C.
Liver extract Lb	649 mgm.	per 100 C. C.
Liver extract Lc	203 mgm.	per 100 C. C.
Liver extract Ld	520 mgm.	per 100 C. C.
Liver extract Le	444 mgm.	per 100 C. C.
Pituitary extract Pa	295.5 mgm.	per 100 C. C.
Pituitary extract Pb	522 mgm.	per 100 C. C.
Pituitary extract Pc	444 mgm.	per 100 C. C.

DISCUSSION

The injection of pituitary extract produced in the female rat striking changes in the ovaries. The number of follicles was greatly reduced with a marked increase in luteous tissue. The follicles present were small, but were normal in their histological structure, there being no evidence of degeneration in either the theca externa or in the granulosa. Many of the corpora lutea seem to have been formed without rupture of the follicle as neither the theca externa and interna show any of the degenerative changes that follow the rupture of the follicle. The ova appear to have been caught by the rapidly proliferating granulosa which forms the lutean cells by replacing the follicular fluid. The cells of the corpora lutea were not arranged in the usual cell-cord manner; but were in numerous cases arranged in a very disorderly way. There were no large follicles filled with fluid; but, as stated above, they were small and surrounded by cells that later in the normal ovary, form the discus proligerus. No minute study of the ova themselves was made; but they appeared normal, the nuclei were intact. No maturation spindles were seen.

So far as known all of the work ever done on the injection of pituitary extracts and its effect on the

ovary, has been published in abstract form in the Anatomical Record, with the exception of that of H. M. Evans whose results were published in the Harvey Lectures, consequently very few of the detailed findings are available. The work of Evans in The Harvey Lectures agrees with the work reported here. He does not give some of the details, nor, does he discuss the causes or mechanism of his results.

The fact that the follicles are all small and that none of them contain fluid may explain the manner of formation of the corpora lutea without rupture of the follicle. As the follicles increase in size, the cells of the granulosa may hypertrophy as fast as the theca externa grown to allow room for their proliferation. The ova thus remain in situ being surrounded by the cells of the lutea. This idea is supported by the fact that cells unlike those of corpus lutea are present within the corpus itself producing an abnormal arrangement of the cells. Further, the cells are not of the usual large, clear cuboidal type, but are smaller with a cloudy cytoplasm and very darkly staining nuclei resembling the cells of the granulosa. The growth of the corpora lutea has taken place at the expence of the ovarian stroma, so that they have almost entirely replaced it.

A. T. Walker (1925) produced atresia in the ovary

of the fowl by the injection intraperitoneally of anterior pituitary extract. He gave evidence that the effects were due to the pituitary extract, and not to a general protein toxic effect, because his controls were injected with muscle extract and that it produced no changes in the ovaries. In order to make a similiar control and to determine whether or not the atresia described by Evans for the rat was a pituitary phenomena, or due to some toxic action exerted by the protein rich suspension, a liver extract was made with about the same number of milligrams of nitrogen per cubic centimeter as the pituitary extract, and injected in a similiar manner into the peritoneum. Both extracts were readily absorbed. In only one rat was any of the material found unabsorbed and walled off in the peritoneal cavity at the time of autopsy. The material when given by mouth loses its effect on the ovary was shown by Bergeim, Larson, and Fisher (1928). The ovaries of the rats injected with liver extract were normal showing none of the changes found in those injected with pituitary extract. This shows that the action was not a general protein toxic action; but that it was a pituitary effect. The results produced by the pituitary extract were not due to any products formed by putrefaction as the extract was made under sterile conditions and was kept in the

ice-box with .1 per cent tri-cresol added as a preservative.

The length and regularity of the oestrual cycle is dependant upon many factors, such as; diet, disturbance in feeding, and ventilation and general hygiene. So that to observe changes in the oestrual rhythm great care must be exercised, not only in the feeding of the animals, but also in the general hygienic conditions of the animals. The rats used in this experiment were fed an adequate diet consisting of table scraps, yellow corn, oats, fresh milk, and an abundant supply of clean fresh water.

The injection of pituitary extract was begun on the twenty eight day of life. There was no delay on the date of the opening of the vagina or in the regularity or length of the oestrual cycle until about the eighty-fifth day of life, when there was a lengthening of the oestrual pause. Complete cessation of the oestrual rhythm did not occur until after the hundredth day of life. Evans and Long (1921) in their studies on the effects of injection on the oestrous cycle of the rat found that there was a cessation of the rhythm after the rats had ovulated three or four times. With smaller doses the cycles occured at longer intervals finally stopping it altogether. Evans began his injections on the fourteenth

day of life while our initial injection was not until the twenty eight day of life. No nitrogen analyses of Evan's extracts have been published. So we cannot say the difference in the effects produced by our extract and his was due to a difference in quantity of protein. The extract prepared by Evans represented slightly more than two grams of fresh anterior pituitary of the ox while our extract represented only about a gram of the gland, per cubic centimeter of the extract. Another difference that may account for our delayed effect of the inhibition of ovulation was that our injections were begun at a much later date than those of Evans and Long. Since at the earlier date the cell nests are just beginning to form the cell cords that have grown down from the germinal epithelium, therefore they might be much more sensitive to injections at that time.

From the changes that occur in the ovary with the cessation of the oestrus cycle after the injection of the pituitary extract, it seems that the ovulation and the maturation of the follicle has a definite stimulating effect on the cyclic changes in the vagina, this is according to popular belief. However, Parkes (1927) has made the surprising statement that the oestrus cycle is not dependant upon the maturation of the Graafian follicle, since the cycle occurs after all of the foll-

icles were destroyed by irradiation with X-rays. So if this is true the pituitary extract must have a destructive action on other cells of the ovary, as well as those of the Graafian follicle which lead to normal ovulation.

The interesting results produced in the ovary by the injection of pituitary extract led us to believe there might be some specific action of this extract on the sex-cells of the male also. The histological characteristics of the testes were not altered by the injection of either the pituitary or the liver extract, the microscopical picture being practically the same in the control pituitary injected animals. There was noticed however much more difficulty in hardening the testes removed from the pituitary injected rats than from either the controls, or those injected with liver extract. They were much softer and the fibrous tunica seemed to fit the under lying structures very loosely. In spite of this softening no difference was shown on microscopic examination. The weights of the testes were all below the average given by Donaldson (1924) for rats of the same weight. The difference were small, amounting to an average of about fifteen per cent. The average weight of the testes of the controls were about thirty per cent higher than the average given by Donaldson. So the testes of our pituitary injected rats are more than forty per cent less than our control animals. The weight

of the males injected with liver extract corresponds almost exactly with those given by Donaldson. At the time of autopsy all of the males were in excellent health except one pituitary rat which died from diarrhea; and one of the males that was injected with liver extract which died of peritonitis.

Although degenerative changes were present in the testes and perhaps to a greater extent than in the control males, according to Oslund (1928) degenerative changes normally occur in the testes and especially in the germ sex-cells where most of the degeneration in the pituitary-injected animals was observed. The injection of foreign material can cause degeneration and intereference with the development of the sperm as has been shown by Oslund (1926).

SUMMARY

The intrapertioneal injection of pituitary extract into the rat was followed by atretic degenerations in the ovary, with an inhibition of the normal maturation of the Graafian follicle, ovulation, and a cessation of the oestrual rhythm. These results were not due to a general protein toxic effect for the control animals injected with liver extract were in every respect normal.

The extract was without effect on the males. After the injection of pituitary extract the testes remained normal both as regards weight and histological structure.

Bell, Blair. Experimental Operations on the Pituitary. Post Operative Symptoms. Quart. Jour. Exper. Physiol. 12,77, 1917.

Bergeim, O., Larson, E., and Fisher, N. F. The influence of Anterior Pituitary Extract of Reproduction and growth. Unpublished results.

Biedl, A. Innere Sekreton 1910. Quoted from The Endocrine Organs, Sharpey-Schafer.

Corner, G. W. Origin of the Corpus Luteum of Sow from both Granulosa and Theca Externa. Amer. Jour. Anat. 26, 117,1919.

Crowe Cushing and Homans. Quart. Jour. Exper. Physiol. 2, 1909. Quoted from Sharpey Schafer, The Endocrine Organs.

Dandy, W. E. and Reichert, F. L. Studies on Experimental Hypophysectomy. Effect on the Maintenance of Life. Bull. Johns Hopkins Hosp. 37, 1, 1925.

Dodd, E. C. and Dickens, F. The Chemical and Physiological Properties of the Internal Secretions. Oxford Medical Publications.

Dott, N. M. An Investigation into the Function of the Pituitary and Thyroid glands. Quart. Jour. Exper. Physiol. 13, 241--283,

1923.

Donaldson, Henry H., The Rat. Memoirs of The Wistar Institute of Anatomy and Biology. No. 6, 1924.

Drummond, J. C. and Cannan, R. K. Tethelin- The Alleged growth Controling Substance of the Anterior Lobe of the Pituitary Gland. Biochem. Jour. (Lond). 16, 53--59, 1921.

Engle, Earle. Quantitative Study of Follicular Atresia. Amer. Jour. Anat. Vol. 39, 1927.

Evans, H. M. The Function of the Anterior Hypophysis. Harvey Lectures 212-235, 1923-24.

Goetsch, E. The Influence of Pituitary Feeding upon Growth and Sexual Development. An Experimental Study. Bull. Johns Hopkins Hosp. 27, 29, 1916.

Hayato, Arai. Number of Ova. Albino Rat. American Jour. Anat. 27, 1919.

His, Wilhelm, Boebachtungen uber den Bau des Saugethier Ereistoches. Arch. f. Mikr. Anat., 64, 361, 1865.

Horsley, V. C. Lancet 1, 3-5, 1886. Quoted from Dodd Dickens. The Chemical and Physiological Prop. of the Int. Secretions.

Koch, F. C. and McMeekin, T. L. Jour. Amer. Chem. Soc. 56,

2066, 1924. Quoted from Practical Physiological Chemistry. Hawk and Bergeim.

Knauer, E. Arch. f. Gynak, 60, 322, 1900. Quoted from G. Corner (1919).

Landau, T. Quoted from Mainzer (1896).

Long, J. A. and Evans, H. M. The Oestrus Cycle in the Rat and its Associated Phenomena. Memoirs of the University of California Vol. 6. 1922.

Marinus, C. J. The effect of feeding Par Tuberlaris and Pars Anterior Propior on bovine pituitary glands upon the early development of white rats. Am. Jour. Physiol. 49, 238, 1919.

Mainzer, F. Deutsch Med. Wooh., Berlin, 1896, Vol. 12, P. 188.

Marie, P. Rev. de Med. Paris, 6, 297-333, 1886. Quoted from Dodd and Dickens. The Chemical and Physiological Properties of The Internal Secretions.

Oslund, R. M. Physiologic Effects of Spermatoxin in Rats, Rabbits and Guinea Pigs. Jour. Amer. Med. Assoc. 86, 1755-58, 1926.

Oslund, Robert M. The Physiology of the Male Reproductive System. Jour. Amer. Med. Ass. 90, 829, 1927.

Oswald, A. Virchow Archives 1902, 169, 444-479.

Pearl, R. and Surface, F. M. On failure of Extract of Pituitary Body (Anterior lobe) to Activate the resting Ovary. Jour. Biol. Chem. 21, 95, 1915.

Robertson, T. B. The Influence of Tethelin, the growth controling principle of the anterior lobe of the pituitary body, upon the Growth of the White Mouse. Jour. Biol. Chem. 24, 347, 1916.

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

Smith, P. E. Genital System Response to Daily Pituitary Transplants. Proc. Exper. Biol. and Med. 24, 337, 1927.

Smith, P. E. Hastening Development of the Female Genital System by Daily Homeoplastic Transplants of Pituitary. Proc. Exper. Biol and Med. 24, 131, 132, 1926.

Smith, P. E. Induction of Sexual Maturity by Pituitary Homeo-transplants. Amer. Jour. Physiol. 80, 1, 1927.

Smith, P. E. and Engle E. T. Induction of Precocious Sexual Maturity in the Mouse by Daily Pituitary Homeo and Hetero-transplants. Proc. Soc. Exper. Biol. and Med. 24, 561, 1927.

Smith, P. E. Result of Feeding Fresh Anterior pituitary to Hypophysectomized Rat. Amer. Jour. Physiol. 81, 254, 1927.

Sorbata, J. Uber die Bildung des Corpus Luteum bie der Maus. Anatomischer Anzieger. 10, 482.

Stockard, C. R. and Papanicolaou, G. N. The Existence of a Typical Oestrus Cycle in the Guinea Pig. Amer. Jour. Anat. 22, 1927.

Sharpey-Schafer, E. The Endocrine Organs. Part 11. (New York)

Smith, C. S. The Alleged Effects on Body Growth and Gonad Development on Feeding Pituitary Gland Substance to White Mice. Amer. Jour. Physiol. 65, 277, 1923,

Schafer, E. A. The Effects upon Growth and Metabolish by the Addition of Small Amounts of Ovarian tissue, Pituitary and Thyroid to the Normal Dietary of White Rats. Quart. Jour. Exper. Physiol. 5, 203-228. 1912.

Von Baer, K. E. De Ovi Mammalian et Hommini Genesi,. Quoted from Bischoff (1843).

Walker, A. T. An Inhibition in the Fowl by the Intraperitoneal administration of fresh anterior hypophyseal substance. Am. Jour. Physiol. 74, 249-256. 1925.

Winiwater, H. Les Debuts de l'atresia folliculaire. Comptes Rendus de la Soc. de Biol. 89, 960, 1923.

Zondek, B. and Ascheim, S. The Anterior Lobe of Pituitary and Ovary. Jour. Amer. Med. Ass. 89, 13, 1927.