EFFECTS OF INJECTING AN OVARIAN RESIDUE EXTRACT INTO YOUNG FEMALE RATS

by

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INTRODUCTION

One of the most baffling questions facing biologists today is that of the function of the interstital cells of the ovary. They come from and are intimately connected with follicular atresia. They are transitory structures which increase in number during puberty and during

pregnancy. It is hoped that when the final analysis of the present work is reached, some light can be thrown on this very interesting but very evasive question.

For the sake of clarity, the author will describe what shall be considered an interstitial cell throughout this thesis. This type of cell is found in the ovary and also in the testis. The male cells are described by Novak (1931) as being in small isolated groups or in short strands embedded in connective tissue. They are polygonal; some are round, rhomboid, or spindle shaped; 12 to 23$_{\mu}$ in length.

The female cells are described by Lipschutz (1924). He writes, "they tend to be spindle shaped, found in nests or cords in the connective tissue ... protoplasm is granulated and contains small droplets of fat which Athias maintains are secretory products which change chemically before leaving the cells." The cells are found in the amphibians and reptiles and are abundant in birds and mammals. Figure 1 of Plate I shows the interstitial cells of the ovary of the white rat.

ACKNOWLEDGMENTS

The origin of the present work dates back to 1929 when Johnson and Wade (1931) injected into ground squirrels a saline-alcohol extract of bovine ovaries prepared by the

Veterinary Division of this college. This extract had been found by McLoed (1929) and Frank (1930) to bring back into reproduction cows whose ovaries had ceased to function. No effect of the injection was noted in the ground squirrels. However, it was suggested to Dr. Johnson by Dr. McLeod that the extract be tried out more extensively on laboratory animals. This work was undertaken by the writer in 1931 at Dr. Johnson's suggestion. No effect of the extract on mice was seen. Aid in purification of the extract was sought from Dr. H. W. Marlow of the Chemistry Department, who had previously prepared a purified extract from sow residual ovaries while working in Dr. Koch's laboratory at the University of Chicago. Some of his extract was tried on mice and when it was observed that it stimulated development of the genital system of female mice it was decided to concentrate our efforts on this extract alone.

The writer is indebted to Dr. Marlow for supplying this extract and also for his aid in the experiments with it. To Dr. Johnson, the writer expresses appreciation for directing the work and for aid in it and in the preparation of the thesis. The author wishes to thank the Chemistry Department for the use of its animal house and laboratory equipment.

REVIEW OF LITERATURE

According to Graves (1931), Berthelde in 1849 laid the foundation for the study of endocrinology by showing that, after castration, young cockerels could be prevented from showing typical castration symptoms by reimplanting the testicle. The success of this early experiment is now known to have resulted from supplying the needed testicular hormone which governs the secondary sex characters. The source of the hormone has been shown to be the interstitial cells of the testicle (Benoit, 1926, 1927).

Graves (1931) in his interesting account of the history of female sex hormone states that "Simon in 1904 was the first to show that the secretory function of the ovary was not entirely dependent upon the ova-producing mechanism as had been supposed since the time of Von Baer. He found that in the ovarian grafts which had taken, the follicles degenerated whereas the interstitial cells retain their integrity. Marshall and Jolly and others have confirmed this experiment." Could it be that the interstitial tissue was the source of the hormone which prevented or repaired the castration symptoms? The question is still unanswered. Novak (1931), after a thorough and exhaustive search of the literature on the question of the interstitial cells both

in males and females, states: "Seldom is there a biological topic that in the main takes on a more theoretical than practical meaning, a subject so warm, in so wide a range of dispute, as the question of the meaning of the interstitial cells." And as one progresses through Novak's paper this becomes more apparent. Histologists, embryologists, cytologists, physiologists, morphologists, gynecologists, and pathologists are all arguing back and forth. The arguments in either case are as theoretical as the argument of the opposition.

Lipschütz (1924) states that the interstitial tissue is less injured by X-rays than is the rest of the ovary and, while sterility results, secondary sex characters are not impaired. He concludes, therefore, with Steinach, whom he quotes, that these results were "caused by an increase of the interstitial cells derived from the follicles." Lipschutz (1924) states another argument in favor of the secretory function of the interstitial cells. Since grafts which are, in the main, composed of interstitial cells, are able to perform the internal secretory function of the ovary they are the source of the female hormone.

Some have argued that, since the male hormone comes from the interstitial cells of the testis, the female hormone must come from the interstitial tissue in the ovary.

Great doubt is thrown upon this view by the work of Jaffe and Marine (1923). They have shown that there is very good reason to doubt the physiological similarity between the interstitial cells of the male and female. By injuring or removing entirely the suprarenal cortex, they were able to cause the ovaries in some 76 per cent of their experimental rabbits to double in size. The increase in size was due to an increase in the amount of interstitial tissue. In the males there was no difference in the amount of interstitial cells in the controls and experimentals. Thus, in rabbits at least there is a compensitory relationship between the interstitial cells of the ovary and the suprarenal cortex, while there is none between the interstitial cells of the testis and the cortex of the suprarenal and the cells are not physiologically similar.

The work of Moore and Price (1932) adds weight to the arguments presented. After working with male and female hormones on the question of sex antagonism they have concluded that the pituitary of the pregnant animal is less active than the pituitary of a non-pregnant animal and that the urinary gonadal stimulating hormone is probably not of hypophyseal origin, but perhaps arises somewhere in the ovarian stroma. Again can it be that the hormone comes from the interstitial cells?

Kingsbury (1914) in accordance with most histologists (i.e. Bailey, 1931) after a rather exhaustive study of the interstitial cells in the ovary of the cat states: "I can find no evidence for regarding the interstitial cells as constituting a morphological intra-ovarian gland. And regarding them as a physiological functioning gland is without sufficient evidence". Wilkerson (1925) after studying the atretic follicles of the mouse, rat and rabbit also found that while there is little doubt that the atretic follicles become interstitial cells eventually, the evidence is lacking for regarding them as a distinct gland.

In concluding the discussion of literature dealing with the question of the interstitial tissue, it may be said that the question of its function is still an open one. The author would refer those interested to complete bibliographies given by Graves (1931) and Novak (1931).

Thus we come to some papers dealing with extracts of the ovarian stroma. McLeod (1929) and Frank (1930) used a saline-alcohol extract to good advantage. Dickens, Dodd and Wright (1925) and Allen and Doisy (1923) have made extracts of the whole ovary and have shown that theelin is present in small amounts outside the follicular fluid. Cortland, Heyle, and Neupert (1930) and Payne, Van Peenan, and Cortland (1928) have shown that the biological activity

of the ovarian residue (ovaries from which only the corpora lutea had been removed in this case) closely correlates the activity of pure theelin as shown by Doisy et. al. (1930).

MATERIALS AND METHODS

The purified extract used was in nearly crystalline form and was dissolved in a little olive oil to a consistency of a thick syrup. The 27 grams of extract used were made from 16 pounds of tissue. The follicular fluid was drained and the corpora lutea were removed before the extraction took place. Proteins were precipitated out, cholesterol, fatty acids also were removed. Its purity was shown by the absence of "protein sores" which our mice showed when the alcohol-saline extract was used. No animals were lost from any injections except one which got out of its cage soon after an operation and was in rather bad condition when found the next day. One very small rat died of unknown cause.

The pure extract was carefully weighed out and then dissolved in a little 95 per cent alcohol. Olive oil was then added in the proportions of 20 cc. of oil to one gram of extract. This was thoroughly shaken up and the alcohol boiled off under reduced pressure so that the temperature of the extract did not rise above 40°C. The extract was

clear, transparent and only slightly darker in color than the original oil. About 500 cc. of this oil solution of the extract was used.

In these experiments rats served as laboratory animals throughout with the exception of the preliminary experiment on mice. In all, about 114 animals were used. The animals were kept in steel wire cages and fed an adequate diet. The cages were sterilized once a week by live steam.

Healthy young rats, 21-23 days old, were selected and marked. Each animal was weighed. Litter mates served as control animals. The experimental animals designated by A received 0.25 cc. of ovarian extract per day, subcutaneously in the back. One group received 0.50 cc. per day. Control animals designated by B received an equal amount of olive oil as a control measure. Daily examinations of all animals was a routine procedure. General health, physiological activity and sexual development were the main points noticed. A careful weight record was kept for each animal. Later, a daily smear record was kept for the experimental females.

EXPERIMENTAL RESULTS

Effect on Weight

On the first few experiments a daily weight record was kept. It was found that controls and experimentals kept

very close together. The effect on weight, therefore, is of little significance. Table I shows the average weights for animals at the start and at the close of the experiments. Every advantage in weight was given the controls. There was

Table I. Average Weights of Young Rats in Grams

	Number of animals	Average weight start	Average weight finish	Average daily gain	Difference
Group I					
Control Females	11	30.4	62.4	2.15	.25
Experimental Females	16	28.3	64.3	2.40	
Control Males	10	26.8	71.2	2.52	.22
Experimental Males	9	25.6	67.4	2.74	
Group II					
Control Females	4	28.75	55.8	2.02	.23
Experimental Females	12	25.75	49.4	1.79	
Control Males	1	26.0	51.0	1.92	.39
Experimental Males	3	25.0	45.3	1.53	

a slight tendency for the experimentals in Group I to gain

more weight than the controls. This is reversed in Group II. It is also interesting to note that in Group I the females gained more weight than the males and in Group II this is again reversed. From this table it can be concluded that there is little effect on the growth of the animal as shown by increase in weight by injecting the ovarian residue extract. Individual weights will be found in the autopsy charts (II and III).

Effect on Males

In this experiment 23 male rats were used. Of this number, 12 were experimentals and 11 were controls. Table II gives the summary of results on the male rats. It will be noted that there is no differences in weight as has been already pointed out and that scrotality (taken as a criterian for physiologic activity) happened as soon in controls as in experimentals, where it was observed at all. The size of the testes is found likewise inconsistent; first the controls and then experimentals having the larger. Therefore it may be concluded that the extract has little effect on the male animal. However, this needs further study.

Table II. Autopsy Chart Males

Animal	Age at start (days)	Age at finish (days)	Weight at start (grams)	Weight at finish (grams)	Scrotality Day	Scrotality Age	Daily injection (cc.)	Total injection (cc.)	Testis size (mm.)	Total gain (grams)	Average daily gain (grams)
Group I - Animals Receiving Extract											
A130	21	31	25.5	46.0			.25E*	2.5E	8 x 5	20.5	1.8
A131	21	31	25.0	48.1			.25E	2.5E	10 x 6	23.1	2.1
A132	21	31	22.2	42.1			.25E	2.5E	8 x 5	19.9	1.8
A142	21	38	25.3	72.0			.25E	4.25E	6 x 3	46.7	2.6
A143	21	38	23.4	75.0			.25E	4.25E	7 x 5	51.6	2.8
A148	21	37	25.5	76.0			.50E	8.00E	9.5 x 6	50.5	2.9
A160	24	41	27.5	84.0	Swollen		.50E	8.50E	8 x 4.5	56.5	3.0
A164	21	36	26.0	63.0	13	34	.50E	7.00E	9 x 6	37.0	2.4
A168	20	36	30.0	65.0	13	33	.25E	7.00E	9 x 6	35.0	2.3
Group II - Animals Receiving Extract											
A188	21	36	26.0	45.0	13	34	.25E	3.50E)			
A190	21	36	26.0	50.0	13	34	.25E	3.50E)	Not autopsied		
A189	21	36	23.0	41.0			.25E	3.50E)			
Group I - Animals Receiving Olive Oil											
B130	21	31	27.9	53.0			.25 0*	2.50 0	8 x 5	25.1	2.2
B141	21	37	24.2	70.0			.25 0	4.00 0	8 x 6	45.8	2.7
B142	21	36	23.4	59.0			.25 0	3.75 0	7 x 4	35.6	2.2
B143	21	38	23.4	70.0			.25 0	4.25 0	8.5 x 5	46.6	2.6
B146	21	37	29.0	90.0			.50 0	8.00 0	9 x 6	71.0	4.2
B147	21	37	26.9	75.1			.50 0	8.00 0	8 x 5	48.1	2.9
B160	24	42	30.0	83.0			.50 0	9.00 0	9 x 5	53.0	2.8
B161	24	41	27.5	85.0	15	39	.50 0	8.50 0	9 x 6	57.5	3.1
B164	21	37	26.0	64.0	13	34	.50 0	7.00 0	9 x 6	38.0	2.5
B168	20	36	30.0	63.0	13	34	.25 0	7.00 0	10 x 6	33.0	2.2
Group II - Animals Receiving Olive Oil											
B188	21	36	26.0	55.0	13	34	.25 0	3.50 0)	Not autopsied		

*E = Extract
0 - Olive Oil

Effect on Females

Effect on Reproductive System. The effects of the extract upon the female reproductive system were very pronounced and clear-cut when compared with control animals. Young females 21 days of age showed the greatest response. The effects on young females 21-23 days old at the start have been tabulated in Table III. Column 2 shows that the first sign of any effect produced by the extract was shown by the opening of the vaginae of the experimentals. It will be noted that at no time did any controls show this sign of effect of the pure olive oil.

As soon as the vagina of an experimental was observed to be open a daily smear record was started and kept throughout the experiment. The numbers on the right side of Table III give the stages of oestrous cycles which were apparent by the vaginal smear, on successive days. The significance of these records will be taken up later.

A figure 1 signifies pro-estrum or a vaginal smear of small and large nucleated epithelial cells; 2 signifies an early oestrous smear with cornified and large epithelial cells; 3 is typically oestrus, showing only cornified cells; 4 is late oestrus and early dioestrus with cornified cells and leucocytes in abundance; 5 is dioestrus or small

Table III. Effects of Extracts on Young Females

Animal	Day vagina opened	Age vagina opened	Uterus	Day after vagina opened on which smears were taken: 1	2	3	4	5	6	7	8	9	10	11	12	13
Group I																
A133	9	30	15 x 2													
A140	11	32	20 x 2	1	5	5	1	5	5							
A144	11	32	17 x 1	5	5	1	5	5	5							
A145	10	31	17 x 1.5	1	2	5	1	5	4							
A146	8	29	20 x 3	1	2	2	2	4	5	5	1	5	1			
A147	8	29	20 x 3	5	5	5	1	2	5	5	2	4				
A154	9	41	22 x 2													
A161	8	32	17 x 2	2	2	2	2	2	2	2	2	2	1-2	2	2	2
A163	7	28	15 x 2	2	2-3	2	2	2	2	2-3	2	2	2	2	4	2
A165	12	32	22 x 1.5	1	5	5	5	1-5	2	2	3	5				
A166	10	30	22 x 2	5	5	5	5	5	3	5	2	2	2	5	5	1-2
A167	12	32	21 x 1.5	5	1	1-2	5	5	2	2	2		4	1	4	
A171	7	32	15 x 2													
A172	7	32	17 x 3													
A173	7	32	10 x 2.5													
A174	7	32	18 x 3													
Control Group I																
B133	Not open		23 x .5													
B140	Not open		17 x .8													
B153	Not open		22 x 1.0													
B154	Not open		22 x .5													
B163	Not open		20 x .1													
B165	Not open		22 x .5													
B166	Not open		22 x .5													
B171	Not open		17 x .5													
B172	Not open		22 x .5													
B173	Not open		22 x .5													
B174	Not open		20 x 1.0													
Group II																
A180	11	33		1	2	5	5	5	5	5	5					
A181	8	32		1	2	2	5	1	3	5	5	5	5			
A182	8	33		4	1	2	4	2	5	5	5	5-1	2			
A183	8	31		2	2	2	4	1	1-2	5	5	1	4			
A184	9	34		1	4	2	1	2	2	2	1	2	4			
A185	8	32		5	2	2	5	1	2	2	5	5	5-1	1		
A186	8	33		5	1-2	2	5	2	2	4	5	1	2			
A187	10	31		5	2	4	4	2	4-5	1	2	5				
A191	11	32		4	5	1	2	2	4	5	1					
A192	9	30		1	2	4	4	5	5	1	5	5	1			
A193	10	31		5	2	4	2	2	2	2	2	2	2			
AA194	9	30		5	1	2	5	2	4	1	2	5	5			
Control Group II																
B180	Not open															
B181	Not open															
B182	Not open															
B188	Not open															

epithelial cells and leucocytes in equal abundance. The smears were taken by means of a small swab and then later stained either with Wright's blood stain or hematin and eosin. Staining each slide gives it permanency and also enables minute cytological examination of the smear. Each smear was studied and the approximate number of each type of cell plotted and from these analyses the stage of the cycle was determined and recorded as shown in Table III.

Autopsy Observations. All animals were killed by ether and the body cavity opened and organs examined as quickly as possible. Table IV gives the results of these autopsy observations on the females. The size of the uterus at autopsy is shown in Table III. In general, all the animals, both controls and experimentals, had some difficulty in absorbing all the oil. This was more apparent in the controls. All animals were active and showed a certain amount of fat, caused by the oil which had been absorbed. This was usually found in the body cavity or found in the intestines and uterus, making the latter difficult to dissect out. The fat deposition was much more apparent in controls than in experimentals which may be attributed to the greater activity which the experimentals displayed throughout the experiment. Figures 2, 3, 4, Plate I, illustrate these gross autopsy findings. The size of the uteri and the fat

Table IV. Autopsy Chart, Females Group I.

Animal	Age at start (days)	Age at finish (days)	Weight at start (grams)	Weight at finish (grams)	Total gain (grams)	Average daily gain (grams)	Daily injection (cc.)	Total injection (cc.)	Mammary glands
				Experimentals - Injected with Ovarian Extract					
A133	21	31	23.1	40.0	16.9	1.5	.25	2.50	
A140	21	37	25.4	65.0	39.6	2.3	.25	3.50	
A144	21	36	20.4	49.5	29.1	1.8	.25	3.50	
A145	21	35	26.7	53.0	26.3	2.8	.25	3.25	
A146	21	37	27.0	75.0	48.0	2.8	.50	8.00	
A147	21	36	27.5	68.0	40.5	2.5	.50	7.50	
A154	32	43	25.0	50.0	25.0	2.5	.25	2.75	
A161	24	44	27.0	95.0	68.0	3.2	.50	10.00	Slight swelling
A163	21	40	25.0	75.0	50.0	2.5	.50	9.50	Apparent
A165	20	40	27.0	77.0	50.0	2.4	.25	5.00	Apparent
A166	20	41	26.0	77.0	51.0	2.3	.25	5.25	Apparent
A167	20	42	29.0	80.0	51.0	2.1	.25	5.50	Slight swelling
A171	25	34	43.0	63.0	20.0	2.5	.50	4.50	
A172	25	35	36.0	56.0	20.0	1.6	.50	5.00	
A173	25	36	26.0	41.0	13.0	1.3	.50	5.50	
A174	25	37	39.0	65.0	26.0	1.9	.50	6.00	
				Controls - Injected with Olive Oil					
A131	21	31	25.0	44.7	19.7	1.8	.25	2.50	
B140	21	36	24.0	54.0	30.0	2.0	.25	3.50	
B153	32	42	40.0	70.0	30.0	3.0	.25 and .50	3.75	
B154	32	42	34.0	61.0	27.0	2.7	Normal Control		
B163	21	40	24.5	89.0	34.5	3.2	.50	10.00	Not apparent
B165	20	40	27.0	76.0	49.0	2.3	.25	5.25	Very slight
B166	20	41	26.0	78.0	52.0	2.3	.25	5.50	Very slight
B171	25	34	43.0	58.0	15.0	1.6	.50	4.50	
B172	25	35	40.0	53.0	13.0	1.3	.50	5.00	
B173	25	36	26.0	50.0	24.0	2.4	.50	5.50	
B174	25	37	29.0	52.0	23.0	1.9	.50	6.00	

deposition around them are apparent in the photographs. These were taken as soon as the body cavity was opened and show typical autopsy findings.

The other glandular organs were found to be normal except in the case of animals A161, A163, A165, A166, and their control litter mates B163, and B165. These animals were kept in the experiment over a period of 20 days or more. This whole group of animals showed mammary development. The experimentals all showed greater development than the controls. These were confirmed by a second observer present at autopsy. The significance of these observations will be discussed later. No autopsy observations are included of Group II because these animals were carried on under different conditions by Dr. Marlow after the smear record given in Table III was taken.

Effect of Extract on Ovariectomized Females. Since the effects of theelin upon the ovariectomized animal are well known (Allen and Doisy, 1923; Doisy et al, 1930) an experiment to determine the effects of the extract on ovariectomized females was carried out. The results of this experiment are given in Table V.

Table V. Results of Injecting Extract into Young Ovariectomized Females

Animal	Age in Days: Start	Finish	Weight in grams: Start	Finish	Injection: Daily	Total	Uterus (mm.): Length	Thickness	Histology of Sections
A152	30	42	34	56	.25 cc. and .50 cc.	3.75 cc. E*	18 x	2.5	1.4 x 1.6 mm. diameter after sectioning; medium high columner epithelium; more glands in mucosa
B 152	30	42	35	57	.25 cc. and .50 cc.	3.75 cc. O.*	22 x	.5	0.64x0.80 mm. diameter after sectioning; low columner epithelium; some glands in mucosa
A153	30	42	34	59	.25 cc. and .50 cc.	3.75 cc. E.	18 x	2.5	1.2x1.5 mm. diameter after sectioning; high columnar epithelium; more glands in mucosa

*E, ovarian extract; O, olive oil

These animals were 22 days old when the operation was performed. They were allowed to rest four days and then injections were begun. In the first half of the experiment the experimental animals received .25 cc. of the extract per day. At the middle of the experiment (sixth day) the dosage was doubled. There were no external developments noted in any of the animals. At the autopsy, the uteri of animals A152 and A153 were large but transparent. They were not at all like the uteri of animals which were in heat when autopsied. The histology revealed that the uteri of animals receiving the extract had about twice the diameter of those of the controls (Table V). They also contained more glands and the epithelium was more columnar than in the controls. These effects on the uterus must be attributed to the theelin content of the extract.

In order to further determine the effects of the extract upon the genital tract of ovariectomized animals the author started an experiment with five adult females.

Table VI is a record of these five normal adult female rats. First a regular smear record was kept for nine days to use as a basis of comparison. Animal A155 shows a cycle of eight days, A156 shows a cycle of at least 6 days and A157 and A158 show equally long cycles. The animals A155 and A158 were then ovariectomized and A157 was operated on

Table VI. Smears of Adult Females

Animal	December 26	27	28	29	30	31	January 1	2	3	January 21	22	23	24	25	26	27	28	29	30	31	February 1	2	3	4	5	6	7	8	9	10	11	12	13
	Smears before ovariectomy									Smears while injected with ovarian extract											Smears after injections were stopped												
A155	5	5	1	2	2	3-4	5	5	5	5	5	1	4	2	2	2	2	2	2	2	5	5	5	5	5	5	5	5	5	5	5	5
A156	5	5	5	5	5	1	2	5	5	0.5 cc. per day Died at operation																							
A157	5	5	5	5	5-1		5	2	5	5	5	5-1	5	5-1	4-5	2	4-5	5	5	2	5	5	5	5	5	Autopsied							
A158	2	2	5	5	5	2	5	5	5	5	5	1	4	2	4-5	5	5	4	5	2	5	5	5	5	5	5	5	5	5	5	5	5	5
A159	5	5	5	5	5	5	5	5	5	5	2	2	5	5	5-1	2	2	5	5	1	2	5	5	5	5-1	2	2	5	5	5-1	2	2
	Pregnant						Birth																									
	Normal									All received 0 Extract											Stopped											Normal

Table VII. Summary of Histological Results

	Atretic Follicles		Open Follicles		Partially Open		Closed Follicles		Interstitial Tissue
	Small to Medium	Medium to Large	Small	Large	Small	Large	Small	Large	
Experimentals	Many	Several to many	25.7_u	41.6_u		38.1_u		32.6_u	Medium to abundant
Controls	Many	Several	26.4_u	41.3_u		27.9_u	23_u		Medium

as a control measure. After resting seven days the animals were started on the second stage of the experiment. Each received 1 cc. of the extract per day. It is seen that A155 has one normal cycle and then a series of oestrous smears, due perhaps to the oestrogenic content of the extract. Animal A157 shows the same type of smear record as before and then it goes into dioestrus. The change to dioestrous record will be taken up later. Animal A158 shows two cycles. Animal A159, whose young had died, was put on the experiment, as a control, shows very regular five day cycles, the extract having no apparent effect. After ten days the extract was no longer given and the animals show an oestrous smear with the exception of A159 whose cycles go on uninterrupted. This experiment gives further evidence that the oestrogenic content of the extract is small in comparison with extracts of the whole ovary, since Payne, Van Peenan and Cartland (1928) report very positive reactions in spayed females to the injection of varying amounts of ovarian residue extract which contained the follicles and, therefore, theelin. The significance of this experiment will be taken up later.

Another experiment was conducted to show whether or not the pituitary was in some way stimulated by the extract. Two litter mates were implanted with pituitary glands from young rats. The experimental received pituitaries from

animals which had received the ovarian extract and were active sexually. The control received pituitaries from litter mates of the previously mentioned donors. Ten implantations were made. The experimental animal showed the vagina to be open at the end of ten days. The control showed no signs of sexual activity. At autopsy the uterus of the experimental animal was 18 x 1.5 mm. while the control uterus measured 21 x 1.5. These findings therefore may be insignificant. Histological study revealed that in the experimental ovary there were developing two very large follicles 75 and 80 $_u$ in longest diameter. The control ovary showed many maturing follicles 60 to 70$_u$ in longest diameter. Both animals showed signs of stimulation due to the implants. Just the significance of the larger follicles and open vagina of the experimental animal, is difficult to say, as the number of animals is too small, but one may interpret these results as showing that the pituitary is being stimulated by the extract.

Histological Observations. The histological effects are not particularly evident at first glance. After a thorough examination first by the author and then by Dr. Johnson, the histological observations were summarized in Table VII. Atresia was prevalent throughout the entire group of animals. There appeared to be a little more

atresia of the large follicles in the experimental than in the control animals. The one constant difference was found to be the size of follicles which were open or partially open. The open follicles of the experimental ovary are further characterized by having a thick granulosa. Animal A161 had the largest follicles measured, 80$_{u}$ in diameter and the wall strongly suggested a tendency toward luteinization. There seemed to be more interstitial tissue in the experimentals.

Plate II, Figs. 1, 2, and Plate III, Figs. 1 and 2 illustrate typical histologic findings. Figure 1, Plate II shows a normal ovary of a 21 day old rat. The ovary is almost entirely composed of small follicles. Figure 2 shows the normal rat ovary at 36 days of age, the age at which most of the animals were autopsied. Figures 1 and 2 Plate III, are ovaries of a control and of an experimental animal respectively. Figure 2 shows the larger partially open follicles typical of the experimental slides. The walls of the open follicles are thicker than the walls of the control shown in Figure 1. Note also in Figure 2 the small number of open follicles present. Atresia is about the same in these two figures (Figs. 1 and 2, Plate III).

The histological studies of the cross sections of the uteri confirmed our previous observations, namely thickened walls in the experimental animals. The epithelial tissue was highly columnar in the experimentals. From these histological observations we may say that there is enough theelin in the extract to produce changes in the uterus or else that the ovary is stimulated to follicular fluid secretion and that in turn influences the uterus.

DISCUSSION OF RESULTS

Premature development of the genital system of young animals has been accomplished by many workers. Allen and Doisy (1923) used impure extract from the follicular fluid. Later Doisy and his co-workers (1930) purified theelin and theelol and found that they had similar physiological activities. Pituitary implants have long been used to produce precocious sexual development in young animals (Smith and Engle, 1927) and Zondek and Aschheim (1927) used urine extracts to accomplish the same results. Collip (1931) was able to induce premature development with a placental extract. Few workers report working with residual ovary extract but those that do (Allen and Doisy, 1923) report that it has no effect. Certain reactions in young female rats injected with ovarian residue extract which have been

reported in this thesis may be attributed to theelin or theelol. Another possibility is that the pituitary is being stimulated which in turn stimulates the ovary and the uterus. The third alternative is the combination of these two factors.

From the work of Doisy and his co-workers (1930, 1931) and from the work of Moore and Price (1931) the effects of theelin and theelol are well known. They affect the uterus only. Theelin works as well in hypophsectomized rats as in rats with intact pituitary (Smith, 1932). Theelin definitely inhibits the ovary and this becomes especially noticeable long periods of injection (Doisy, Curtis and Collier, 1931). After precocious development has occurred, oestrous smears persist as long as two to five days in young rats which have received injections with theelol (Doisy et al, 1930). With these effects of theelin and theelol in mind let us examine the results reported herein and see whether or not theelin or theelol can be said to be the sole active principle in the extract.

Theelol develops sexual precosity in 2 to 10 days. The animals receiving the extract which was used in this experiment did not show signs of development till at least three or four days and the vaginae did not open usually till ten days after. However, it may be that the theelin or theelol

content may be too small to act sooner. The smear records of the animals which stayed in oestrus may show more effects of theelin or theelol injections. The fact that the other smear records do not show oestrous smears may be said to show that the theelin content is too small to keep the animal in heat. The larger uteri of the experimentals and the experiments with ovariectomized females seem to indicate farther that theelin or theelol is the causative principle.

On the other hand, the longer reaction time for the ovarian extract used in this experiment seems to indicate that some other organ or gland is being stimulated. This organ may be the pituitary. The histologic studies of the ovaries of the animals which give greatest support to the theelin, theelol hypothesis are the strongest argument against the theory. A161 whose smear record was that of nearly complete oestrus shows the greatest follicular development observed throughout the experiment. The ovaries of the rest of the experimental animals show a tendency to have larger follicles and show greater potentialities of growth (Table VII). This is indicative of anterior pituitary stimulation. The fact that atresia, a characteristic of young growing ovaries, was about the same in both controls and experimentals, gives further support to the theory that some other principle than theelin is causing the changes reported in this thesis, since theelin inhibits the ovary.

Theelol in small amounts gives some increase in follicular size and corpora lutea are present. We found no true corpora lutea, but A161 showed a tendency to form them. Theelol causes the vaginae of ovariectomized young females to open in three to seven days. Our experiments showed that by injecting large amounts, the vaginae of young ovariectomized females failed to open although the uterus enlarged. The implantation experiments which seemed to indicate greater activity of the experimental pituitary gives further evidence that the pituitary seems to be involved.

If the pituitary be accepted as the dynamo which governs the rhythm in the sex organs then the smear records in Tables III and VI show strong indication that this organ is stimulated for a time at least.

Long and Evans (1922) report that "but slightly more than half of a miscellaneous stock of animals gives oestrus cycles of such unfailing regularity that a discrepancy of more than 48 hours in length of any one of them did not occur, and that, furthermore even in these instances cases of an inexplicably longer cycle interpolated in a long series of regular cycles occurred. We are thus acquainted with the necessity not only for a very superior hygiene but also for an exact individual oestrous history of every

animal upon which reliable data as to experimental physiology of the sexual system are desired." The author has attempted to obtain an individual smear record and reference to Table III will show that many of the animals are well within range of normal variation. This range has rather wide limits. The average length of the cycle is 4.8 days but of the group that they worked with, Long and Evans found 65 animals which showed a cycle of three days and 57 animals whose cycle was 13 days.

Animal A140 shows a regular three day cycle; A144 shows a six day cycle; A147 shows a five day cycle; and A165 shows a five day cycle. Others show interrupted cycles. The cause of the continuous oestrous smear of A161 apparently was the presence of large follicles, already mentioned, which probably secreted a large amount of oestrin or theelin. In Group II we find again some animals showing regular cycles and others with irregular cycles. But in only two cases do the cycles ever come close to the 13-day cycle which Long and Evans set as a normal upper limit. This smear record shows one of two possibilities. The oestrogenic content of the extract is too small to cause cornification except in one case, or that there is another principle which is causing the ovary to respond in a semblance of normal function. Collip (1931) gets the

same type of smear record with the placental extract. This relationship will be taken up later.

The fact that in the adult animals in Table VI we find that the animals, both ovariectomized and normal, go into dioestrus immediately after the injections stop shows that if theelin is present it is in minute quantities. Further, animal A157 at autopsy showed complete luteinization which suggests strongly that the luteinizing factor is being stimulated (Katzman, Levin and Doisy, 1931). The results do not vindicate a statement that the anterior pituitary is the sole cause of the results reported but rather it seems that the results warrant the hypothesis that the extract contains a minute amount of theelin or theelol, or both, and an unknown principle which is causing the above quoted results.

Briefly then what is probably the cause of physiological and morphological results reported? The extract used probably contains a minute amount of theelin or theelol. This is not hard to imagine since it is practically impossible to dissect out or drain all the follicles. Theelin content may account for (1) the opening of vaginae, (2) the larger uteri, (3) the œstrous smears of certain animals. But the extract probably also contains an unknown

principle which acts through the pituitary, which would explain, (1) the tardy opening of vaginae, (2) the rhythm in the majority of smear records, (3) the success of the implants, (4) the larger follicular development in the experimental ovaries, and (5) the appearance of diestrous smears after a certain number of cycles had been gone through with, as has been shown by further experiments by Dr. Marlow, and also the complete luteinization of animal A157.

The extract seems to simply speed up the process of puberty. May not the unknown principle, if there be one, be an extract from the interstitial cells? And to conjecture even further, since Collip (1931) is the only author reporting effects like the ones reported in this thesis, may not that hormone possibly have its precursor in the interstitial cells which are known to increase while the hormone with which he works, increases during pregnancy? Further experimentation will reveal the answer.

CONCLUSIONS

An ovarian residue extract has been injected into young female rats with the following results:

1. Young female rats receiving the extract became sexually mature in ten days.

2. These young females, with two exceptions, went through an approximately normal cycle, as shown by a daily smear record. This is unique as far as other workers have reported.

3. The uteri of experimental animals were larger in diameter than were control uteri. This may be due to theelin present in the extract, or it may be due to the extract's effect upon the pituitary which may stimulate ovarian function.

4. A discussion of the relative merits of three possible explanations is given.

5. The extract was found not to be effective on growth as revealed by increase in weight and on young male rats.

6. Certain explanatory experiments are taken up in detail to help determine the active principle or principles found in the extract.

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EXPLANATION OF PLATES

PLATE IA

Fig. 1. Interstitial cells in upper right. Oil immersion. X1400.

Fig. 2. Uterus of experimental (A) and uterus of control (B) showing difference in size.

PLATE IA

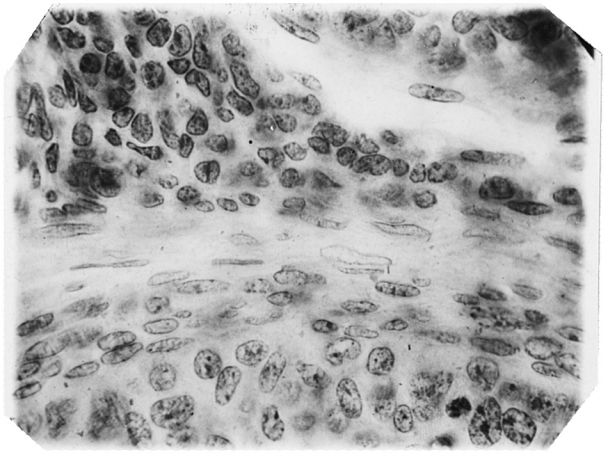


Figure 1.

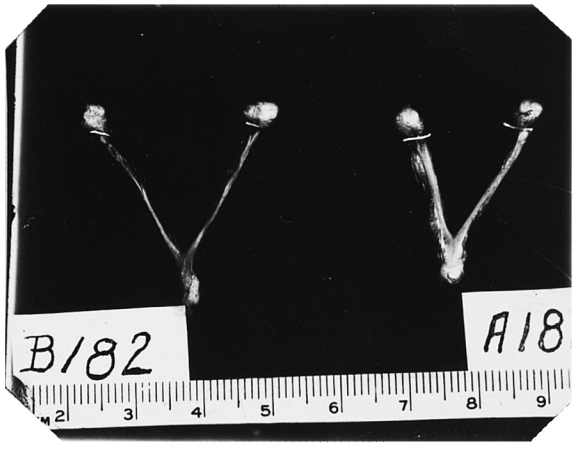


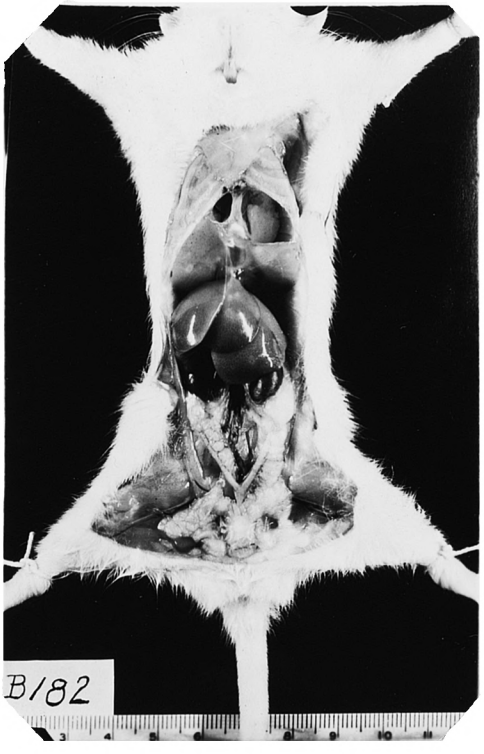
Figure 2.

PLATE IB

Fig. 3. Gross structures control animal (B) showing typical fat deposition.

Fig. 4. Gross structures experimental (A) showing typical autopsy findings.

PLATE IB



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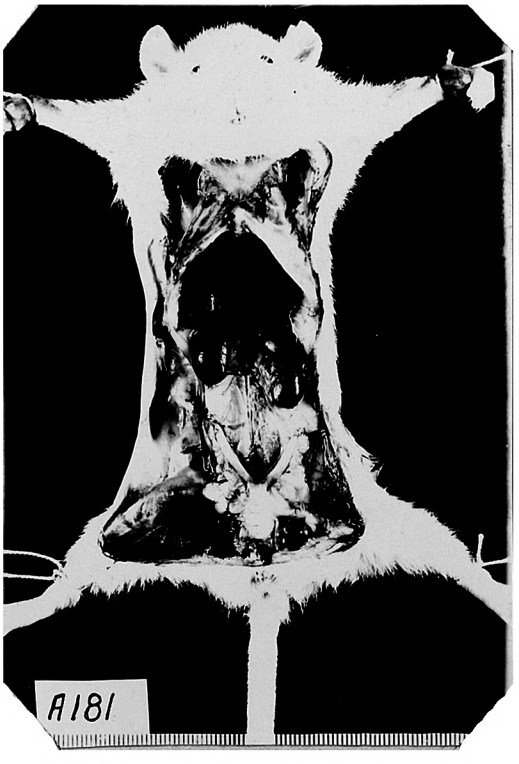

Figure 4.

PLATE II.

Fig. 1. Section of normal ovary 21 day rat. X342.

Fig. 2. Section of normal ovary 36 day rat. X342.

PLATE II.

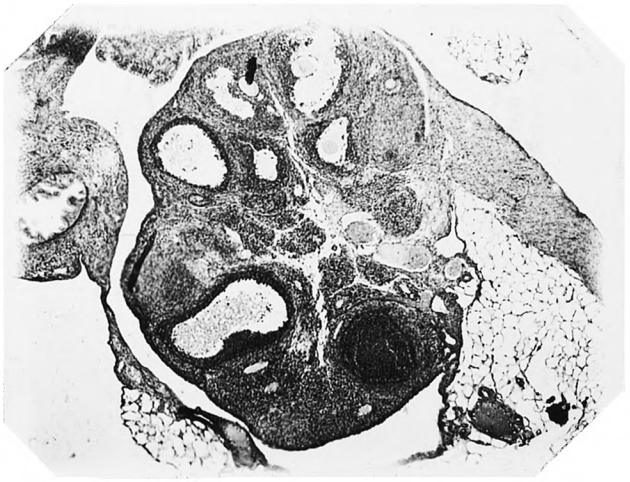

Figure 1.

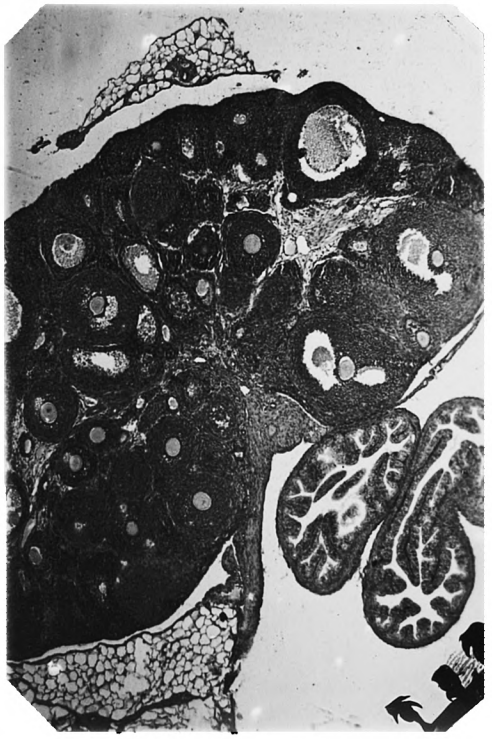

Figure 2.

PLATE III.

Fig. 1. Section of control ovary. X285.

Fig. 2. Section of experimental ovary. X450.

PLATE III.

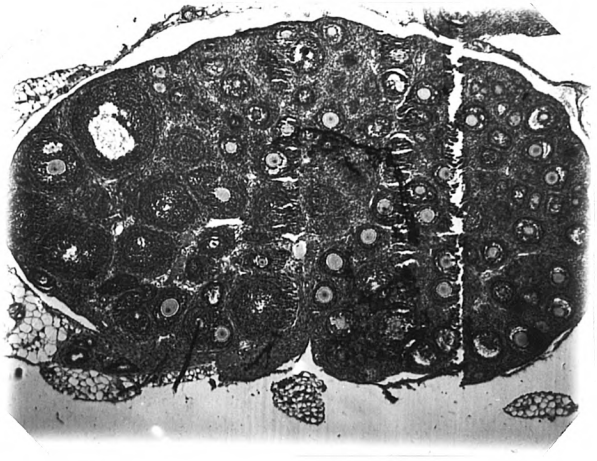

Figure --

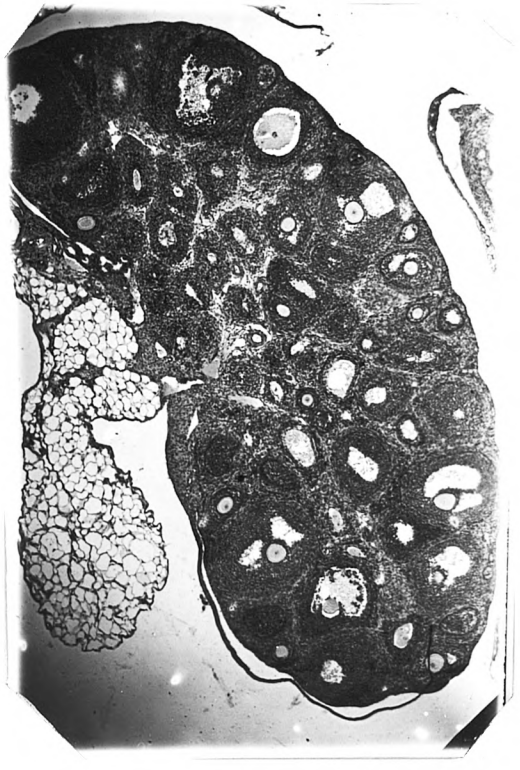

Figure 2.