THE EFFECT OF SUBCUTANEOUS INJECTIONS OF ANTUITRIN-S ON THE SEXUALLY INACTIVE ADULT MALE GROUND SQUIRREL

by

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INTRODUCTION

The ground squirrel is an annual-breeding animal which exhibits a periodicity in its sexual cycle. During a portion of the year it is sexually inactive. A seasonal variation in the amount of gonad-stimulating hormone secreted by the anterior pituitary gland seems to be an

influencing factor (Moore et al., 1934). The external influences which regulate this periodicity within the pituitary itself are still unknown.

In the work conducted by Johnson, Gann, Foster and Coco (1934) on the physiology of reproduction of the ground squirrel, Citellus tridecimlineatus arenicola (Howell), an attempt was made to stimulate these animals to sexual activity during the inactive period by pituitary implants. The work herein reported aimed to accomplish the same end by the use of the gonadotropic substance of human pregnancy urine. This is contained in high concentration in the commercial extract, antuitrin-S*.

The chief aims of this investigation are: (1) to find out the histological and physiological effects of antuitrin-S injections on the male gonads and accessory organs and (2) to compare these results with those reported by other workers who have used the same principle on different animals.

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* For brevity, antuitrin-S will be represented by AS and pregnancy urine by PU.

REVIEW OF LITERATURE

This review will be composed of three parts: first, a brief discussion of the nature and occurrence of the gonadotropic substance of pregnancy; second, a presentation of the results of experiments employing it; and third, a statement of the general situation as regards the sexually inactive adult ground squirrel.

The gonad-stimulating substance was discovered in the urine of pregnant women by Zondek and Aschheim (1928). They found it to appear soon after conception and to rise at once to a high level which is maintained until the eighth month of gestation. Thereafter, the hormone declines gradually in quantity and disappears at about eight days post-partum.

According to Smith and Engle (1934), Zondek long maintained that this substance was of pituitary origin and named it "prolan". As a result, this term has come to signify in the German literature either (1) the gonad-stimulating principle of pregnancy urine or (2) the sex hormone secreted by the anterior pituitary gland itself (Allen, 1932, p. 776). However, in this country the name "prolan" is used in a more restricted sense, i.e., to designate only the former of the two mentioned substances.

Many of the Mammalia develop a special cell type in the pituitary gland during pregnancy, known as Erdheim-Stümme cells, which some workers have considered as a possible source of this gonadotropic substance of pregnancy urine. The only animals in which this principle has been demonstrated in the blood and urine are the human, great ape and mare (Allen, 1932, p. 792). Consequently, these cells are probably not the source of the substance in question. Collip et al. (1931) believe that emmenin, the gonadotropic substance which they have extracted from the human placenta, is non-pituitary in type and is truly a placental hormone.

The presence of a gonad-stimulating principle in urine is extremely limited in the Mammalia. A substance similar to PU, insofar as it stimulates the ovaries of immature rats, has been demonstrated in the blood serum of gestating mares (Cole and Hart, 1930) and in the urine of pregnant apes. Snyder and Wislocki (1931) have shown the inability of the urine of pregnancy of cats, dogs, rats, rabbits and monkeys; and Leonard (1931), that of the cow to elicit ovulation in the rabbit.

A gonad-stimulating hormone also appears in human female urine concurrently with certain pathological conditions including hydatidiform mole, genital carcinoma and chorio-epitheliomata (Allen, 1932, p. 781 and 782). The

detection of its presence is of extreme diagnostic value. According to Smith, Engle and Tyndale (1934), Zondek reported its presence in the urine of ovariectomized women and of those past the menopause but these workers, as well as Leonard and Smith (1933) have detected a physiological difference in the properties of castrate and pregnancy urines and choose to call the former "follicle-stimulating" urine.

Considerable work has been done on various animals to determine the influence of injections of whole urine of pregnancy and extracts of it on the male genital system. These experiments included: (1) attempts to stimulate the immature rat, mouse, monkey, pigeon, duck and fowl to premature spermatogenesis; (2) efforts to replace by PU the stimulative influence lost in hypophysectomy; and (3) attempts to alleviate human clinical cases of cryptorchidism and underdevelopment of the male genitalia. The above points will be reviewed in the order listed.

Many investigations have been carried on using PU on immature animals. Fels (1927) induced an increase in size of the accessory sex glands by injecting serum of pregnancy. Brouha, Hinglais and Simmonet (1929) obtained the same effect in addition to gonadal hypertrophy in the rat, mouse and guinea-pig with PU.

Certain investigators using varying doses of prolan or PU have brought about some localized destruction of the germinal epithelium (Bourg, 1931; Boeters, 1930,1931; Borst, Döderlein and Gostimirović, 1930). Borst reported no acceleration of germinal epithelial development after 5 to 10 days treatment with PU but did obtain an hypertrophy of the interstitial tissue and growth of the accessory organs. Definite injury was obtained with certain urines only. Boeters induced spermatogenesis up to and including spermatid formation with small doses but large doses of prolan (10,000 R.U. per day) caused lysis of the germ cells. Borst et al. administering 30-200 R.U. daily, likewise obtained interstitial cell hypertrophy and, in certain animals, increased mitoses with larger doses.

Neumann and Péter (1932) treated male mice with a total of 2000 R.U. of prolan A and B from the twentieth to seventy-sixth day of life and caused inhibition of maturity. Neumann (1931) found small doses of prolan A and "Prähormone" without effect on the interstitial cells or tubules while larger doses caused symplasm formation in the latter.

Kraus (1930) observed that the amount of prolan A and PU required to induce an increase in interstitial cells and hypertrophy and hyperplasia of the accessory glands, often caused appearances of regression in the tubules. He,

therefore, concluded that this degenerative effect was secondary and was a pressure atrophy caused by the increased amount of interstitial tissue. On the other hand, Boeters (1931) believes that testicular weight is always increased, and that interstitial growth is secondary.

Engle (1929) treated immature male rats with PU and caused marked hypertrophy of the genitalia, an increase in the interstitial cell mass, varying degrees of destruction of the germinal epithelium but no acceleration of spermatogenesis. He also observed a closer similarity between the effect of fresh AP implants and PU injections in the male than in the female, although they differ in the tendency of the latter to induce cytolysis of the germinal epithelium and a definite increase in the interstitial cells. Later, Engle (1932a) combined PU with an extract of sheep AP and caused testicular enlargement due to tubular growth and to an increase in the interstitial cell mass which was uniform in the monkey but not so regular in the rat. There was no acceleration of spermatogenesis but an increase in the size of the seminal vesicles. In some tubules the mitoses

seemed unaffected while in others a degeneration occurred with clumped masses in the lumina.

Moore and Price (1931) with urinary hebin (Wallen-Lawrence and Van Dyke) caused a slight increase in the gross measurements of the testes of immature rats, little or no increase in tubular diameter and little or no acceleration of spermatogenesis, but a decided hypertrophy of the interstitial cells and enlargement of the prostate and the seminal vesicles. The latter two glands exhibited a precocious attainment of secretory differentiation. These investigators also obtained a suggestive earlier appearance of spermatozoa but did not consider it significant. The same results followed fresh hypophyseal implants. In adult males, hebin resulted in a marked enlargement of the prostate and the seminal vesicle; the testis, however, showed only a slight increase in interstitial tissue. These workers emphasized the Leydig cells as the probable source of the male hormone.

Dharmendra (1931) obtained hypertrophy of the testicles, the seminal vesicles, Cowper's gland and the penis. He is one of the few definitely to report descent of the testis induced by PU in the immature rat.

Molien, D'Amour and Gustavson (1933), using urinary hebin in immature rats, brought about similar effects,

namely, no hastening of spermatogenesis but growth of the testicular interstitial tissue and an increase in the weight of the gonads and in the size of the seminal vesicles.

Butcher (1932) compared the effects of whole urine of pregnancy and an extract of it (antuitrin-S) on the reproductive tract of the immature rat. The former inhibited spermatogenesis while the latter accelerated it. Both methods of treatment lead to an hypertrophy of the interstitial cells and of the accessory glands and to an enlargement of the seminiferous tubules.

Smith and Leonard (1934), on the whole, agree with the results thus far presented in that PU (antuitrin-S and follutein) does not hasten maturity and does cause an increase in the interstitial cell mass, but they found no injury to the seminiferous tubules in either mature or immature male rats. These workers observed the infrequent appearance of intra-tubular columns of typical spermatogenic cells.

Since the testes of the pigeon and dove serve as an extremely sensitive test object for the AP sex hormone, they were tried as test objects for the various gonad-stimulating urines (Riddle and Polhemus, 1931; Evans and Simpson, 1934). Neither urine of human pregnancy, of human

menopause, of pregnant mares, nor of a case of embryonal carcinoma of the testis stimulated the bird testes. However, a urinary hormone has been found in a case of testicular tumor which did give maximum acceleration in growth of the pigeon testis. Schockaert (1933) found PU extracts without any effect on the male gonad of the duck and fowl.

Bourg (1930a and 1930b) used a different approach and performed a series of experiments from which he concluded that PU stimulates the interstitial cells to secrete the male hormone. This investigator first irradiated the testes of immature rats which destroyed the germ cells and then he treated the animals with PU. The interstitial cell mass hypertrophied and the seminal canals matured precociously.

Work on replacement therapy in hypophysectomized rats with PU has been limited to three groups of workers. Smith and Leonard (1934) caused enlargement of the accessories and hypertrophy of the interstitial tissue. In adult rats, spermatogenesis was maintained by immediate treatment after hypophysectomy. Delayed treatment in both immature and mature rats caused marked increase in testicular weight, enlargement of the seminiferous tubules and an increased activity of the germinal epithelium. Injections into immature hypophysectomized rats begun at the time of the

operation produced such an effect that spermatogenesis continued only to spermatid formation.

Collip, Thomson and Selye (1933) obtained somewhat different results when they used the anterior pituitary-like substance of the human placenta. This increased the interstitial tissue of hypophysectomized rats and enlarged the accessories but did not check the degeneration of the germinal epithelium.

After observing from evidence presented by other experimenters that the gonadotropic substance of PU and the sex hormone of the AP differed fundamentally in nature, Evans, Pencharz and Simpson (1934) attempted to find in the anterior pituitary secretion a principle which might activate PU, or act with it to produce a more normal effect. To this end, after considerable preliminary work, they extracted from fresh glands a fraction free from the growth and the gonad-stimulating hormones, and injected it alone and in combination with PU into hypophysectomized male rats. With either extract alone, or with both combined, spermatozoa were produced. These workers concluded that pregnancy-prolan stimulates the Leydig tissue while the synergist is primarily concerned with the stimulation of the production of the male germ cells. Evans, Meyer and Simpson (1931) had previously believed that PU either con-

verted the growth hormone into a gonad-stimulating one or activated a prohormone. However, this belief was refuted by Leonard (1932) who obtained increased stimulation of immature rat ovaries by combining PU and the hypophyseal sex hormone.

Inasmuch as in the human, descent of the testis occurs at the time of birth when estrin and the gonadotropic substance are in high concentration in the maternal circulation, Engle (1932b) devised an experiment with monkeys which was to have far-reaching clinical application to cases of cryptorchidism. He injected extracts of PU and AP together into immature male Macacus monkeys and successfully induced descent and enlargement of the testes with preliminary development of the scrotum. The interstitial cell mass was increased. Several medical men have since used the treatment to good advantage in causing descent of the testis and with varying degrees of success in relieving the condition of aspermia (Sexton, 1934; Brosius, 1935; Rubinstein, 1934).

Considering the ground squirrel more specifically, spermatogenesis has been accelerated by implants of rat pituitaries (Johnson, Gann, Foster and Coco, 1934). Recently Moore et al. (1934) have published an incomplete report of the stimulation of the testis to the production

of male hormone and the induction of spermatogenesis with antuitrin-S. At least, the "residual cells" metamorphosed into spermatozoa.

Moore (1926) showed that the mammalian scrotum serves as a thermoregulator and that until the gonad descends, the higher temperature of the abdominal cavity inhibits spermatogenesis. This work has, in general, been confirmed by Phillips and McKenzie (1934).

The foregoing literature may be summarized under the following points: (1) the pregnancy urine gonadotropic substance appears to act primarily on the Leydig cells of the testis, stimulating them to grow and to secrete the male hormone which in turn results in an hypertrophy and increased activity of the accessory glands; and (2) PU causes very little, if any, acceleration of spermatogenesis in the normal immature animal and does induce some stimulation in the hypophysectomized rat and sexually inactive ground squirrel.

MATERIALS AND METHODS

Animals Used

Healthy, adult male ground squirrels, free from excessive fat, were selected for the experiment. These animals also exhibited sexual inactivity, at which time the

testes are cryptorchid and all of the genitalia in a restive condition.

Operative and Experimental Technique

With some changes, the operative technique is the same as that employed by Johnson, Gann, Foster and Coco (1934). At the beginning, each animal was anesthetized and opened through the linea alba. The tunic of the right testis was punctured and a few seminiferous tubules squeezed out by means of tweezers and then fixed. At the same time each testis was measured in three dimensions by means of a millimeter scale. The animals were permitted to rest for two days after which time the experimental animals received daily subcutaneous injections of antuitrin-S along the back. Five Rat Units of the extract diluted to .5 cc. with Locke's physiological solution constituted the daily dosage. With the exception of the first four injections of saline into animal No. 939, a different syringe was used for the controls.

On either the fifth or ninth day, the right testis was removed through an abdominal incision made to the right of the first opening. In order not to lose the effect of previous treatment, daily injections were resumed on the next day and continued until a total of either 12 or 16 had

been administered, after which time an autopsy was made on the animal. The left testis, seminal vesicles, prostate and Cowper's glands were excised, measured and fixed. After removal to 70 per cent alcohol, all organs were weighed to the nearest milligram. Besides the seminiferous tubules excised at the beginning of the experiment serving as a control for the animal from which they were taken, four males received identical treatment including the operations, except that they were injected daily with .5 cc. of Locke's solution in place of the extract. This made it possible to ascertain any effect the operations alone might have on the reproductive tract.

Daily observations were made to determine the descent of the testes, formation of scrotum, degree of pigmentation and swelling of the penis.

Both the Locke's solution and the extract were kept at the same temperature and the use of separate hypodermic syringes for each insured against accidentally injecting extract into a control.

Milk, sprouted oats and a specially prepared meal constituted the diet during the experiment.

The average diameter of the seminiferous tubules was determined by taking the widths of ten tubules on a given transverse section by means of a calibrated ocular microm-

eter and then averaging them. Care was exercised to select only those tubules which had been cut transversely.

Histological Technique

All tissues were fixed overnight in Bouin's solution, to which had been added a few crystals of uric acid. The picric acid was later removed from the tissues by repeated washings with a saturated alcoholic solution of lithium carbonate. Paraffin served as the embedding medium; sections were cut at ten microns and then stained with Kornhauser's (1930) hematin staining method with eosin-bluish as counterstain.

EFFECTS OF EXTRACT INJECTIONS

Effect on the Testis

(1) Macroscopic. Antuitrin-S caused a striking enlargement of the testes when an increase of one mm. in each of two directions was considered significant. As shown by Table 2, statistical treatment confirms this conclusion. The change in size was most noticeable after the first four injections but continued throughout the course of the experiment.

The enlargement of the male gonads was due primarily to an increase in the size of the seminiferous tubules.

The control tubules from the experimental animals averaged 130_u in diameter, while those of the left testis, after 12 injections, averaged 198_u and after 16 injections, 224_u, a gain of 52 per cent and 72 per cent, respectively. Table 2 shows an approximate correlation in the ratios of enlargement of the testes at 4 or 8 injections as compared with the same testes after receiving no injections and with the controls at 4 and 8 injections, and with the ratio of increase in tubular diameter during the first 4 to 8 injections of antuitrin-S. However, the tubules of the control animals which received saline also showed a slight increase in diameter of 10 per cent after 12 injections and of 8 per cent after 16.

After twelve injections, the average weight of the left testes of the animals treated with antuitrin-S, showed a 273 per cent increase over the average weight of those from the controls. Similarly, the left testes excised after 16 injections exhibited a 219 per cent increase over the controls.

(2) Microscopic. Whereas, the control seminiferous tubules exhibited primary spermatocyte spiremes as the most advanced stage of spermatogenesis and no lumina (Fig. 1), the most striking occurrence after four injections was the formation of lumina in practically all tubules.

This seemed to be the first effect of the extract on the germinal structures.

From this point on, the histological picture becomes somewhat confusing. Under further treatment, the cavities in some tubules became abnormally enlarged (Fig. 3), the lumina of which were lined by a single layer of primary spermatocytes and a single layer of spermatogonia or sometimes by Sertoli cells and spermatogonia. These tubules were rarely stimulated to spermatogenesis.

On the contrary, as indicated in Table 1, other tubules remained in a more normal condition and underwent germinal development. All of the experimental animals, after at least 12 injections, showed secondary spermatocytes. After 16 injections three, and possibly four, animals had produced spermatozoa. In addition, the right testis of No. 855 contained spermatozoa on the fifth day but when an autopsy was made developing spermatozoa were the most mature germinal element apparent. It must be emphasized that when maturation divisions appeared, they were usually found in only a few tubules. Similarly, spermatozoa were few and generally were not present.

Among the controls only one animal, No. 939, underwent any sexual development. It was seen from the control seminiferous tubules that this animal was more advanced

Table 1. Effects of Antuitrin-S and Saline Injections.

No.	Year of birth	Date experiment begun	Testis: Size in Millimeters: Injections 0	Injections 4	Injections 8	Injections 12	Injections 16	Testis: Average Diameter of Tubules (μ): Injs. 0	Injs. 4	Injs. 8	Injs. 12	Injs. 16	Wt. (Mg.)	Histology*: Injections 4	Injections 8	Injections 12	Injections 16	Descent	Accessory Organs: Prostate: Size (mm.)	Prostate: Wt. (Mg.)	Seminal Vesicles: Av. size (mm.)	Seminal Vesicles: Av. Wt. (mg.)	Cowper's: Av. size (mm.)	Cowper's: Av. Wt. (mg.)	Pig.	Remarks
														Experimental Animals												
			14x5.25x5	16x7x7									414													
857	1933	9-17-34	14x6x5	16x7x8		19x9x8		139	188		175		678 549	1		1,2,3		2	8.25x7x4	133	17x7x6	297	6x6x5	94	2	
			15x6x6.25		19x9x8.25																					
860	1933	9-17-34	15x6.25x5		19x8.75x8		21x10x9	125		193		258	755 465		1,2		1,2,3,5,6	2	10x8.25x5	189	19.25x8x6	438	9x7x5	205	1	
			14.25x6x6		16x8.50x8																					
858	1933	9-17-34	14x7x6		16x8.75x8		17x11x8.50	125		187		207	736 563		1,2		1,2,3,4,5,6,7	2	8x7.50x4	147	18.75x6x3	301	8x7x5.12	160	2	If spermatozoa, undeveloped
			16x7x5.75	20x9x7.50																						
859	1933	9-17-34	16x6x6	19x8x8		22x10.25x8		125	169		199		980 452	1		1,2,3		2	8x7x4	118	17x9x3.87	296	8x7x4	148	2	
			13x6x6.25		17x7x8.25																					
942	1933	10-22-34	13x6x6.50		17x9x7.75		18x10x8	126		187		217	805 395		1,2		1,2,3,6	2	9x7x4.75	124	21x5.75x4	365	7x6x4.75	135	2	
			13x7x6.25	17x9x7																						
873	1933	10-29-34	13x5.75x6	18x7x7		17x8x7.75		144	187		214		552 452	1,2		1,2,3,5,6		1-	6x6x3.50	68	21x4x3.25	166	6x5.25x4	80	2	
			14x6.75x7	17x8x7																						
855	1933	11- 2-34	14x6x6.50	17x8x7		19x9.25x9		122	162		190		813 495	1,2,3,5,6,7		1,2,3,5,6		1-	8x6x4	100	17.25x6x4	205	7x5.25x4	92	2	Spermatozoa in 5 tubules; thicker epithelium
			11x6x5.75		14.50x8x8																					
935	1934	11- 6-34	11x6x6		16.50x8x8		17x10x9	152		186		221	763 414		1,2		1,2,3,4,5,6,7	2	7.25x6x4	71	16.87x5x3	164	5.37x4x3	42	1	Spermatozoa few, undeveloped
			14x5x5.25	16x7x7.75																						
944	1934	11-14-34	14x5x4.75	16x8x7.25		17x9x7.50		129	176		194		669 607	1,2		1,2,3,6		2+	7x5x3	71	14x3.37x3	78	4.87x4x3	35	2-	
			12x7x6		19x9x7.75																					
936	1934	11- 7-34	13x6x6.50		19x9x8.50		19x8x8	120		194		227	897 320		1,2,3,4,5		1,2,3,4,5,6,7	2	8.50x7x4	119	16x6x5.12	308	6x6x4.75	72	2-	
			12x6x5	14x7.50x7																						
938	1934	11- 7-34	12x6x5	14x7x6.50		16x8x8		124	180		209		542 442	1,2,3,4		1,2,3		2	8x6x5	103	15x5x6	237	6x4.87x4	71	2	
			13x5.50x5		16x9x7.25																					
952	1934	11-20-34	13x6x5		16x8x7.75		16x9x9	131		202		215	662		1,2,3		1,2,3,5,6,7	2	7.50x7x4	103	14.62x4x4	187	6.62x4x3	62	2	Very few spermatozoa
														Control Animals												
			14x6x5.75	.	13x6x5.50								252													
939	1934	11- 9-34	14x6.25x6	.	13x5x5.75		12x6.75x6	141		143		149	280 197		1-		1,2	3	2x2.25x1	4	4.62x2x1	4	1.75x1x1	2?	3	
			12x5x6		13x6x6																					
945	1934	11-14-34	12x5x6.50		13x6x5.50		12.25x6x5	135		---		150	210 302		---		1-	3	2x2.75x1	3	4x2x1	4	1.62x1x1	2	3	
			12x6x5.50	13x6.25x6																						
882	1933	9-18-34	12.75x6x5	13x6x6		13x6x5.75		114	152		125		241 165	---		---		3	3x3x2.50	19	6x2x2.25	13	2.50x2x2	8	3	
			12.50x4x4	11x5x5.25																						
951	1934	11-20-34	12x5x4.50	12x5.50x4		10x5.25x5		108	139		121		132	---		---		3	2x1.50x1	2	3x1.75x1	4	1x1x1.37	2	3	

*Unless otherwise noted, all tubules removed at beginning and control testes showed primary spermatocyte spiremes and no lumina.

Testis Histology: 1, lumen formed; 2, first maturation division; 3, secondary spermatocytes; 4, second maturation division; 5, spermatids; 6, developing spermatozoa; 7, spermatozoa.

Testis Descent: 1, Complete descent and complete scrotum; 2, testis laterad to penis or slightly posterior; 3, no indication of descent.

Scrotal Pigmentation: 1, completely pigmented -- black; 2, slightly pigmented; 3, not pigmented.

Table 2. Effects of Injections on Male Genitalia.

Line number	Number of glands	Glands or tubules	Injections No.	Injections Kind	Range of Vol., Diam. or Weight	Mean of Vol., Diam. or Weight	S. E. of mean	S.D. of distribution	Lines compared	Diff. means	S. E. difference	Diff. / S. E. Diff
1	24	Testes	4 & 8	AS[1]	588-1368 mm^3	1000.4 mm^3	41.52	203.05				
2	24	Testes	0	AS	240-588 mm^3	406.4 mm^3	35.13	171.8	1 vs 2	594.0	54.38	10.92[2]
3	8	Testes	4 & 8	Sal*	240-468 mm^3	378.0 mm^3	30.14	84.4	1 vs 3	622.4	51.2	12.12
4	8	Testes	0	Sal	192-504 mm^3	349.5 mm^3	32.42	90.8	3 vs 4	28.5	42.4	.67
5	12	L. Testes	12 & 16	AS	952-1890 mm^3	1381.8 mm^3	79.53	275.2				
6	12	L. Testes	4 & 8	AS	588-1368 mm^3	1002.4 mm^3	56.15	194.3	5 vs 6	379.4	97.3	3.89
7	4	L. Testes	12 & 16	Sal	250-432 mm^3	358.0 mm^3	33.6	67.3	5 vs 7	1023.8	86.3	11.86
8	4	L. Testes	4 or 8	Sal	240-468 mm^3	355.7 mm^3	41.9	83.9	7 vs 8	2.7	53.7	.05
9	12	Tubules	0	AS	120-152$_u$	130.1$_u$	2.8	9.8				
10	12	Tubules	4 & 8	AS	162-202$_u$	184.2$_u$	3.2	10.9	9 vs 10	54.1	4.2	12.88
11	4	Tubules	12 & 16	Sal	121-152$_u$	136.2$_u$	6.7	13.5	10 vs 11	48.0	7.3	6.5
12	12	Tubules	12 & 16	AS	175-258$_u$	210.5$_u$	5.88	20.0	11 vs 12	74.3	8.9	8.34
13	12	Prostates	12 & 16	AS	68-189 mg	112.1 mg	9.68	33.56				
14	4	Prostates	12 & 16	Sal	2-19 mg	8 mg	3.22	6.44	13 vs 14	104.1	10.2	10.2
15	12	Cowper's	12 & 16	AS	35-205 mg	99.6 mg	14.39	49.8	15 vs 16	96.1	14.44	6.65
16	4	Cowper's	12 & 16	Sal	2-8 mg	3.5 mg	1.29	2.59				
17	12	Sem.Ves.	12 & 16	AS	78-438 mg	253.4 mg	27.54	95.40	17 vs 18	247.2	27.59	8.95
18	4	Sem.Ves.	12 & 16	Sal	4-13 mg	6.2 mg	1.80	3.60				

1 - Antuitrin-S

2 - Saline

* - 2.7 is considered a significant figure.

than any of the others at the beginning of the experiment in that it possessed incipient lumina in some tubules. When an autopsy was made, a few anaphase and metaphase stages of the first maturation division were observed. In addition, No. 945 showed the formation of slight lumina in some tubules.

In practically all experimental animals, the nuclei of the germinal cells of the left testes did not stain well as compared with those of the right testes from the same animals and with those of the left gonads from the controls. This combined with an infrequent "vacuolization" of the germinal epithelium constitutes the only effect observed which might have been degenerative.

No noticeable change was observed in the interstitial tissue with the exception that the individual cells may have undergone hypertrophy.

Effects on the Accessory Genital Organs

The seminal vesicles, prostate and Cowper's glands of the sexually inactive ground squirrel are small, their acini much reduced in size or totally absent, and the secretory epithelial cells are low cuboidal and inactive (Figs. 4 and 6).

(1) Macroscopic. All of these structures changed markedly when the male hormone output of the testis was

increased under the influence of antuitrin-S (Figs. 5, 7 and 8). They showed a great increase in size and weight over the controls. The change in weight was variable but approximately proportional to the duration of the experiment. After sixteen injections the experimental seminal vesicles averaged 253 mg.; the prostates, 112.1 mg.; and the Cowper's glands, 99.6 mg. On the other hand, the same organs from the controls averaged 6 mg., 7 mg., and 3.5 mg., respectively. These differences proved statistically significant (Table 2).

(2) Microscopic. The acini enlarged as they became filled with secretion from the activated secretory epithelium. The cells of this tissue changed to a high columnar type and in those of the Cowper's gland and prostate, the secretory granules were frequently visible.

DISCUSSION

Since previous work in this laboratory has shown that individual ground squirrels vary considerably in their sexual development at any given time, the operative procedure described on Page 15 was designed to enable the experimenter to give adequate consideration to this important factor. The seminiferous tubules taken from the right testis at the beginning of the experiment served as

a control for the animal from which they were removed.

The importance of considering the degree of descent of the testes cannot be overemphasized in the face of work already reviewed (Moore, 1926; Phillips and McKenzie, 1934). These workers demonstrated that the testes must be located in a region of comparatively low temperature as is found in the scrotum in order that spermatogenesis might be carried on. The abdominal approach in removing the right testis was preferred to the scrotal approach in order to avoid complications in the latter region which might hinder testicular descent and, also, to avoid any discoloration which might obscure possible pigmentation of the scrotum caused by antuitrin-S. Nevertheless, the abdominal approach called for extreme caution. If the incision were not made far enough cephalad the edges of the opening through the peritoneum were likely to adhere to the portion of the right fat body from which the gonad had been cut. If this occurred, intra-peritoneal adhesions and, frequently, inflammation followed. Thus, any increased body temperature in the region of the testes might inhibit spermatogenesis. The degenerative effects on the germinal epithelium reported by many workers treating immature animals with PU (Allen, 1932, pp. 775-776) may have resulted partially from the inability of the gonadotropic principle to induce descent because of somatic immaturity. Dharmendra (1931)

definitely reported descent after treatment of young rats with PU.

An examination of Table 2 shows that although practically complete descent occurred in only two cases, the gonads of all experimental animals underwent partial descent, which made it possible for at least one end of the testis to have been located in the cooler region of the scrotum or in the lower portion of the inguinal canal.

An endocrine situation exists in the sexually inactive ground squirrel which is somewhat analogous to that in the hypophysectomized animal, insofar as it pertains to the absence of the AP gonad-stimulating hormone. The induction of spermatogenesis herein reported is in agreement with the replacement studies of Smith and Leonard (1934). On the other hand, Collip et al. (1933) were unable to restore spermatogenesis in the hypophysectomized rat with the gonadotropic substance from the human placenta. The induction of spermatogenesis in the sexually inactive ground squirrel with antuitrin-S reported by Moore et al. (1934) is confirmed. Table 1 shows that spermatogenesis is more readily induced as the normal time of sexual activity is approached. This period is about the middle of December for laboratory ground squirrels (Johnson, Foster and Coco, 1933). This might indicate that there is some physiological change preparatory to the appearance of sexual activity

which may be due to the secretion of the gonad-stimulating hormone in very small amounts.

With considerable regularity, the nuclei in the germ cells of the left testes from the experimental animals, removed after 12 or 16 injections, did not stain as well as those of the right testes excised after 4 or 8 injections. It does not seem reasonable to ascribe this poor staining reaction to a degenerative effect, since equal volumes of Bouin's were used to fix each testis and it may well be that the increase in volume of the left testis between the middle and termination of the experiment resulted in an insufficient amount of fixative for this testis.

Moore et al.(1934) reported compensatory hypertrophy of the remaining testis during anoestrus after unilateral castration of the ground squirrel. It is evident from Table 1 that there is no evidence of such an occurrence among the four controls of this experiment. In spite of the fact that four animals may be an insufficient number upon which to base a final statement, it is clearly indicated that for the period of this experiment, possible compensatory hypertrophy is so negligible that it need not be considered.

SUMMARY

1. Daily subcutaneous injections of 5 R. U. of antuitrin-S caused partial descent, marked enlargement and an increase in the weight of the testes.
2. The seminiferous tubules were increased in diameter and formed lumina which were often abnormally large.
3. Spermatogenesis was induced and spermatozoa were formed.
4. The extract caused the secretion of the male hormone with a consequent enlargement and secretory differentiation of the seminal vesicles, prostate and Cowper's glands.
5. These results are in agreement with most published works dealing with adult animals.

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EXPLANATION OF PLATES

PLATE I.

Fig. 1. A transverse section of a seminiferous tubule from No. 938 at the beginning of the experiment which shows primary spermatocytes in the spireme stage and no lumen. X320.

Fig. 2. Seminiferous tubules from the right testis of No. 938 after 8 injections of antuitrin-S. First maturation divisions are visible. X320.

PLATE I.

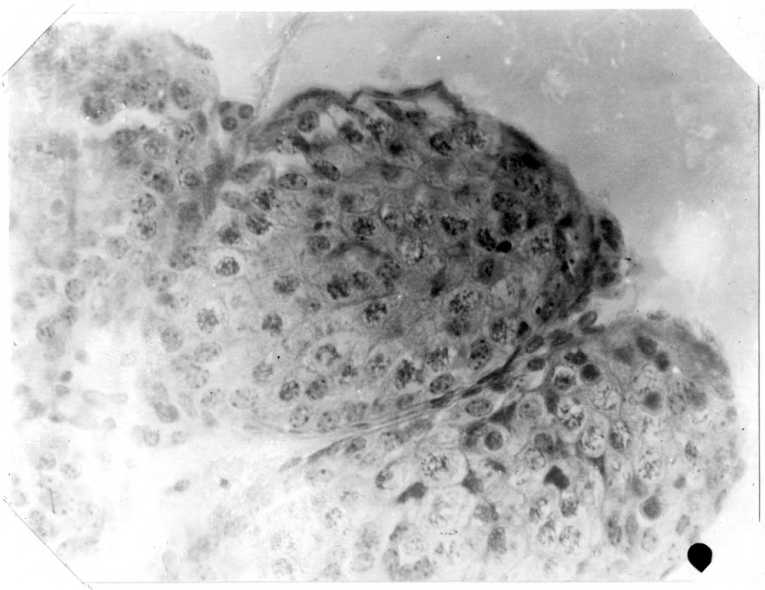

Figure 1.

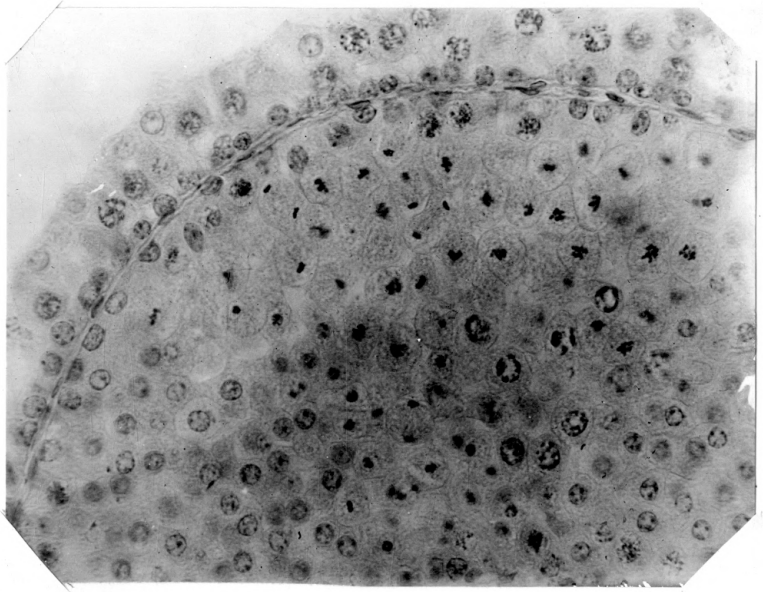

Figure 2.

PLATE II.

Fig. 3. A longitudinal section of a seminiferous tubule from the left testis of No. 938 after 16 injections which shows the abnormally enlarged lumen. X320.

Fig. 4. Section of seminal vesicle of control No. 939. Acini are small and secretory epithelium is low cuboidal and inactive. S50.

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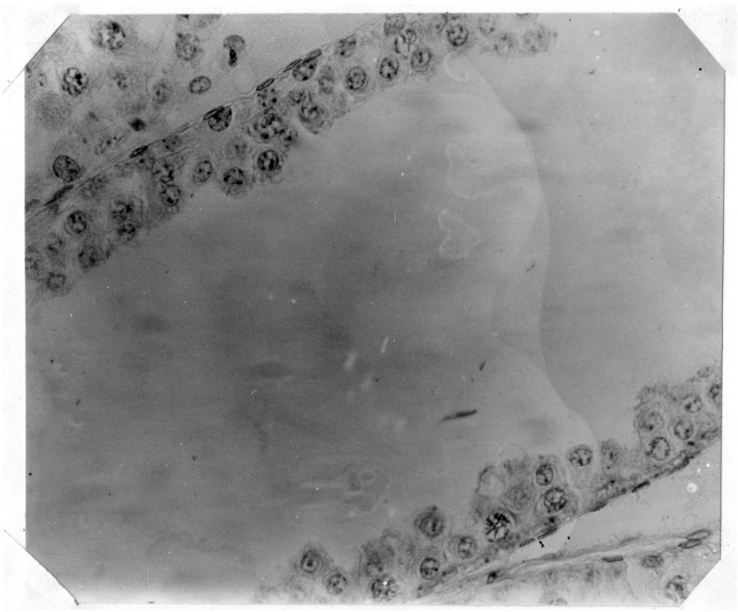

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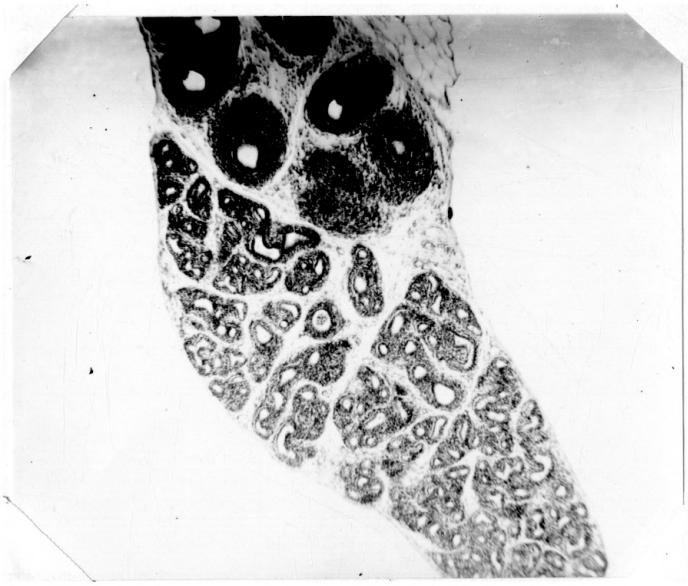


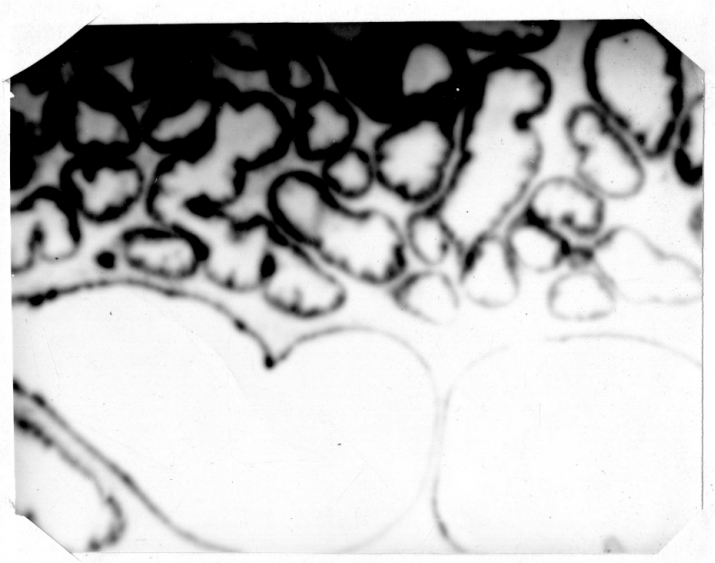
Figure 4.

PLATE III.

Fig. 5. Seminal vesicle of No. 938. Acini are large and filled with secretion. Secretory epithelium is high columnar and active. X50.

Fig. 6. Prostate from No. 939. Acini are small and secretory epithelium is undifferentiated. X320.

PLATE III.



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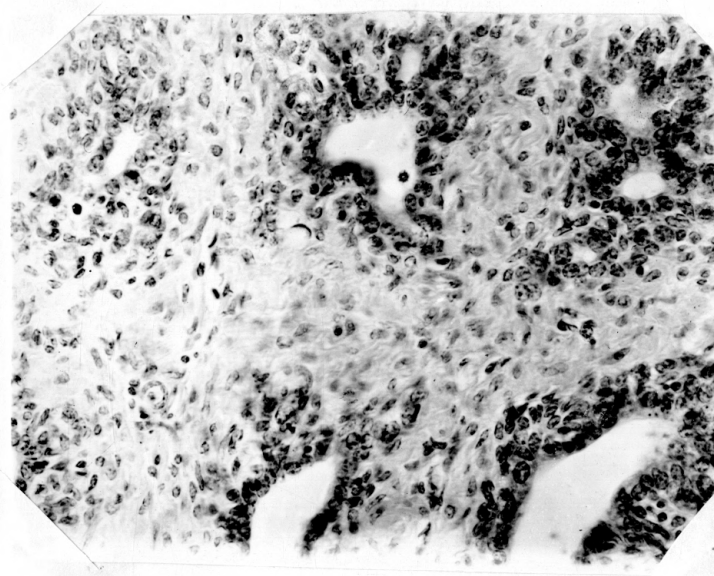


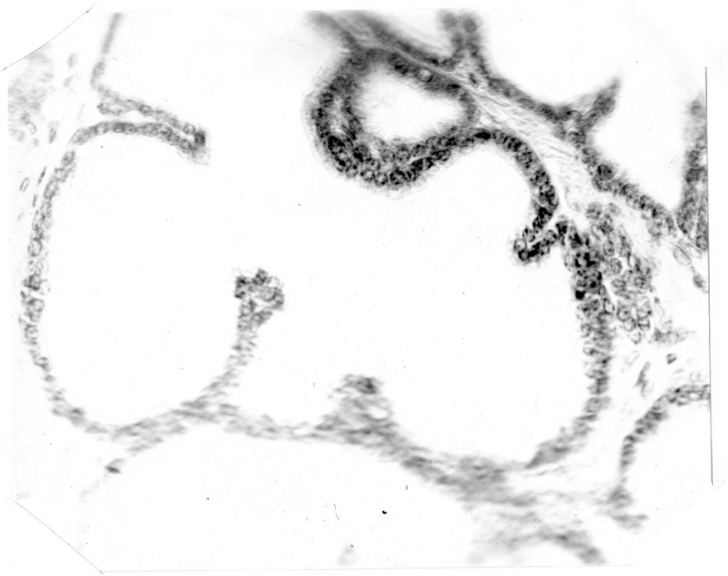
Figure 6.

PLATE IV.

Fig. 7. Prostate from No. 938. Acini are large with secretion and secretory epithelium is columnar and active. X320.

Fig. 8. Cowper's gland of No. 938 showing large acini with secretion and, also, secretory epithelium differentiation. X320.

PLATE IV.



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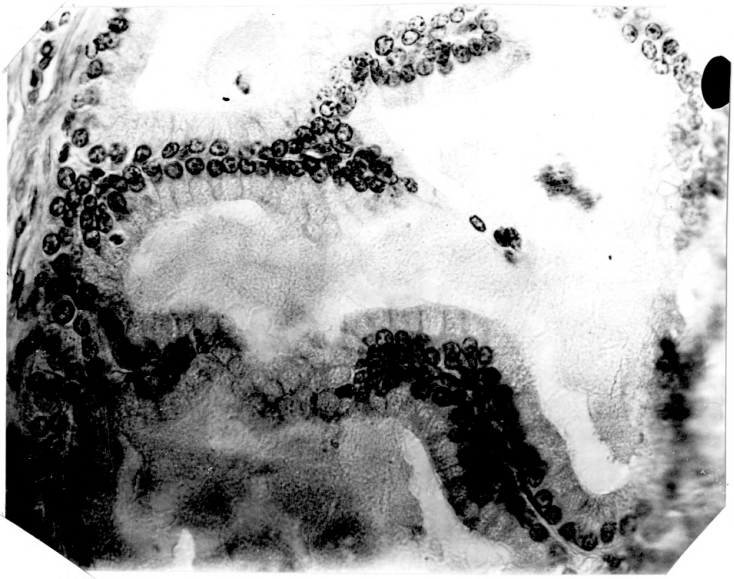

Figure 8.