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The influence of Theelin on the histology and physiology of the primary and accessory sexual organs of the Albino rat

Violet Alice Garrett
Atlanta University

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THE INFLUENCE OF THEELIN ON THE
HISTOLOGY AND PHYSIOLOGY OF THE
PRIMARY AND ACCESSORY SEXUAL ORGANS
OF THE ALBINO RAT

A THESIS
SUBMITTED TO THE FACULTY OF ATLANTA UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF ARTS

BY
VIOLET ALICE GARRETT

DEPARTMENT OF BIOLOGY

ATLANTA, GEORGIA
JUNE 1936
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A crystalline substance was obtained from the urine of pregnant women by Doisy and Butenandt, independently, in 1929. Doisy has given it the name, "theelin", which is derived from the Greek term, "theelus", meaning female. Its function is the maintenance of the accessory genital organs and the secondary sex characteristics of the female in full functional condition.

Since males and females differ in so many essential respects, it was natural to expect differences in the reaction of the two sexes to theelin. It is the purpose of this paper to check and attempt a verification of certain facts already established and to compare the effects of theelin on males and females thus:

1. Effects of theelin injections in the immature female rat as related to:
   (a) Production of "heat" or the "oestrus" cycle
   (b) Effects on the vagina, uterus, and uterine tubes

2. Effects of theelin injections on the immature male rat, as related to:
   (a) Development of the testes
   (b) Descent of the testes into the scrotal sac
Since the principal action of theelin is to induce growth in the tissues of the accessory genital organs of the female, it seems natural to conclude that this same hormone was probably the effective agent responsible for growth of these organs which results in the attainment of puberty. The immature rat is especially well adapted for experimental tests of this kind because the vagina does not open until the attainment of puberty. Rats and mice also have the shortest known oestrus rhythm among mammals, four or five days for both growth and degeneration phases. During this oestrus period the vaginal epithelium cornifies, providing a clear-cut end point, which can be followed in the living animal. For these reasons the investigator decided to use the immature rat as a test animal.
CHAPTER II
REVIEW OF LITERATURE

The hormone, theelin, is produced in the follicles. Its principal function is its influence upon accessory sex organs and secondary sex characters of the female, keeping them in full functional condition. This was demonstrated by Allen and Doisy, 1923, by injections of liquor folliculi from follicles of hog ovaries. The test animals used were mice and rats. Several injections of the active substance induced the accelerated growth, hyperemia, and secretion in the genital tract characteristic of the period of oestrus. The experimentally induced conditions were equal in degree to the maximum attained in the normal animal under ovarian influences. While in this induced oestrus condition the test animals experienced typical mating instincts, at times taking the initiative in courtship, and accepting coitus in a normal way. Therefore, sex instincts are ultimately dependent upon this hormone (Allen and Doisy and collaborators, 1924). In other rats the full oestrus growth reactions of the vagina may be obtained without evidence of mating reactions (Hemmingaen, 1929).

The most important of the reactions to this substance is an increase in growth in the accessory genital organs, vagina, uterus, uterine tubes, and mammary glands. The epithelial
tissues show a high incidence of cells in mitotic division. The epithelium of the vaginal wall is completely replaced at each oestrous period. Secretion from glandular tissues and a change in contraction rate and amplitude of muscular tissues of the genital tract follow the wave of growth. These processes culminate in oestrous, the normal period of sexual activity of lower mammals, which can be induced experimentally in ovariectomized animals by injections of the ovarian follicular hormone (Allen and Doisy, 1924). The temporary cessation of secretion of this hormone or a decrease below a threshold level fails to sustain this hyperplastic condition and much of the new growth resulting from the endocrine action degenerates and is removed, returning the accessory genital organs to the castrate or resting condition. After the hormone is exhausted, degenerative changes set in and most of the tissue resulting from its action during the preceding period is removed. Thus the alternate absence and presence of the follicular hormone is sufficient to supply the causative mechanism of the oestrous cycle (Allen and Doisy, and collaborators, 1924.)

In immature animals transformations in the genital tract may be induced both in the normal and in the ovariectomized rats by injections of the ovarian follicular hormone (Allen, Edgar and Doisy, E.A. 1924). Thickening of the vagina and cornification of the epithelium result in opening of the

---

1 Allen Edgar, Sex and Internal Secretions, Baltimore: Williams and Wilkins Company, 1932
2 Ibid., p. 404.
vaginal orifice. The tiny infantile uterus begins rapid growth and differentiation; besides enough secretion forms to dis- tend the uterus greatly.¹

Unusual effect of ovarian extract was demonstrated in the pocket gopher by Hisaw, 1925. He was able to cause resorption of the pubic bones, leaving the pelvic girdle open in front in young females and males with injections of ovarian extract.

The action of the ovarian extract in the immature animal gives exactly the same histological results in the accessory genital organs as are obtained from implants of anterior pituitary tissue or injections of active extracts of that gland (Smith and Engle, 1927). Implants of anterior lobes in immature female rats and mice produce changes in structure that are characteristic in the vagina and uterus of the normal rat and mouse at puberty and maturity. These changes are identical to those reactions to the ovarian follicular hormone. The conclusion then that the anterior pituitary hormone stimulates the accessory genital tissues to growth is obvious. That the anterior pituitary substance has no direct action upon the accessory genital organs is shown by negative results with ovariectomized animals.² It thus must act through the ovaries by stimulating their endocrine activity.

Evans and Simpson, (1928), associate two distinct substances with the mammalian anterior hypophysis: one a growth

¹ Edgar Allen, Sex and Internal Secretions, Baltimore: Williams and Wilkins Company, 1932. p. 415
² Ibid., p. 417.
hormone and the other a maturity-provoking hormone. The maturity-provoking hormone stimulates the development of the gonads.

Injections of ovarian extract in normal adult females are successful in accentuating and maintaining growth in the accessory genital organs. Continued administration of more than minimal doses may eliminate completely the degenerative phase of the cycle in the vagina of the normal animal as determined by the cell contents of the vaginal smear. The uterine mucosa is maintained on a high functional plane.

Injections of the follicular hormone into senescent albino rats, after the wave of sexual activity, have resulted in the typical wave of oestrus growth in the accessory organs similar to that already described in ovariectomized animals (Slonaker, 1927). Such injections have no stimulating effect on the ovaries. There is no evidence to indicate that this substance will rejuvenate the senescent female.

The adult male shows few distinctly harmful effects from injections of moderate doses of ovarian follicular hormone (Bugbee and Simond, 1926). In the immature male, however, it is possible to inhibit the normal growth of the spermatogenic tissues with relatively small amounts of ovarian hormone (Golding and Ramirez, 1928). The testes remain infantile, sperm are not developed, and descent of the testis into the scrotum is inhibited. The testes can be held in this state of development for several weeks, well past the normal time of attainment of puberty. Very soon after the cessation

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1 Edgar Allen, Sex and Internal Secretions, Baltimore: Williams and Wilkins Company, 1932. pp. 419-420
of injections, however, the retarded testes begin to grow very rapidly and descend into the scrotum. Two weeks after cessation of hormone treatment the testes have recovered from the inhibiting effects, normal, mature cells in all stages of spermatogenesis are found, and sperm are present in the lumen of the seminiferous tubules. These inhibitory effects may be explained by a depressing action upon the anterior pituitary as far as its production of secretion essential to normal growth and function of the testes is concerned.

Doisy, Clement, Veler, and Thayer, (1930), reported the isolation of the crystalline follicular hormone before a meeting of The Thirteenth International Physiological Congress at Boston, August 1929. Upon further study they found that in the preparation of the hormone, two crystalline forms could be obtained: one, a leaflet type of crystal, appeared when a solution of the hormone was precipitated with water. The injection of solutions of the crystals produces the oestrus response of ovariectomized rats.

Kunde, D'Armour, Carlson, and Gustavson, (1930), reported that injections of the female sex hormone cause secretory activity of the uterus, swelling of the vulva, hemorrhagic discharge from the vagina, and accentuation of mating instincts in the dog.

According to Butendandt and Marrian, (1931), Doisy and his fellow workers reported the isolation of a crystalline substance out of pregnancy urine that was identical with one they reported in 1929. There are two active substances in pregnancy urine; their formulas are C_{18}H_{22}O_2 (called Theelin
by Doisy, di-hydroxy Oestrin by Marrian, and Progynon by Butendandt) and C$_{18}$H$_{24}$O$_3$ (called Theelol by Doisy, tri-hydroxy Oestrin by Marrian, and Hormone Hydrate by Butendandt). Theelin is a Hydrate of Wheelol.

The exact nature of the action of theelin upon the genital tissues is still not understood. "Since we consider hormones to be metabolic products of cells, secreted directly into the blood stream or tissue fluids, it seems reasonable to look for action directly upon the cells which are involved. It seems possible that such action may be centered on the mechanism regulating the blood supply. There is little doubt but that these hormones directly stimulate the vascular control mechanism, for a hyperemia attendant upon the development of oestrus conditions has been recorded by the earliest investigators in this field".¹

A normal rhythm of alternate dilation and constriction causing blushing and blanching of tissue exists in the uterus of mammals. During oestrus this vaso-dilation occurs more often. Markee, (1932), studied experimentally the rôle of vascular control in theelin by transplantation of uterine mucosa to the anterior chamber of the eye. Injections of theelin into immature uterine tissue transplants bring about the oestrus type of vaso-dilation within thirty minutes after the first injection. The blushing and blanching persists in a proportional amount to the hormone injected.

¹Edgar Allen, Sex and Internal Secretions, Baltimore: Williams and Wilkins Company, 1932. p. 425
CHAPTER III
MATERIALS AND METHODS

One of the major events upon which the isolation of the follicular hormone depended was the discovery of Ascheim and Zondek, (1927), of the great concentration of the follicular hormone existing in the urine of pregnant women. After the isolation of theelin and theelol the vaginal smear procedure of bio-assay was used by many investigators. This caused much confusion, for almost every investigator introduced his own ideas into the bio-assay procedure. Many variables existed. Mice and rats were used; some investigators used a single injection and others used as many as eight. Curtis and Doisy, (1931), defined the rat unit as, "the minimum quantity that causes establishment of the vagina orifice within ten days in three out of five animals receiving the hormone in six doses in three days, beginning when the animals are eighteen days old." "Theelin is approximately twice as active as theelol in adult spayed rats, whereas theelol is six or seven times as active as theelin in immature female rats." Using the vaginal smear method of assay on eighteen day old rats approximately 0.16y of theelol causes opening of the vagina within ten days following the first injection; 1.08y of theelin suffices. Theelol, with subcutaneous injections assays at least 1,500 rat units per mg. Orally it is one-third to one-half as active. With subcutaneous injections of eighteen day female rats theelol assays about
6,000 rat units per mg.

Allen obtained best results with subcutaneous injections.\(^1\) Aqueous solutions of Theelin are more effective when administered in divided doses (Dodds, 1929).

The hormone, theelin, was employed in the investigations reported in this thesis. It was obtained from the Parke, Davis and Company, which distributes it under license from St. Louis University. Each lot of the product is tested and approved by the Biochemical Laboratory of St. Louis University before being released for sale. The hormone was received in glaseptic ampoules of 50 rat units per cc. Each ampoule was diluted, when needed, with 7.2 cc. of distilled water for injection material. This gave a potency of 3 rat units per 1/2 cc., which was equivalent to the rat unit of Curtis and Doisy.

The albino rats used were obtained from the Breeding and Laboratory Institute, 1567 Third Avenue, New York City, New York. These were inbred for several generations and used when needed, thus insuring uniformity of material. The animals were kept in breeding cages made of galvanized-iron wire netting, resting in a galvanized-iron pan. This material was used because it may be readily sterilized. Once a week the cages were cleaned with a stiff brush, provided with fresh nesting tissue and clean food dishes. Once a month everything was washed, sterilized, and disinfected. Such sanitary precautions were observed because, even with

the best of care, rats may be infested with parasites or in-
fectious diseases.

The food consisted of cracked corn, rolled oats, and
lettuce. In order to prevent vitamin diseases the lettuce was
provided daily; cod-liver oil was mixed with the cracked corn
once every two weeks; and a dropping bottle containing yeast
dissolved in water was placed in each cage once a week. As rats
are dependent upon the constant availability of food for good
health, a dog biscuit or slice of toasted bread was kept in
their cages at all times. A closed drinking-water bottle,
fitting with a rubber cork pierced by a glass tubing drawn out
to a small opening, was placed in each cage. This was done be-
cause water standing in an open dish in a rat cage becomes
quickly contaminated with urine and feces and is thus unfit
for drinking.

Every animal was numbered, marked, and a record kept with
regard to date of birth, sex, individual number, and descrip-
tion. The marking system used for identification purposes was
the clipping of hair in minute spots on either the head,
fore-limbs, hind-limbs, back, or stomach.

The first series of experiments was begun July 5, 1934.
Animals of a single litter were kept in one cage and all cages
were kept under like conditions. Control groups were formed
from each litter and kept under the same conditions as the
experimental groups.

Subcutaneous injections of three rat units per 1/2 cc.
of theelin were made in the abdominal region of the females,
twice per day and for three successive days, beginning when the animals were eighteen days old. Records were kept daily of the opening of the vagina. Vaginal smears of each animal (experimental and control) were made by sampling the contents of the vaginal lumen twice daily for twelve successive days, beginning when the vagina opened. The various types of cells were identified after spreading them in a drop of water on a slide (a platinum wire was used) and staining with eosin or methylene blue. The cells found indicated the condition of the vaginal epithelium. In rats and mice cornification of the vaginal epithelium during oestrus provides a clear cut end point which can be studied in the living animal. The stages of the oestrus (heat) cycle are diestrus, proestrus, oestrus, and metaestrus.\(^1\) During the diestrus, interval, the vaginal epithelium is thin and many polymorphonuclear leukocytes are found in the vaginal lumen. In the proestrus, preparatory, the epithelium becomes several layers thick, proliferate, and begins to cornify. Leukocytes are rare. The oestrus, heat, phase is characterized by cornification of the upper epithelial layers. The nuclei no longer stain, leucocytes are absent, and only cornified cells are present in the smear. The metaestrus stage consists of a sloughing off of the cornified layers, infiltration of leucocytes, and a return of the vaginal epithelium to its minimum.

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A positive smear consists of non-nucleated, cornified scales which stain a bright red with eosin. The negative smear consists of a few nucleated, epithelial cells and many leucocytes.

Tissues of the vagina, uterus, and uterine tubes were removed from (1) the eighteen day old rat, before injections were started, (2) from experimental animals at opening of the vagina, (3) from controls at normal opening of the vagina, (4) from experimental animals when the vagina of the controls opened, (5) from controls at the normal opening of the vagina. The tissues were fixed in Bouin's fluid and stained with Heidenhain's Iron-hematoxylin. This stain is used in the study of cell division and in determining the finer structures of the nucleus. The method used for preparation of tissues and slides was according to Guyer.

In the males injections of theelin were also started July 5, 1934. Injections of three rat units per 1/2 cc. were made daily over a period of several weeks or several months. Daily records were kept of the descent of the testes in both experimental animals and controls. Tissues were removed from (1) eighteen day old rats before injections were started, (2) from controls having descended testes, (3) from experimental animals still injected with theelin when the testes of the controls descended, (4) from experimental animals still injected weeks after the testes of the controls had descended.

The tissues were then fixed in Bouin's fluid and prepared for histological examination, the same procedure being followed as that used on the female tissues.
CHAPTER IV
RESULTS

Experimental Results in Females.— The first experiments were started July 5, 1934. There were seven females in this litter, A. Two were used as controls. The five experimental animals were given injections of three rat units per 1/2 cc. of theelin twice per day for three successive days. The injections were made subcutaneously in the abdominal region. During the afternoon of the fourth day after the first injection, July 8, 1934, a congested vulva and open vagina was noted in all experimental animals. The secretion in the vulva was a colorless, viscous mucus evident in the vaginal orifice for about two hours only. After two hours the fluid dried up, leaving a pasty substance in its place, which was gradually rubbed away by the animal itself. Vaginal smears were positive, indicating the establishment of a typical oestrus reaction. The vagina of the two control animals was still closed and immature in appearance. The experimental animals and one control were anesthetized with ether and incisions into the abdominal cavity made. Observations showed the vagina, uterus, and uterine cornua of the control infantile. Those of the experimental animals were hyperemic and considerably distended. These structures were removed and prepared for histological examination. The vagina of the remaining control did not open until July 22, 1934. The animal was thirty-five days old, the age at which
normal opening of the vagina should occur. As in the case of the prematurely opened vaginas of the experimental animals, the vulva was congested upon opening and the smear was positive.

Experiments on litter C were started November 8, 1934. There were four experimentals and two controls. After the injections of theelin, the vagina of three experimentals opened during the evening of the fourth day after the first injection, November 11, 1935. The smears were positive. The vagina of the fourth experimental animal did not open until the morning of the fifth day after the first injection, and the smear made was negative. This indicated only a partial reaction to theelin. The investigator attributed this to a slight loss of the injected hormone, due to the movement of the animal when one injection was made. The vagina of the controls opened when the animals were thirty-five days old, November 25, 1935, the smears being positive.

Experiments on litter D were started February 15, 1935. There were five experimental animals whose vaginas opened four days after the first injection. The vagina of one control opened when the animal was thirty-five days old. The vagina of the other control did not open until the animal was thirty-six days old, March 24, 1935. The smear was positive. Normal opening of the vagina of albino rats occurs, usually when the animal is thirty-five days old. However, no accurate standardization regarding age of the opening of the vagina and attainment of puberty can be given. The range is between thirty-five and ninety days (Long and Evans, 1922).
### TABLE I

**EFFECTS OF THEELIN INJECTIONS UPON NORMAL ALBINO RATS**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Age in Days</th>
<th>Injections (First Day)</th>
<th>Injections (Second Day)</th>
<th>Injections (Third Day)</th>
<th>Total Vagina</th>
<th>Age at Oestrus</th>
<th>Oestrus</th>
<th>Smear</th>
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<tr>
<td>A-1</td>
<td>18</td>
<td>7/5/34</td>
<td>7/6/34</td>
<td>7/6/34</td>
<td>2.1</td>
<td>7/22/34</td>
<td>+</td>
<td></td>
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<tr>
<td>A-C</td>
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<td>B-C</td>
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<tr>
<td>D-1</td>
<td>18</td>
<td>2/1/35</td>
<td>2/4/35</td>
<td>2/1/35</td>
<td>21</td>
<td>2/1/35</td>
<td>+</td>
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<td>D-C</td>
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<td></td>
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<td></td>
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<td>F-C</td>
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<td>H-1</td>
<td>18</td>
<td>6/2/35</td>
<td>6/2/35</td>
<td>6/2/35</td>
<td>21</td>
<td>6/2/35</td>
<td>+</td>
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<td>9/2/35</td>
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<td>M-1</td>
<td>18</td>
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<td>1/27/36</td>
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C represents the control animal of each group or litter; R.U. stands for rat unit; + means positive.
In Table I is listed data on the effects of theelin injections upon nine different litters of albino rats. About seventy-five female rats were used. Litter sisters are grouped together. The animals were eighteen days old when the first injections were given. Injections of three rat units per 1/2 cc were given for three successive days. This made a total number of eighteen rat units received by each experimental animal. During the afternoon of the fourth day after the first injection congested vulva and open vagina were noted in animals A-1, D-1, F-1, H-1, J-1, and K-1. Smears made when the vagina opened were positive, indicating the establishment of a typical oestrus reaction. Control animals were still sexually immature. The vagina of controls A, B, F, J, K, and N did not open until the animals were thirty-five days old, two weeks after the opening of the experimental animals. The vagina of control D opened when the animal was fifty-six days old, control H at fifty days, and control M at forty-nine days of age. Since puberty ranges from forty to ninety days, this was not an altogether unnatural phenomenon.

Vaginal smears made from the control animals indicated that they pass through a complete oestrus cycle every four days. As the vagina opened, positive smears were obtained. These smears indicated that the animals were passing through oestrus phase of the cycle. Smears made twenty-four hours later were negative, indicating that the animals were passing through the metaoestrus phase. On the third day, thirty-six hours later, the smears were still negative and indicated that the animals were passing through the diestrus stage. On the fourth day, forty-eight hours later, the smears were also
negative and showed that the animals were passing through the proestrus phase. By the fifth day another positive, oestrus, smear was obtained, thus the cycle was starting over again. In Table 2 are listed data on the effects of theelin injections upon the oestrus cycle of the albino rat. Eighteen rat units of theelin caused the vagina of immature rats to open at the age of twenty-one days. Smears made at the opening were positive, indicating a typical oestrus response to theelin. These smears were made twice per day until the animals were about forty-five days old. After the oestrus smear, obtained when the vagina opened, a metaestrus smear was obtained on the second day, and a diestrus smear on the third day. On the fourth day, instead of getting a proestrus smear, which would have indicated that the oestrus rhythm was about to start over again, a diestrus smear was still obtained. Smears for the fourth day, eighth day, and twelfth day after the first oestrus smear were all negative. On these days the oestrus phase of the cycle should have occurred, and a positive, cornified, epithelial smear should have been obtained. After the first positive smear another one was not obtained until the animals were at least thirty-five days old. On this day normal opening of the vagina should occur and a typical, positive, oestrus smear should be obtained. Smears of animals A-1, B-1, D-1, F-1, H-1, K-1, N-1, and N-1 were all positive. On the second day a metaestrus smear was obtained, a diestrus smear on the third day and a proestrus smear on the fourth day. By the fifth day another oestrus smear was obtained in the same animals. Every four days thereafter the smears showed a return to the oestrus condition. After the first
TABLE II

EFFECTS OF THEELIN INJECTIONS UPON THE OESTRUS CYCLE IN THE ALBINO RAT

<table>
<thead>
<tr>
<th>Animal</th>
<th>R.U.</th>
<th>Date</th>
<th>Age</th>
<th>4 Days</th>
<th>8 Days</th>
<th>12 Days</th>
<th>Later</th>
<th>Later</th>
<th>Later</th>
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<tr>
<td>A-1</td>
<td>11</td>
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<td>21</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>4/15/38</td>
<td>35</td>
<td>+</td>
</tr>
<tr>
<td>B-2</td>
<td>17</td>
<td>8/1/38</td>
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<td>+</td>
<td>+</td>
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<td>-</td>
<td>+</td>
<td>10/23/38</td>
<td>35</td>
<td>+</td>
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<tr>
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<td>35</td>
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<td>+</td>
<td>+</td>
<td>10/23/38</td>
<td>35</td>
<td>+</td>
</tr>
</tbody>
</table>

C equals control; R.U. equals rat unit; + equals positive; - equals negative.
positive smear, obtained at the age of twenty-one, rat J-1 did
not show another positive smear until it was fifty days old.
Smears made every day after that indicated a typical phase of
the oestrous cycle and every four days thereafter a positive
smear was obtained.

Experimental Results in Males. Injections of theelin were
also started in the males July 5, 1934. Three rat units per 1/2
cc were given each experimental animal, starting when the
animals were eighteen days old, for a period of at least forty
days and not longer than one-hundred and four days.

Table 3 shows the effects of theelin injections upon the
normal, male rat. The first injection, in all cases, was given
when the animal was eighteen days old. The testes of all control
rats, except animals H-C and K-C, descended when the animals
were thirty-four days old. The testes of rat H-C descended when
the animal was forty-five days old and that of animal K-C at
forty days. Rats A-6 and B-5 received a total of 108 R.U. over
a period of five weeks and one day. During that time there was
evidence of descending testes and by the fourteenth day the testes
had completely descended into the scrotal sac. Rats D-4 and
F-6 received a total of 118 R.U. over a period of six weeks;
rats H-8 and I-12 received a total of 273 R.U. over a period of
thirteen weeks; rat J-4 received a total of 261 R.U. in twelve
weeks and three days; rats K-5 and M-4 received 210 R.U. in
ten weeks; rat N-5 received 78 R.U. in eight weeks and four
days. In all of these cases the testes did not descend into the
scrotal sac as long as injections of theelin were given. Ten
days after the last injection was given evidences of the
descending testes could be determined; and by the fourteenth
<table>
<thead>
<tr>
<th>Animal</th>
<th>First Injection</th>
<th>Last Injection</th>
<th>Total R.U.</th>
<th>Testes</th>
<th>Descended</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Date</td>
<td>Age</td>
<td>Date</td>
<td>Age</td>
<td>R.U.</td>
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<tr>
<td>A-C</td>
<td>7/5/34</td>
<td>18</td>
<td>8/10/34</td>
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<td>108</td>
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<tr>
<td>B-C</td>
<td>11/1/34</td>
<td>18</td>
<td>12/4/34</td>
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<td>108</td>
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<tr>
<td>D-C</td>
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<td>18</td>
<td>3/29/35</td>
<td>60</td>
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<tr>
<td>F-C</td>
<td>4/13/35</td>
<td>18</td>
<td>5/22/35</td>
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<td>118</td>
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<tr>
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<td>5/24/35</td>
<td>109</td>
<td>273</td>
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<tr>
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<td>18</td>
<td>6/24/35</td>
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<td>273</td>
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<td>K-C</td>
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<td>2/11/36</td>
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<td>210</td>
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<td>M-C</td>
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<td>2/3/36</td>
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<tr>
<td>N-C</td>
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<td>18</td>
<td>2/2/36</td>
<td>78</td>
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</tbody>
</table>
day they had completely descended into the scrotal sac. The size of the testes and genital organs of control animals was twice that of injected rats.

**Histological Picture in Females.**—Sections of the uterus and vagina of litter N represent the conditions of these organs in all animals. The uterus of control animal N-C is infantile, anemic, and the walls are collapsed (Fig. 5). The uterine stroma and epithelium is undifferentiated and embryonic (Fig. 6). A few simple tubular glands have begun to develop. The lumen of the uterus is slit-like.

The uterus of injected animal N-1 is hyperemic and distended with secretion (Fig. 7). Typical low, columnar, secretory epithelial cells with nuclei arranged along the basal membrane are present (Fig. 8). These epithelial cells have enlarged and their cytoplasm is filled with secretory droplets, which are discharged into the lumen of the uterus. The uterine blood vessels are prominent.

The vagina of control animal N-C is closed by a solid cord of epithelium, four to eight cells in thickness (Fig. 9). Leucocytes are found in and under the epithelium. A mitotic figure is found in two or three places (Fig. 10). This indicates that some growth is taking place.

The vagina of experimental animal N-1 is open and typical of an animal at its first oestrus (Fig. 11). The vaginal epithelium has grown to twelve to fifteen layers in thickness. The granular layer is cornified, its outer two or three layers are sloughed off into the lumen (Fig. 12). This layer contributes the cells of the oestrus smear. The germinal
epithelium contains many mitotic figures this indicates that rapid growth is taking place.

Figure 13 indicates the vagina of animal I-1 after the effects of theelin have worn off. The vagina remains open, but the epithelium is at its minimum thickness, two to three cells. The epithelial cells are of the stratified squamous type. Many leucocytes are found in and under the epithelial layers (Fig. 14).

**Histological Picture in Males.** Sections of the testes of animal D-8 represent the conditions of the testes of an immature, eighteen day old rat. The testes are infantile and sperm are not developed. The germinal epithelium consists of many small, embryonic, epithelial cells. The seminiferous tubules have no distinct lumens (Fig. 16). Many small epithelial cells, arranged radially, are present. These cells are the future Sertoli cells. Primary sex cells are scattered between the small epithelial cells. Between the tubules are found broad strands of interstitial tissue.

Figure 17 represents the condition of the testes of animal M-C, a control rat at the beginning of puberty. This animal was thirty-four days old when the testes were removed. The germinal epithelium consists of small, cuboidal cells, arranged in a single layer in some places or several layers. A small lumen is present in many of the seminiferous tubules. The indifferent epithelial cells of the tubules have decreased in numbers. The sex cells between the epithelial cells have increased in numbers and have the characteristics of spermatogonia. The interstitial cells are arranged in strands between the tubules.
Figure 18 represents the condition of an adult testis, animal I-8. This animal was one-hundred nine days old when the testes were removed. The seminiferous epithelium rests on the inner surface of the basement membrane. The interstitial tissue contains interstitial cells, blood and lymph vessels, thin collagenous fibres, and fibroblasts. The interstitial cells, scattered between the tubules, are irregularly polyhedral or elongated. Some cells have one nucleus, others have two nuclei. The nucleus contains chromatin granules and one or two nucleoli. The seminiferous tubules are composed of Sertoli cells and sex cells arranged around a lumen. The Sertoli cells are slender structures, attached perpendicularly to the basement membrane. The nucleus is oval in shape and contains a nucleolus which is also oval in shape. The nucleus is imbedded in cytoplasm containing many granules. All stages of spermatogenesis are found in the tubules. Each tubule, however, has the same combinations of generations along its periphery. Bunches of nearly mature spermia are seen near the lumen of the tubule to the lower left of the figure. Whorls of mature spermia are present in the lumen of the seminiferous tubule in the middle of Figure 18.

Figure 19 represents the condition of the testes of experimental animal H-8. This animal has been injected with theelin for thirteen weeks, receiving a total of 273 R.U. This animal is of the same age as control animal I-8 of Figure 18. The germinal epithelium is very thin, consisting of one or two layers of small cells. The seminiferous tubules have no distinct lumens. Their epithelium consists of two or three cells arranged radially. Primary sex cells fill the remaining space. No mature
spermia are found. Strands of interstitial tissue are present between the tubules.
CHAPTER V
CONSIDERATION OF RESULTS

Injections of theelin into immature female rats result in rapid growth of the vaginal epithelium, which causes the opening of the vagina prematurely. This occurs four days after the first of a series of injections. The epithelial layers become cornified, the outer two or three layers are sloughed off into the lumen. Smears made at this time indicate that the vagina passes through a typical oestrus reaction, similar to a normal animal experiencing its first oestrus. Smears of a later date indicate that after the cornified cell stage, the epithelium is infiltrated with leucocytes. The vaginal epithelium is returned to its minimal thickness, two to four layers. The vagina remains in this condition until puberty is reached, when it undergoes the characteristic cornification and degeneration phases periodically, every four days.

The tiny infantile uterus, under the influence of theelin injections, begins rapid growth and the secretion of a clear fluid greatly distends it.

Thus it seems that theelin induces, in the immature female, the accelerated growth, hyperemia, and secretion of the genital tract characteristic of the period of oestrus in the normal animal at puberty.

Injections of theelin into immature male rats inhibits the growth of the testes, which are retained in the abdomen.
The testes and genital organs of control animals are much larger and the testes descend into the scrotum during the experiment. The testes of injected animals remain infantile and sperm are not developed. This state of development can be maintained for several weeks, even months past the normal time of the attainment of puberty. Two weeks after the cessation of injections the retarded testes begin to grow very rapidly and descend into the scrotum. These glands are normal, containing all stages of spermatogenesis.

From these considerations it seems that theelin tends to accelerate growth and development of the genital organs of the female and to retard the growth and development of the genital organs or testes of the males.
CHAPTER VI
CONCLUSIONS

1. The injections of theelin into immature female rats induces a condition in the genital tract similar to that of an animal experiencing its first oestrus.

2. This result may be obtained in four days by six successive injections of three rat units each, as early as twenty-one days of age. This is fourteen days before the normal time.

3. The uterus becomes hyperemic and distended with secretion.

4. Rapid growth greatly thickens the vaginal wall, causing the formation of a heavy cornified layer. This results in the opening of the vaginal orifice.

5. After the hormone is exhausted, degenerative changes set in and most of the tissue resulting from its action during the proceeding period is removed.

6. Injections of theelin into immature male rats retards the development of the genital organs or testes.

7. The testes can be held in this state of development for at least thirteen weeks. Fourteen days after cessation of treatment the testes descend into the scrotum and sperm are developed.
Fig. 1 - Oestrus smear, obtained upon opening of vagina. Cornified epithelial cells, nuclei don't stain, leukocytes absent. This smear was obtained in normal animals at the age of 35 days and in animals under the influence of theelin.

Fig. 2 - Metaestrus smear, 24 hours after the oestrus smear, few cornified scales with stainable nuclei, many leukocytes. $\times 440$

Fig. 3 - Diestrous smear, 36 hours after the oestrus smear, an occasional epithelial cell with many leukocytes. $\times 440$

Fig. 4 - Proestrus smear, 48 hours after the oestrus smear. Cornified cells with stainable nuclei, few leukocytes. This type of smear was not again obtained in injected animals until they were 35 days old. This is the age the first oestrus smear is obtained in the normal animal. $\times 440$

A Leitz compound microscope was employed in all the histological studies. Objective and ocular combinations were chosen to give the magnifications indicated in the legends.
Fig. 5 - Control: Uterus (cross section) infantile, anemic; lumen slit-like. Rat N-C. \( \times 100 \)

Fig. 6 - Control: Uterus. A higher magnification of Fig. 5. Uterine stroma and epithelium is undifferentiated and embryonic. \( \times 440 \)

Fig. 7 - Injected: Uterus (cross section) hyperemic and distended with secretion. Rat N-1. \( \times 100 \)

Fig. 8 - Injected: Uterus. A higher magnification of Fig. 7. Low, columnar, secretory epithelial cells, cytoplasm full of secretory droplets, stroma cells spindle-shaped. \( \times 440 \)
Fig. 9 - Control: Vagina (Saggital section). The vagina orifice is closed by a solid cord of epithelium, four to eight cells thick. The vaginal orifice does not open until 17 days later. Rat N-C (18 days old). $\times 100$

Fig. 10 - Control: Vagina. A higher magnification of a section of Fig. 9. Mitotic figures, leucocytes in and under the epithelium. $\times 440$

Fig. 11 - Injected: Vagina (Saggital section). Animal in an experimental oestrus condition from injections of theelin. This hormone caused rapid growth, thickening, and cornification of the epithelium, which resulted in the opening of the vagina prematurely. Rat N-3. $\times 100$

Fig. 12 - Injected: Vagina. A higher magnification of a section of Fig. 11. Epithelium 12 to 15 layers thick; granular layer is cornified, outer 2 or 3 layers are sloughed off into the lumen, epithelium has many mitotic figures. $\times 440$
Fig. 13 - Injected: Vagina (cross section). Thick walled epithelium, with heavy cornified layer being sloughed off into the lumen; a typical oestrous condition. Rat H-2, 22 days old.

Fig. 14 - Injected: Vagina (Sagittal section). After effects of theelin have worn off. Vaginal epithelium at minimal thickness, 2 or 3 cells. ×100
Fig. 13

Fig. 14
Fig. 15 - Immature testis (cross section). Arrangement of the seminiferous tubules. Rat D-8, 18 days old. $\times 130$

Fig. 16 - Immature testis. A higher magnification of a section of Fig. 15. Sperm not developed, seminiferous tubules have no distinct lumens, primary sex cells in tubules. $\times 440$

Fig. 17 - Control: Section of testis (cross section) at beginning of puberty. Small lumen in seminiferous tubules, sex cells have appearance of spermatogonia, epithelial cells of the tubules resemble Sertoli cells. Rat M-C, 34 days old.
Fig. 18 - Control: Section of adult testis (cross section) Seminiferous tubules have mature spermia in the lumen. All stages of spermatogenesis in tubules. Rat I-8, 109 days old.

Fig. 19 - Injected: Section of testis (cross section) under the influence of theelin for 13 weeks. This animal is of the same age as control animal of Fig. 18. Seminiferous tubules have no distinct lumens; primary sex cells present, no mature. No mature spermia nor stages of spermatogenesis. Rat H-8, 109 days old.
BIBLIOGRAPHY


