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## A Study of theelin

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A STUDY OF THEELIN

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SENIOR THESIS PRESENTED TO THE COLLEGE OF MEDICINE

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## DEFINITION

Theelin has been defined as keto-hydroxy estrin; the specific ovarian hormone capable of inducing hypertrophic changes in the uterine mucosa and in the vagina. The term "theelin" is a trade name; synonyms are estrin, amniotin, menformon, progynon, the female sex hormone, and estrogen.

## HISTORY

Theelin was first found in large amounts in pregnancy urine in 1927 by Ascheim. Within a few years it was obtained in the crystalline form by Doisy and his co-workers, the crystals being exhibited by Doisy at the International Congress on Physiology, held at Boston in 1929. At the same, but independently, it was isolated by Butenandt, whose publication appeared a little before that of the American workers. (Novak, 1935). It has since then been isolated by Lacqueur in 1931, and by Zondek in 1932. (Ehrenstein, 1936).

Frank (1935) reviewed the earlier work done by investigators on the existence of gonadal hormones. Indirect proof of such a secretion was first offered by Berthold in 1849. He restored the sexual characteristics of castrate cocks by the transplantation of testes. Later in the nineteenth century, various men demonstrated the existence of an estrogenic hormone by the transplantation of ovaries into ovariectomized animals. In 1905, Halban, on a purely clinical basis, stated

that the placenta was an organ of internal secretion. In 1912, Isovesco definitely established the estrogenic potency of placental extract by its reaction on the uterus of castrate or immature animals.

In 1923 Allen and Doisy published specific tests for qualitative and quantitative determination of the ovarian follicular hormone. They suggested standardization by the examination of vaginal smears from castrated rats previously injected with test solutions. Therefore, although the existence of the hormone was established, its function and chemical nature had not been intensively studied until it was found in quantities by Ascheim in 1927.

Recently Marker, Kamm, et al. (1936) prepared theelin synthetically. Biologically this is significant, but clinically it can be obtained at lower cost from natural sources.

Smith in 1926 discovered the important fact that neither the ovary nor the testis function, however, without stimulation by the gonadotropic factor of the anterior pituitary. At the present time, the pituitary is thought to be the fundamental endocrine gland, although it, in turn, may be influenced by other endocrine organs.

As this study continued, it was found that the sex hormones could be identified in various body tissues and fluids. This discovery has made it possible to use the hormone content as a

basis of various diagnostic tests, which should in turn be used to indicate the need for endocrine therapy.

Most of the work at present has been directed at the actual hormonal content of various tissues and fluids. Little work has been done, however, on the conditions governing its secretion and on the factors determining the presence of the hormone at a given site.

At present, theelin is prepared commercially, the unit used being the international unit of the Permanent Committee on Biological Standardization of the Health Organization of the League of Nations. It is the estrus producing activity contained in .1 microgram of a standard preparation of theelin. This amount is one-third of the original rat unit preparation of Allen and Doisy. Most of these preparations are obtained from the urine of pregnancy, the placenta, or amniotic fluid.

Estrogenic agents, however, have been obtained from a large variety of sources. The following is a list of reported sources:

- (1) Follicular fluid.
- (2) Corpus luteum.
- (3) Ovarian stroma.
- (4) Blood of non-pregnant women at certain stages of the menstrual cycle.
- (5) Blood of pregnant women.

- (6) Urine of non-pregnant women.
- (7) Urine of pregnant women.
- (8) Placenta.
- (9) Fetal membranes and amniotic fluid.
- (10) Male blood.
- (11) Male urine.
- (12) Certain plants. (Green leaves, fruit blossoms, fir trees, oil from fir trees, plant blossoms, etc.)
- (13) Other organic materials such as petroleum and lignite.

Schiller (1936) stated that the distribution of theelin upholds the idea that it is not a specific female sex hormone, but is a general growth hormone present in animals and plants.

Recently MacCorquodale, Thayer, and Doisy (1935) of St. Louis reported the isolation in crystalline form of an estrogenic substance from the follicular fluid of hog ovaries. This is the first time a pure estrogenic preparation had been isolated from the ovary itself. The product obtained by them had approximately the same estrogenic potency as dihydroxyestrin. (Theelin is keto-hydroxyestrin and theelol is trihydroxyestrin.) This substance had four to eight times the activity of theelin. The evidence for the composition is not yet complete; the findings are based upon the similarity in estrogenic activity and in the melting points. The results do not exclude the possibil-

ity of other active estrogenic compounds being in the follicular fluid.

#### COMPOSITION

Theelin belongs to the lipoidal type of hormones along with the corpus luteum hormone, the male sex hormone, and the adrenal cortex hormone. Its formula has been determined by a number of workers to be C-18, H-22, O-2. It is one of a group of chemical substances built up around the phenanthrene group (three six-membered rings). Phenanthrene itself is quite inactive, but the various sex hormone derivatives possess different types and degrees of physiologic potency.

The male hormone differs molecularly from the female hormone only by a molecule of water and an atom of carbon. Zondek (Novak, 1935) has suggested that in both sexes the male hormone is first produced, being converted by dehydration into the female type, probably under the influence of the metabolic processes which many consider of importance in sex determination.

Witschi and many biologists favor the theory that a germ cell developing in the cortex will become an oocyte; that in the medulla, a spermatocyte. This is clearly established in such animals as the frog. If this is true, it would not be surprising to find that the character of the sex hormones was subject to environmental modifications. (Novak, 1935).

The mechanism by which estrin transforms into progesterin is



unknown. It may depend on the ovarian-pituitary relationship such as determines the change of granulosa into lutein cells. This occurs pathologically when ovarian tumors of the granulosa cell group occasionally metamorphose into lutein-like cells. This morphological change is reflected in the appearance of a decidual type of endometrium such as is produced, so far as is known, only by progestin. (Novak, 1935).

There has also been discovered a close chemical relationship between the sex hormones and the sterol group of chemicals, particularly the so-called carcinogenic substances, such as certain tar derivatives. Cholesterol is included in this group. According to Ehrenstein (1936) the bile acids are formed by the oxidation of sterols which shortens the side chain of the molecule. If shortened further, the corpus luteum hormone is obtained; this is a di-ketone, chemically called progesterone. By further oxidation the entire side chain may be split off to finally yield dehydro-androsterone, a substance both ketonic and alcoholic in nature. This in turn may be transformed either into the male sex hormones, called androsterone and testosterone, or into one of the four estrogenic hormones.

The study of estrin chemically has brought out the following facts:

Schiller (1936) stated that theelin is (1) very soluble in both water and fat--about 10,000 units can be taken up in water

and 50,000 units in oil; (2) theelin is the most stable of all the hormones as it can be boiled in water or in acid, and it will stand heat and light and retain its potency for 10-15 years.

The hormone as described by Doisy (1932) is "an unsaturated keto-monatomic alcohol having a molecular weight of 270 and a formula of C-18, H-22, O-2. The hydroxyl is phenolic in character since theelin gives the phenol color reactions and readily forms an ether when treated with dimethyl sulfate."

The alpha form, as described by Robson (1935) is an oxyketone. It has a constant benzoate of 217.5 degrees C. melting point which has a potency greater than that of the original hormone. Butenandt (Robson, 1935) suggests that the benzoate is fairly slowly decomposed in the body as it brings about a prolonged estrus when injected into a test animal. Such a property should make this form of estrin particularly suitable for clinical use.

The hydroxide (Robson, 1935) of the hormone (C-18, H-24, O-3) isolated by Marrian (1930) and Doisy and his co-workers also in 1930, has an action which is also more prolonged than that of the alpha hormone.

A beta hormone has been produced along with the alpha hormone when water is split off the hydroxide. This substance has specific physical properties, its melting point being two degrees higher than that of the alpha hormone.

Robson also states that other substances than these have been capable of producing estrus when injected into ovariectomized mice. Such a substance is 1-keto, 1:2:3:4 tetra hydroxyphenanthrene, as described by Cook, Dodds, and Hewett in 1933.

#### RELATION OF THEELIN TO THE FEMALE SEX CYCLE

As theelin is considered the female sex hormone, it would be best to explain its position in the female sex cycle and its relationship to menstruation.

Menstruation is considered to be an endocrine phenomenon dependent on the interaction of the hormones from the anterior pituitary gland, the thyroid, the adrenals, and the ovary. Under the influence of a gonadotropic hormone secreted by the anterior lobe of the hypophysis, a primordial cell of the ovary becomes a mature Graafian follicle, with subsequent ripening of the ovum. The follicle produces theelin which stimulates proliferation and growth of the uterine endometrium. This production continues up to and beyond the time of ovulation, about the fourteenth to eighteenth day of the menstrual cycle, at which time the endometrium stimulated by the theelin has obtained its optimum growth. A corpus luteum is now formed by the granulosa and theca interna cells of the follicle. This elaborates its own hormone, progestin, which controls the development of the characteristic premenstrual changes of the endometrium. At the same time, the production of estrin is

continued, causing a progressive inhibition of the anterior lobe of the pituitary (Moore and Price, 1932), so that the amount of ovary-stimulating hormone is gradually diminished. Finally, the amount of this hormone diminishes to a point insufficient to maintain the corpus luteum. As this structure degenerates, there is a cessation of theelin and progesterin production and thus a breaking down of the endometrium. The resulting discharge from the uterus accompanied by a bloody flow about the twenty-eighth day constitutes the objective manifestation signifying the completion of the cycle. With the absence of theelin the anterior pituitary gland again puts out its sex hormone to stimulate the development of a follicle for the next cycle. "The normal cycle depends, therefore, on properly developed and matured gonads that respond to the cyclic stimulation of a normally functioning hypophysis." (Therapeutic Notes, 1935).

Markee (1932) recently reported the process of menstruation as observed in the living animal. This was accomplished by the transplantation of small bits of endometrium into the anterior chamber of the eye where they became vascularized. He concluded that the ovarian follicular hormone was the essential hormone for menstrual hemorrhage. Although normally progestational transformation takes place, it is not necessary as sometimes hemorrhage occurs without ovulation, and therefore without cor-

pus luteum formation. He produced experimental hemorrhage by injections of theelin alone.

He also found that estrogenic substances are not effective for artificial menstruation in the hypophysectomized animal.

He believes that a decrease in the level of the follicular hormone after the induced growth seems to be the ovarian cause of menstruation.

#### STANDARDIZATION

The bio-assay first introduced by Allen and Doisy in 1923 employs the ovariectomized white rat as the test animal. Adult females, two and one-half to three months old, are selected and about two weeks after ovariectomy given a sensitizing series of injections to determine their ability to respond positively. Parke-Davis and Co., makers of theelin commercially (Therapeutic Notes, February 1937), use twenty rats at each dosage level in testing the finished products. When using aqueous solutions, the dose is given subcutaneously in three injections at four hour intervals. Oil preparations, which give a slower response, are given in a single injection.

A "rat unit" is the highest dilution which will return a positive test on the morning of the third day in three-fourths of the test animals used.

Vaginal smears are taken at 24, 32, 48, and 54 hour intervals after the first injection. The results are stated in terms

of International Units.

In the rat, the entire cycle lasts about four to five days. If the cycle is any longer, the resting or di-estrus stage is correspondingly lengthened. In this method of standardization the cycle is divided into five stages with characteristic changes in the uterus and vagina taking place.

STAGE I Normally this lasts about twelve hours, during which time the follicles are enlarging. The uterus is markedly engorged, while the vaginal cells are swollen and partially detached. Numerous mitoses are seen in the basal layer. A vaginal smear shows only the nucleated epithelial cells from the superficial vaginal layer.

STAGE II (early estrus). This stage covers about twenty hours. Ovulation now occurs as the follicles have reached their maximum enlargement. The uterus is no longer markedly congested. The endometrial cells, no longer cuboidal from fluid compression, are of the high columnar type and exhibit mitosis. The superficial vaginal cells have sloughed off, leaving the stratum corneum from which come the cornified cells of the vaginal smear. The cells at this stage show no tendency to clump together; the majority have lost their nuclei.

STAGE III (late estrus). This is a twelve hour stage during which the corpus luteum is formed, the ova being in the fallopian tubes. The endometrial cells remain columnar, but

show vacuolar degeneration. Coincidentally leucocytes invade the epithelium. In the vagina, both the stratum corneum and stratum granulosum are completely stripped to form the "cheesy" type of smear seen at this stage. The character of the smear is due to the clumping of the cornified cells.

STAGE IV (metestrus). This lasts about six hours. Corpus luteum formation is continued. Only very small follicles are found in the ovary. The vacuolar degeneration of the endometrium reaches its peak with abundant leucocytic infiltration. Ordinary squamous cells line the vagina with extensive leucocytic infiltration throughout. The smear contains some leucocytes as a result of their extensive invasion of the vagina. There are also a few nucleated epithelial cells.

STAGE V (di-estrus). The usual duration is fifty to sixty hours. The corpora lutea continue to grow and follicles of various sizes are present in the ovary. The endometrial cells are in a resting condition, showing no evidence of the vacuolar degeneration. The vaginal cells are low; leucocytes continue to invade and pass through the epithelium into the vaginal lumen. At the close of the period there are squamous changes and mitotic activity resembling the pro-estrus stage. The smear is principally leucocytes, with a few nucleated epithelial cells and mucus.

Rats and mice are convenient to use for the bio-assay as

(1) they have a short estrus rhythm of 4-5 days, and (2) there is a sharp endpoint of the vaginal reaction. (Allen, 1932).

Tissues and smears from a rat thirty days after castration are similar to those seen in the di-estrus stage. The endometrial and vaginal cells are small and leucocytes invade the epithelium although to a less degree than seen in the normal di-estrus animal. The smear is scanty but similar to the di-estrus smear.

Corresponding material from a spayed animal which has received theelin shows conditions resembling those described under Stage III. This is the reaction used to test the potency of the theelin.

The results may be affected by the number of injection and the medium in which the substance is dissolved. The activity is lowered when a single dose is given; when the substance is dissolved in oil. Deansley and Parkes (1933) show that solutions in oily media are unsatisfactory for standardization as the material may remain unabsorbed for several days after the injection. Divided injections in an aqueous medium appears to be the most suitable method so far devised.

Frank and Goldberger (1935) studied the sex hormone blood test. The method had been to desiccate the venous blood specimen with anhydrous sodium sulphate and then extract it with ether, chloroform, or benzene. They noted a great increase in estro-



genic activity when an alcoholic extraction was adopted. Their explanation was that some of the estrogenic compounds are ether insoluble. Their technic was as follows:

1. Fifty c.c. of blood were dehydrated with anhydrous sodium sulphate.
2. The powder was then twice extracted with 200 c.c. of 95% alcohol.
3. The alcohol fractions were combined and evaporated to dryness on a water bath.
4. The residuum was taken up in 5 c.c. of olive oil and injected into spayed mice.
5. The bio-assay was done according to the Allen and Doisy method.

Neustaedter (1936) modified the Frank-Goldberger test by using benzol and olive oil to dissolve the lipid residue on the grounds that it is not always possible to emulsify the lipid residue with water. His results closely approximate those of Frank and Goldberger.

Fluhmann (1936) employed a method which eliminated the chemical extraction used by the former investigators. He obtained venous blood, centrifuged it, and discarded the cells. This method is possible since Kemp and Bjergaard, as quoted by Fluhmann, showed that estrin is equally distributed between the red blood cells and the plasma.

The results of this method differ from those of Frank and Goldberger; however they show much similarity to the total daily urinary excretion of estrin at various stages.

Curtis and Doisy (1931) found that theelol was more effective in causing canalization of the vagina of normal immature rats, but that theelin was more potent when dealing with ovariectomized adult rats.

#### PRODUCTION

The source of estrin in the human body is generally conceded to be the ovary and probably the placenta. The best known means of determining the secretory activity of an organ is to compare the hormone content of the arterial blood entering the organ with that of the venous blood leaving it. No such estimations have as yet been attempted on the ovary. The actual hormone content would not, however, necessarily indicate a secretory activity.

It was first thought that the Graafian follicles secreted the hormone, but as estrus occurs after X-ray treatment and consequent follicle destruction, the solid substance of the ovary must also secrete the hormone. Allen, Pratt, Newell, and Bland (1930) determined that the liquor folliculi and granulosa cells contain considerable amounts of the hormone while the normal stroma tissue of the cortex takes little part in the secretion. Robson (1935) quotes Zondek in saying that in the non-pregnant

woman the ovarian cortex contains no estrin, but during pregnancy parts of the cortex may contain this hormone. Allen et al. (1930) studied the corpora lutea and found that it takes part in producing this hormone. Large amounts were found early in the menstrual cycle (13th-17th days), and during the first third of gestation. However, at full term the corpora lutea gave negative results--indicating that at that time it does not participate in producing the large amounts of estrin found in the placenta, blood, and urine. Zondek (Robson, 1935) showed that the placenta contained large amounts, as do also fetal tissues and urine of the new-born up to the fourth day after birth.

Fluhmann (1936) in discussing the source of theelin gives as proof of gonadal origin (1) the cyclic increase and decrease during the menstrual cycle which points to a close association with follicular activity, and (2) the preadolescent appearance at the time secondary sex characteristics develop. He states, however, that the occurrence of estrin in the blood of castrates and postmenopausal patients points to an extra-ovarian source. Other investigators do not find this latter phenomenon to be true. Fluhmann uses a different method of extraction, giving the same cyclic rise and fall, but in greater quantities. He does not attempt to further elucidate the possibility of an extra-ovarian source, and his work, if it proves to be true, will reopen the discussion of the source of estrin.

Robson (1935) reviewed the essential changes taking place in the ovary to establish its cyclical function,

Before puberty the ovary is small and undeveloped. With sexual maturity, two changes occur, (1) maturation of the ovarian follicles with ovulation and (2) formation of the corpus luteum.

(1) Follicular maturation occurs rhythmically during the whole period of sexual activity. Evans and Swezy (1931) have shown that, contrary to popular belief, the ova are not all formed at birth. They claim that ova arise after puberty from the germinal epithelium by invaginations which are cut off from the epithelium and pass through the tunica albuginea. Some of the cells develop into sex cells, the remaining form the "follicle" cells. With each maturation many follicles may undergo the preliminary stages of growth, but finally only one of the follicles becomes greatly enlarged. It approaches the surface of the ovary, breaks, and the ovum is extruded. The final growth and subsequent extrusion, or ovulation, is accompanied by destruction of the remaining follicles, usually beginning in the granulosa cells. Such waves of follicular maturation occur at definite intervals, varying with the species. It continues during pregnancy, but the final stages of growth and ovulation do not occur.

(2) Physiologically the corpus lutea are formed in the

follicles after ovulation. In the non-pregnant individual, the corpora lutea exert their activity for about half the duration of the menstrual cycle. Its degeneration is immediately followed by the onset of menstruation. The corpus luteum of pregnancy exerts its activity during the whole of gestation, and may be essential for the maintenance of pregnancy.

Hammond and Marshall in 1925 (Robson, 1935) studied the formation of the corpus luteum in the rabbit. After ovulation, hemorrhage occurs within the cavity, but the follicular epithelial cells are nearly all retained. These cells hypertrophy to eight times the original diameter, but they do not divide. They are then penetrated by a mass of connective tissue from the theca along with blood vessels. Subsequently there is an increase in the lutein content and a corresponding reduction in the protoplasm of the luteal cells.

Studies as to the secretory activity of the ovaries have been based upon ovariectomy and subsequent injections of the ovarian extracts (Allen, 1928). It has been found that removal of the ovaries results in cessation of all manifestations of estrus with ultimate atrophy of the sex organs. However, with transplants of the ovary all the phenomena of estrus were restored, providing the grafts were soon vascularized. As it was immaterial to what part the grafts were implanted, it was shown that (1) the ovarian control was not exerted primarily through

the nervous system, and (2) the ovary did not primarily exert its own activity through the nervous system.

The injection of follicular fluids and ovarian extracts also caused estrus-like changes in the vagina and uterus as already described.

#### PHYSIOLOGICAL EFFECTS OF THEELIN

Primarily this hormone is a growth hormone, acting on the female genitals and the mammary glands. There are also certain secondary sex changes which depend on the presence of an estrogenic substance. Juhn and Gustavson (1929) report plumage changes as studied in the brown leghorn. Parkes (1930) and Allen (1927) found reddening and swelling of the "sexual skin" in chimpanzees, baboons, and monkeys.

Specific changes take place as follows:

VAGINA--the vaginal reaction has already been described under the method of standardization. Allen (1924) showed that in either rats or mice a new vaginal epithelium is grown in less than five days. From twelve to twenty layers of stratified epithelial cells are grown. By means of vaginal smears this growth can be followed; the completion of the growth is established by the appearance of cornified epithelial cells and the lack of the leucocytes seen in earlier smears.

UTERUS--the uterine changes have also been described under the process of standardization. Allen (1924) described

large numbers of mitotic figures in the endometrium, and a serous fluid which is secreted and then retained in the uterus to cause distension of that organ. Reynolds (1931) found that both amplitude and rate of contractions were partially under the control of the hormone. Robson (1933) demonstrated that uterine muscle had a greater rhythmic activity after theelin injections. Abnormal hyperplasia may follow repeated doses (Burch, 1931), but the progestational type of uterus is never produced.

Campbell and Hisaw (1936) and Lubin and Clarke (1936) show that theelin excites uterine motility while corpus luteum extract, progestin, inhibits the motility. They recommend the use of progestin for afterpains and for dysmenorrhea.

**BLOOD VESSEL CHANGES**--Markee (1932) implanted bits of endometrium in the eyes of rabbits and observed the effect on the capillaries. He found that theelin caused vasodilatation. If no theelin was given the ovariectomized animals, there was atrophy and vasoconstriction.

**FALLOPIAN TUBES**--Seckinger (1924) found that contractions of the tubal musculature of the pig were regulated by the sex hormone. The development of ciliated cells of the tubes also depends on this hormone.

**OVARIES**--Leonard, Meyer, and Hisaw (1931) injected estrogenic substances and studied the effects on the ovaries themselves. There is no stimulation; in fact there may be retarda-

tion if large amounts are injected into immature animals.

**MAMMARY GLANDS**--The mammary gland depends upon theelin for growth stimulation. Turner et al. in 1932 stimulated the growth of rudimentary male glands by theelin and also by theelol. Theelin alone stimulated growth in the guinea pig, but in other mammals progestin must be given along with the theelin. Experiments by Allen (1927) and Allen and Turner (1933) showed that theelin caused repair of the gland following castrate atrophy, following ovariectomy in immature, and in male monkeys.

**NIPPLES**--Allen in 1927 also showed that theelin brought about growth in the epithelial covering of the nipple, both in the male and in the female.

**NERVOUS PHENOMENA**--Sexual drive is also influenced by the follicular hormone, as shown by Allen et al. (1924).

The spontaneous activity of a normal rat and of a rat that had been ovariectomized were measured by voluntary running in a rotary cage. The ovariectomized animal had no such periods unless they were induced by ovarian transplants (Wang, Richter, and Guttmacher, 1925) or by injections of theelin (Richter and Hartman, 1934). Herren and Haterius (1931) showed a change in reflex actions--a prolongation--when there was a high level of the hormone present.

Werner et al. (1934), in studying involuntional melancholia showed that this was also influenced by the level of the hormone



in the blood. It was also found that the estrous reaction of the uterus requires more hormone than does that of the vagina. (Marrian, 1930).

BIRTH CANAL--Hisaw in 1925 and 1929 studied resorption of the pubic bones and its relationship to theelin. This occurs under the influence of theelin before the first ovulation. At the time of parturition the pelvic ligaments are relaxed under the action of theelin followed by a corpus luteum hormone called relaxin.

ABORTION--Pregnancies have been interrupted by the injections of the impure ovarian follicular extracts. If the preparations are purified, much more can be tolerated. Normally, however, the blood shows a high estrogenic content during pregnancy.

INFLUENCE ON THE ANTERIOR PITUITARY GLAND--Theelin exerts a depressing action on the anterior pituitary, especially in relation to the gonad-stimulating power of the gland. This was illustrated by Moore and Price in 1932 and by Meyer et al. in 1932. After castration there are changes in the basophilic cells of the hypophysis (Addison in 1917), and an increase in the gonadotropic hormone as shown by Emery (1932), and Engle (1929). If theelin is injected, such changes do not take place and even normal activity is lowered (Nelson, 1933). Other anterior pituitary activities which are affected are growth-promoting (Spencer, Gustavson, and D'Amour, 1931), lactogenesis

(Nelson, 1934), and diabetogenesis (Nelson and Overholser, 1934).

RELATIONSHIP TO TUMORS AND ATYPICAL GROWTHS--That there is some relationship between certain types of tumors and the estrogenic supply has been demonstrated by Novak and Brawer in 1934. They found that granulosa cell tumors secrete large quantities of theelin and may cause re-establishment of the menses after the menopause.

Loeb (1919) removed the ovaries from carefully inbred mice subject to spontaneous mammary tumors. In the ovariectomized animals, the percentage of tumors was reduced.

Overholser and Allen (1935) and Engle and Smith (1935), gave continued theelin injections and studied the cervical reaction. Growths were produced which resembled early cancerous lesions.

#### THEELIN IN THE BLOOD STREAM

The review of the theelin content of the blood will be grouped as to studies of the normal findings in the female, the occurrence in the male, and manifestations in disease and during pregnancy.

##### A. NORMAL FEMALE

1. Prepubertal--Allen and Doisy (1923) and Frank and Gustavson (1925) found little or no estrogenic factor in the blood. Fluhmann, however, in 1936, by his method of standardization found that theelin appeared between the ages of eight and

ten years, before the secondary characteristics are manifested.

2. Adult (menstruating)--Frank, 1931 (Robson, 1935) stated that 1 M. U. could be demonstrated in 40 c.c. of blood from seven days before the menses until the onset. This amount equals about 25 M. U. per liter. At the time of the onset of the flow, the estrogenic factor disappeared from the blood in from 2-6 hours. With the method used, the minimum amount which could be determined was 1 M. U. in 40 c.c. of blood. Frank and Goldberger (1935) by using the alcohol extraction method, which has been mentioned, found the same general type of curve but got a more delicate reaction. They found a positive test with 40c.c. of blood twenty-one days before the onset of menses; with 30c.c., a positive report between the twenty-first to the fourteenth days; and with 10c.c. of blood, a positive from the seventh day to the onset of menstruation.

Fluhmann (1936) reports a rise eight to eighteen days before the onset with a secondary rise within four days or actually at the time of menses. In the cycles which are regular, but thirty-one to forty days in length, the greatest concentration is somewhat earlier in the interval, usually fourteen to twenty-one days before the onset of menstruation.

3. Physiologic Menopause--Frank (1935) finds that although estrogenic substances may be present for one or two years after the menopause there is normally none found after

the climax is fully developed. Fluhmann (1931) reports increased gonadotropic hormone after the menopause--a blood content of 500 rat units per liter in contrast to the normal finding in the cyclic female of twenty-five rat units per liter. As this hormone has been shown to increase in content after castration, it is reasonable to assume that there is a cessation of theelin content of the blood. However, Fluhmann in his later work of 1936, reported a cyclic rise and fall of estrin to be demonstrable in all cases.

#### B. MANIFESTATIONS IN DISEASE

1. Underfunction of the ovaries--Under secretion may be associated with such clinical syndromes as amenorrhea and oligomenorrhea. Frank (1931) distinguishes three types of amenorrhea, namely (1) the "subthreshold" type with reduction of both blood and urine content; (2) acyclic blood with normal urinary secretion; (3) absence of the hormone in both the urine and the blood.

Fluhmann describes some cases similar to those of Frank as does Siebke (Robson, 1935), but in the majority of his cases there was a rise every two to three weeks equal to that observed during midintervals in normal.

2. Artificial Menopause--After surgical or X-ray castration there is an extreme degree of underfunction. Fluhmann reports an increase of the gonadotropic factor as early

as ten days after operation. In his 1936 studies he finds that estrin is present in amounts comparable with normals. This points to an extra-gonadal source of theelin, although none has been offered.

3. Absence of vagina--Frank, Goldberger, and Spielman (1934) report a normal cycle in such cases and suggest this type of diagnosis for a person of questionable sex. The male may show estrogenic factors in the blood stream, but there is no cycle present.

4. Overfunction of the ovaries--Frank (1931)<sup>o</sup> describes a variety of conditions such as precocious puberty, menorrhagia, or metrorrhagia in which the estrin level of the blood is increased. In premenstrual tension with marked nervous and vascular symptoms there is a rise of the blood content to almost a pregnancy level. This is probably due to elevation of the urinary threshold. (Frank, 1931).

5. Manifestations in Organic Disease--There is apparently no theelin increase in the presence of fibroids or pelvic inflammation, even when accompanied by excessive bleeding although Davis (1934) mentions that arguments have been advanced to establish a relationship between ovarian hyperactivity and fibroid formation.

Estrogenic substances have been found in ovarian carcinomas, sarcomas, adrenal carcinomas, and chorionepitheliomas.

(Frank, 1932 and Frank, 1934).

### G. MANIFESTATION DURING PREGNANCY

1. Normal--It is known that after about the second month the theelin content rises above normal. Cohen, Marrian, and Watson (1935) reported that the ether insoluble fraction increases throughout pregnancy, until the time of labor when the ether soluble fraction again predominates. Zondek (quoted by Frank, 1935) believes that there are 200-300 M. U. at term.

2. Abnormalities--With fetal death, there is a marked reduction of theelin within twenty-four hours in contrast to pregnancy tests which may persist for several days. (Frank, Goldberger, and Spielman, 1933).

D. PRESENCE IN MALES--Theelin is sometimes found in the blood of males. Goldberger and Frank (1928) demonstrated this in three out of forty-seven cases. Geschickter (1934) and his co-workers suggest that a continuous increase of estrogenic or prepituitary-like factors could be the cause of gynecomastia of the males.

### EXCRETION OF THEELIN

The elimination of theelin from the body is to a great extent via the urine, although some theelin is found in the feces. It has been assumed that the amount excreted varies directly with the amount produced, although no work has been

done on this problem.

The activity of the kidney may vary and be reflected in the blood concentration of the theelin.

No one, as yet, knows whether all the hormone is excreted or whether it is partially destroyed in the body. If it functions as a catalyst, it probably escapes destruction; otherwise it may quantitatively be reduced before it is excreted.

A. EXCRETION DURING THE MENSTRUAL CYCLE Estrin excretion begins at puberty. It has been studied by Frank and Goldberger (1930). Normally about 1500 M. U. are excreted during a cycle. The periods of maximum excretion correspond to the times of ovulation and pre-menstruation. Zondek in 1931 (Robson, 1935) studied the fecal excretion of theelin. They found that the fecal excretion curve closely followed the urinary excretion curve and so concluded that the rate of excretion depends primarily on the rate of production and not on the variations of renal function.

B. EXCRETION DURING PREGNANCY. As mentioned before, Ascheim and Zondek in 1927 first discovered the excretion of large amounts of theelin during pregnancy. Smith (1927) has also studied this phase of excretion. She finds that early in gestation about 300-600 M. U. per liter are excreted. After the eighth week, this figure gradually rises until as many as 20,000 units per liter are excreted at the end of pregnancy.

Robson quotes Runge, Hartman, and Sievers (1932) who observed that occasionally the urine contained more than 100,000 M. U. per day, and suggested that such large concentrations of hormone are necessary to keep the uterus in a "tonic" state.

The excretion reaches normal about the end of the first week of the puerperium, although nothing is known as to the influence of lactation or uterine involution on the concentration.

C. EXCRETION AT THE MENOPAUSE. Zondek, 1930 (Robson, 1935) observed a definite initial increase in theelin elimination during the menopause. At this time there is not only about 200 mouse units per day present, but also there is a marked hypertrophy of the uterus. Later only small quantities, if any, are present in the urine. Kurzok (1932) observed urinary theelin in post-menopausal patients.

D. EXCRETIONS UNDER ABNORMAL CONDITIONS. With underfunctioning ovaries, as has been mentioned under the paragraph concerning the blood concentrations, there may either be normal or reduced excretion.

Frank, Goldberger, and Spielman (1934) find from 4,000 to 10,000 M. U. excreted during a cycle in cases of overfunctioning ovaries. Excessive bleeding with fibroids or pelvic inflammations, however, is not generally thought to be associated with increased estrogenic excretion.



## CLINICAL APPLICATIONS

The follicular hormone is used clinically in the form of Theelin for hypodermic injections and Theelol for oral administration. The former was first isolated in 1929 by Doisy; the latter was isolated the following year by Doisy and Thayer. Marrian and Parkes (1930) determined the amount of theelin needed to bring about full estrus in the mouse--about 200 M. U. By using a weight for weight basis Parkes in 1932, calculated that an average human should need about 500,000 M. U. Werner and Collier (1933) were able to produce bleeding in ovariectomized cases by 28,000 R. U. of theelin. It must be remembered that this would probably be a larger dose if it was expressed in mouse units. The benzoate previously mentioned, is more metabolized or eliminated and so is active for a longer period. Zondek (Robson, 1935) finds a need for larger doses when the hormone is given by any other method than hypodermically--such as via the stomach, shaved skin, or the rectum.

Schiller (1936) states that the dosage cannot be too high as the excess overflows in the urine and feces. Of course, continuous large injections would cause a hyperplasia of the endometrium.

Although clinically the hormone has been used for a great variety of disturbances, only the following will be discussed: menopausal disturbances, sterility, amenorrhea, dysmenorrhea,

gonorrhoeal vaginitis in children, hemophilia, migraine, premature infants, pruritus vulvae, and involuntional melancholia.

**MENOPAUSAL DISTURBANCES.** During the first menopausal stage, there are comparatively large amounts of theelin present in the body; later there is a definite theelin deficiency and an increased secretion of the gonadotropic hormones of the anterior pituitary. This endocrine imbalance is apparently responsible for the menopausal symptom complex. Kurzrok (1932) claims success with theelin treatment only when there is no theelin being excreted in the urine, that is, after the initial theelin rise. Hawkinson (1935) claims a good response to such therapy except in those cases excreting theelin--which he believes are only 10% of the cases. Several authors have recorded marked improvement for menopausal disturbances; Sevringhaus (1935); Geist and Spielman (1932); and Houghton and Neville (1936). The latter found that average treatment required about two years, that adjunctive thyroid treatment reduced the dosage of theelin, and that the average requirement of one ampoule (1000 or 2000 units) per week was less expensive than the usual amount of bromide substitute.

Theelin has also been reported as an aid in menopausal hypertension (Schaefer, 1935); climacteric hypersensitiveness to sun and effort (Goldberg, 1935); and menopausal epilepsy (Schaefer and Brosius, 1933).

Werner and Collier (1933), in studying patients with surgical menopause noted increase in the size and sensitiveness of the breast, increased libido, and a general improvement of subjective symptoms with injections of theelin.

STERILITY. In such cases, of course, the spermatozoa should be studied and mechanical and inflammatory conditions excluded. Frank (1931) found a sub-threshold or negative estrin blood concentration in the majority of menstruating sterile women. Oftentimes the sterility is combined with amenorrhea and its typical hormonal changes. In these cases theelin has been used for treatment, although its value has not been definitely determined.

AMENORRHEA. A number of observers agree that theelin treatment is beneficial in cases of secondary amenorrhea; the menstrual habit is, in the majority of cases, re-established after the treatment is discontinued. Gardiner-Hill and Smith (1931), Haultain (1933), and Kincaid (1931), all give favorable reports on the subject.

Parke-Davis (Therapeutic Notes, 1937) recommend the injection of 2000 units of theelin every other day for a period of three weeks. During the last two weeks they also recommend intramuscular injection of corpus luteum. Haultain (1933) used dosages as high as 10,000 M. U.

With cases who have never menstruated--those patients with

primary amenorrhea--results have not been as satisfactory. Menstruation may be produced, but as the ovaries are not themselves stimulated, no permanent benefits will result.

Hamblen in 1931, however, reports encouraging results in so treating a limited number of cases of the primary type of amenorrhea.

**DYSMENORRHEA.** Experimentally theelin has been tried in these cases where it was believed there were no mechanical or psychogenic factors causing the dysmenorrhea. Bailey (1935), recommended a series of injections immediately following menstruation. Kennedy (1932) also endorses this type of treatment. Hawkinson (quoted by Robson, 1935) estimated that sixty to eighty percent of virgins suffering from functional dysmenorrhea were relieved. His method was a series of injections starting on the sixteenth day of the menstrual cycle.

Campbell and Hisaw (1936), mentioned previously, favor the theory that theelin is the cause of painful uterine contractions. They have treated their cases with a series of five daily injections of 6-8 rabbit units of progestin, which they claim acts antagonistically to theelin.

**GONORRHEAL VAGINITIS IN CHILDREN.** Many investigators have found theelin to be the most satisfactory treatment of this condition. Lewis in 1933 reported very favorable results; he has shown that theelin induces a proliferative growth in

the vaginal mucosa and so produces the adult type of mucosa which resists the gonococcal infection. Since then Brown (1934), Miller (1935), Lewis and Adler (1936), Lewis and Weinstein (1936), and Te Linde and Brawner (1935) have all enthusiastically endorsed this therapy. Lewis and Weinstein concluded that the important factor was to render the vaginal secretions markedly acid. They determined the dosage necessary by measuring the acidity of the vagina.

Te Linde and Brawner used the theelin in the form of gelatin capsules. The oil solution is generally preferred to the aqueous solutions as it does not necessitate daily injections. Theelol by mouth is often desirable.

**HEMOPHILIA.** In the limited number of cases studied, theelin has been of value in controlling hemorrhage due to hemophilia. Kimm and Van Allen (1932) reported the reduction to normal of the laboratory reading of blood coagulability. Birch (1932) reported success during hemorrhage, but did not advocate its continued use.

**MIGRAINE.** Treatment in migrainous cases is based on the decreased urinary output of estrin as studied by Glass (1936). He reported relief in 80% of the cases. Thomson (1932) previously reported successful treatment.

**PRURITUS VULVAE.** Zondek (1936) used theelin in the form of a salve and reports prompt relief. Rust (Therapeutic Notes,

1937) described marked improvement in twelve out of thirteen cases.

INVOLUTIONAL MELANCHOLIA. Such cases are treated psychotherapeutically and small doses of theelin and antuitrin-S are used as adjuncts. Improvement in a few cases has been reported by Harris (1934), and Sevringhaus (1933). Werner and his associates (Therapeutic Notes, 1937) compared theelin-treated cases with non-treated cases and decided that theelin administration was rational and effective.

PREMATURE INFANTS. Schiller (1936), on the assumption that theelin is a general growth hormone, administered the hormone to premature babies. He gave 40-50 units twice daily for 3-4 months. He emphasized the fact that it must be given within the first ten hours. He cited one case of twins in which the smaller one was given theelin; it soon outweighed the other twin.

#### SUMMARY.

1. Theelin is keto-hydroxy estrin: a specific ovarian hormone. It is the most stable of all the hormones.
2. Doisy and his co-workers were the first to isolate the crystalline form; the crystals were exhibited in Boston in 1929.
3. Marker and Kamm and their co-workers prepared theelin synthetically in 1936. The chemical formula is C-18,

H-22, O-2.

4. The sources of the follicular hormone are many and varied; commercially it is most convenient to prepare the product from pregnancy urine.
5. The secretion of theelin depends on a normally functioning hypophysis; the phenomenon of menstruation depends on the interaction of the gonads and the hypophysis.
6. The bio-assay has been described. Rats and mice are used for the standardization process because of their estrus rhythm of 4-5 days and the sharp vaginal endpoint that is produced.
7. Studies on ovarian activity have been based on ovarian transplantation or ovarian extract injection into immature or castrate animals.
8. Theelin exerts its effect principally as a stimulant of the sex organs. It has been studied in relationship to tumors.
9. Theelin is normally found in the body between puberty and the menopause; the highest concentrations are found during pregnancy.
10. Theelin is excreted both in the urine and in the feces; it is believed that the excretion directly reflects the rate of production although it is not known if the substance is stored or utilized in the body.

11. Clinically, theelin has been used for many conditions, oftentimes without a thorough study of the endocrine needs of the patient. It can be used hypodermically, orally, rectally, or as a salve.
12. Encouraging clinical results have been reported with the use of theelin in gonorrhoeal vaginitis of children, menopausal disturbances, and with premature infants.



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