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May 29, 1935.

I hereby recommend that the thesis prepared under my supervision by ESTHER BOGEN TIETZ

entitled THE ESTROGENIC HORMONE IN MENTAL DISEASE.

be accepted as fulfilling this part of the requirements for the degree of Doctor of Philosophy

Approved by:

Robert P. Mathews

Dino Yashiro

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THE ESTROGENIC HORMONE IN MENTAL DISEASE.

A dissertation submitted to the

Graduate School

of the University of Cincinnati

in partial fulfillment of the

requirements for the degree of

DOCTOR OF PHILOSOPHY

1935

by

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THE ESTROGENIC HORMONE IN MENTAL DISEASE.

TABLE OF CONTENTS.

THE ESTROGENIC HORMONE AND MENTAL DISEASE.

INTRODUCTION

1 Menstruation and emotional instability

The frequent association of mental and emotional disturbances with the functioning of the reproductive system in women is commonly observed. Dr. Hoskins refers to the "shrewish propensities of the victims of ovarian insufficiency". Dr. Meaker associates the depressions of adolescence with hypoplasia of the generative tract. Thruout history constitutional and psychic affects of the menstrual cycle have been noted and many books such as that of Dr. Jacobi were written about it. Dr. Novak reports that 19% of psychotic women demonstrate an amenorrhea, even tho in that period when the reproductive processes should be active. He referred to the widespread changes that may occur in relation to menstrual disorders. To those familiar with mental patients it is a commonplace observation that many of them show exacerbations before, during or after the flow and the past histories of many of these patients refer to nervousness or irritability concurrent with the menstrual cycle long before the onset of a frank psychosis. Strachan and Skottowe found a large percentage of menstrual disorders in psychotic patients but did not observe any improvement in the mental condition following treatment of the gynecologic disorder. Reference is frequently made to the fact that suicides in women appear to be almost exclusively in adolescent, menstruating or menopausal cases and crimes appear to bear the same close relationship. Just why the menstrual period should have such disastrous effects is a matter of serious importance to the community as well as to the

physician interested primarily in psychiatry.

2. The gonads in Schizophrenia.

Schizophrenia is a malignant, mental disease attacking young adults primarily. It is practically never seen below the age of twelve and rarely begins after the third decade of life. It causes a split in the smooth coordination between thinking and outward behavior. The patients lose ability to express interest or emtion and become incoherent in speaking and actions in many forms. Their activity is frequently of a childish nature, quite unpredictable, due to the influence of mental complexes easily discernible in their speech and behavior. The course is usually progressive but some patients demonstrate improvement or complete recovery. The association of this disease with disorder in the reproductive tract is frequently made, not only because of the time of onset, that is at puberty, but because of the childish emotionality of the patients who rarely make a normal adult hetero-sexual adjustment. There are, however, more convincing evidences in the hand of pathologists such as Mott who observed the smallness of the gonads as did Kretschmer, Frankel and Parhon. Fauser believed these cases showed an immunity to extracts of the sex glands, but his studies were not confirmed. More recently Hoskins and Krogerer emphasized the apparent inactivity of the ovaries and testicles of schizophrenics and Malony expressed the opinion that the symptoms were aggravated by castration, which had been carried out in an attempt to alleviate the condition.

3. The Menopause and psychic disorders.

The striking relationship of the menopause with mental and emotional instability causes some individuals to

feel that it is a natural result of the so-called "change of life". Involutional psychosis are recognized in the newly accepted Classification of Mental Disorders used thruout this country. Norbury describes the irritability, sleeplessness, hostility and aggressiveness demonstrated at that period as due to a lack of direction which seems to leave the mind "Neutral", Paranoid, psychoneurotic and nihilistic ideas are commonly observed in these patients who may change from agreeable delightful matrons into suspicious, grasping, quarrelsome and at times obscene spinster-like creatures.

The changes following childbirth are not so characteristic nor frequent but some cases do occur suddenly following the birth of a child that can be given no other diagnosis than post-puerperal psychosis regardless of the fact that they may present depressed, manic or schizoid symptoms.

4. Suggested Mechanisms for these relationships.

It would appear at first that these very clear cut relationships between the reproductive system in women and mental disease would find an immediate and simple explanation. But altho the problem has been attacked from many angles it is still obscure. Chadwick, in a recent monograph, related the psychologic phenomena associated with menstruation to taboos and other inherited primitive mental patterns but gave no notice to the importance of concommitant physical changes. Macht, on the other hand, believed he had demonstrated the existence of a menotoxin in the blood, urine and perspiration of menstruating women. He noted that his extracts of the latter materials inhibited the growth of seedlings; but recently Freeman found that carefully controlled experiments did not uphold these findings, and

Sachs stated that probably the phyto-toxic effects observed were due to increases of lipoidal material in the extracts. Aschner, however, believed the menotoxin and other noxious toxins were responsible for mental disease associated with the menstrual period.

The study of metabolism during the menstrual cycle in women has yielded some interesting results, which might shed some light on the problem. Increases in the basal metabolism, oral temperature and pulse rate were observed by Griffith to occur premenstrually. He also noted the lowered systolic blood pressure towards the end of the intermenstrual period. These findings have been confirmed by others, notably by Benedict, Wakeman, Nixon and Matters, who found the same drop in temperature and basal metabolism occurring with the menses also.

Chemical studies have been somewhat sketchy and not always in agreement. Nixon, Okey and Boyden, who have contributed a great deal to the study of the metabolic changes in the menstrual cycle, noted the drop in the blood sugar during the third week and its peak during menstruation. More recently their work has been confirmed by Block and Bergel who noticed a reduced sugar tolerance during menstruation and remarked that it was not due to liver damage or toxins but to autonomic imbalance. Griffith and his associate in a series of papers on metabolism reported on the findings obtained by studying 3 women for a year. Their study of the urinary output indicated that the urea, creatinine, amino acids, total N and chlorides all increase in the urine during the inter-menstrual period and decrease during the flow. Ammonia, phosphate and organic acid are less in the inter-menstrual period and rise during the flow. Gillett also demon-

demonstrated a nitrogen retention during menstruation; and Herley found a decrease in water and chloride excretion.

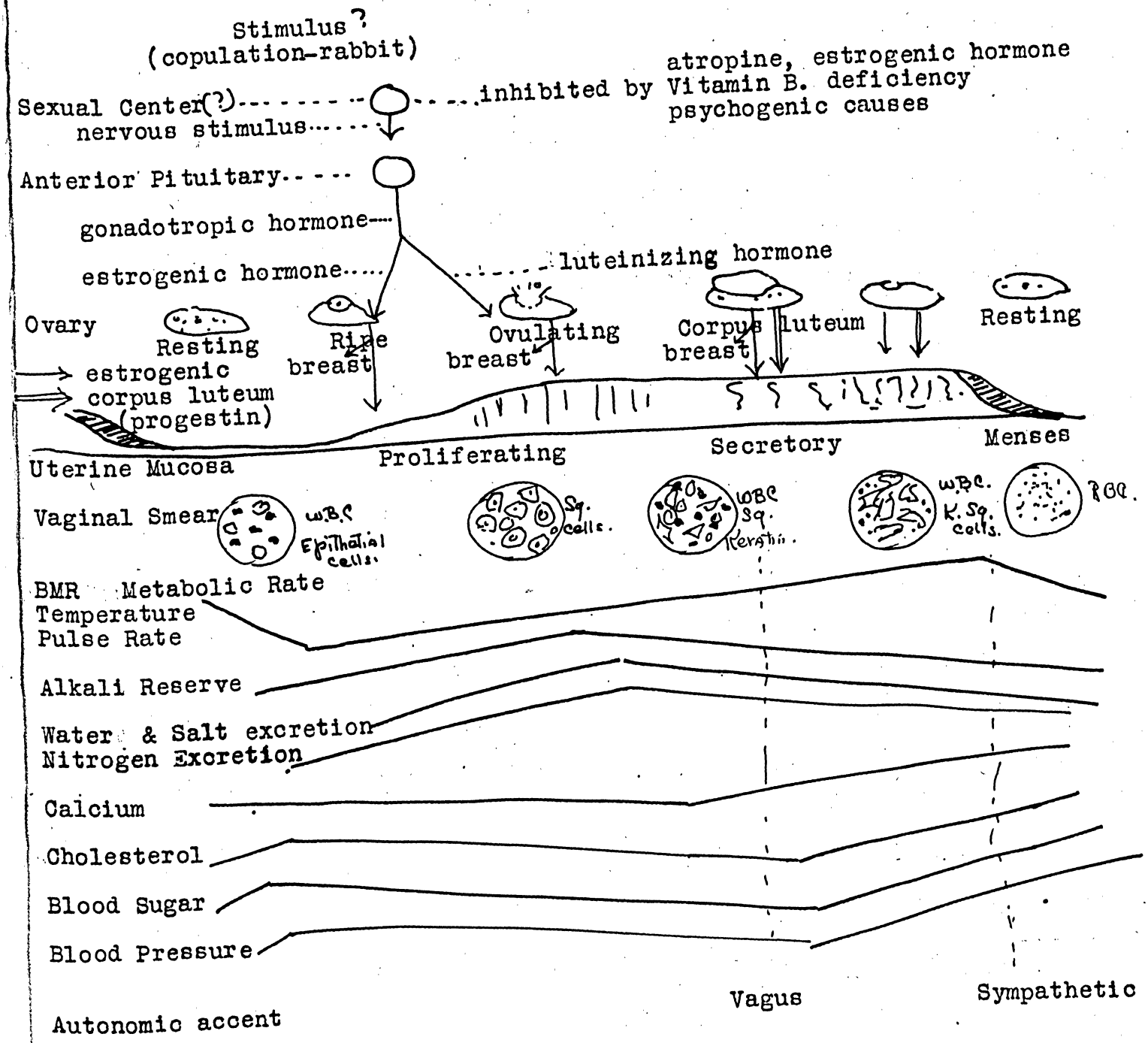
In the study of diseases of many sorts a relationship to the menstrual cycle appears and they will be given here briefly in the hopes that they may cast some light on the problem. Jackson and Thompson noted that the majority of the patients with agranulocytic angina were women, and that the drop in white cells occurred regularly with the onset of menstruation. In a male case they found extraordinarily large amounts of the estrogenic hormone, but when the drop in blood cells occurred, no estrogenic hormone could be recovered from the urine at all. Nixon gives a long list of diseases having a similar relationship, among them being epilepsy, hysteria, migraine and anemia. Tuberculous women have axacerbations at the menstrual period and show pre-menstrual temperature rises. Gastric ulcers are accompanied by increased pain and acid secretion at definite stages of the cycle, and herpes and urticaria are frequent pre-menstrually. Diabetic women show increased difficulty in utilizing sugar at the time of menstruation.

The forgoing information does not, however, appear to give any satisfactory explanation for the marked mental and emotional upsets apparently related to the menstrual or rather ovarian cycle. That these two processes had any relation whatever was not even suggested until Plüeger suggested that the ovary influences menstruation thru a nervous mechanism. In 1899, however, Knauer noted the humoral nature of this ~~con~~-control, forshadowing later endocrine studies. In 1908 Hitschman and Adler described the cyclic changes occurring in the endometrium and later

Schroder and Meyer showed the relation of these to activities in the ovaries. Following the discovery of the changes in the vaginal smear of the guinea pig and their relation to the ovarian activity in that animal by Stockard and Papanicolaou in 1917, however, the studies of the physiologic mechanism of the female generative system developed rapidly. After Long, Allen, Hartman and Corner had studied the cycles in a number of other laboratory animals, this method was applied to the recognition of the particular substance that caused the oestrus changes, the so-called estrogenic hormone. In 1923 Allen and Doisy prepared the pure hormone from the liquor folliculi and altho some called it folliculin for that reason, these authors named it "theelin". This material is responsible for the initiation of the proliferated changes in the uterus, breasts and vagina accompanying ovarian activity. The studies of Smith and Engle and of Aschedm and Zondek point to the pituitary as the stimulus to the gonads, the pituitary being the motor of the sex glands, as Butenandt puts it. Allen, Corner and Hartman contributed further steps studying the effects of this gonadotropic hormone of the pituitary on the uterus, as well as those of the estrogenic hormone, and Hisaw, Corner & Allen contributed studies of the corpus luteum hormones, the chief of which, progestin, prepares the uterine lining for implantation of the developing ovum. Some of the steps are still not unraveled but the accompanying chart shows the known relationships and indicates how the cycle is self limited and therefore periodic. The salient points to be noted are:

1. The ovary does not begin to produce ripe ova until stimulated by the maturing fraction of the gonadotropic hormone

Relation of the menstrual and Ovarian Cycles and their Hormonal Control. Physical and Chemical changes noted in the cycle.



elaborated in the anterior pituitary gland, and reaching the ovary thru the blood stream. As the ovum matures the cells lining its follicle produce the estrogenic hormone some of which is also poured in the blood stream.

2. The estrogenic hormone acts on the breasts(proliferation of the ducts) causes proliferation of epithelium with keratization of the outer cells of the vagina and thickening of the musculature and hyperplasia of the mucosa of the uterus, and has an inhibiting effect upon the anterior pituitary, thru a nervous mechanism.

3. The luteinizing fraction of the gonadotropic hormone stimulates the cells lining the follicle to produce a corpus luteum. This results in more estrogenic hormone as well as the new, corpus luteum hormone (progestin) to be added to the blood stream. The corpus luteum hormone acts principally to prepare the uterus for implantation by stimulating secretory changes and further proliferation of the mucous, but also lessens the irritability of the uterine muscles.

4 The estrogenic hormone is quickly destroyed in the body (Zondek) and as soon as no more is produced, the fall of the hormone content causes dissolution of the thickened endometrium of the uterus.

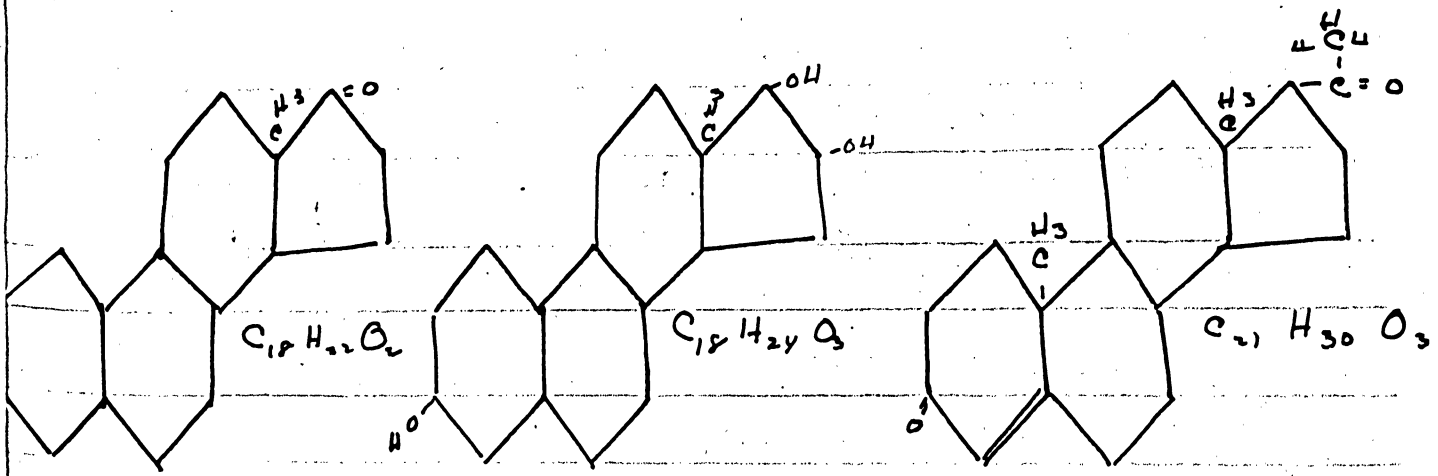
5. If the ovum be fertilized and becomes implanted it begins to produce luteinizing hormone of its own and thus helps prevent regression of the corpus luteum, until the placental attachments begin to loosen. The placenta also manufactures large amounts of the estrogenic hormone; and since pregnant women excrete large amounts of both these

hormones in their urine, this is the usual source from which they are prepared at the present time.

In the above description there is mention only of the generative affects of these hormones and no indication of any mechanism by which they might cause widespread constitutional affects is offered. A few investigators have offered suggestions to elucidate this action. Frank reported that he found three times as much theelin in the blood of women with a "pre-menstrual tension" as he found in normals, suggesting thereby that the estrogenic hormone might be responsible for the irritability observed. Saethre, Oestericher, Harris and Severinghaus on the other hand found that the disturbances of involutional cases in whom they found diminished estrogenic hormone responded quickly and satisfactorily to the administration of the hormone in a great number of cases. Injection of the estrogenic hormone was observed to increase motor activity in rats.

That hormones can greatly affect behavior is shown in the experiments demonstrating that the maternal instincts in rabbits (Tietz) and birds and rodents (Riddle) responded to pituitary injections. The latter has isolated the particular hormone responsible for this and name it "prolactin". Bards hypothalamic cats demonstrated in a graphic way the affect of the ovaries on behavior for they discontinued their display of sham rage during the oestrus period. Incidently his experiments proved that the sexual center, postulated by Butenandt, must be in the hypothalamus ordcaudad to this since his animals continued to go through normal sexual cycles. Domestic female cats that have been spayed are said to de-

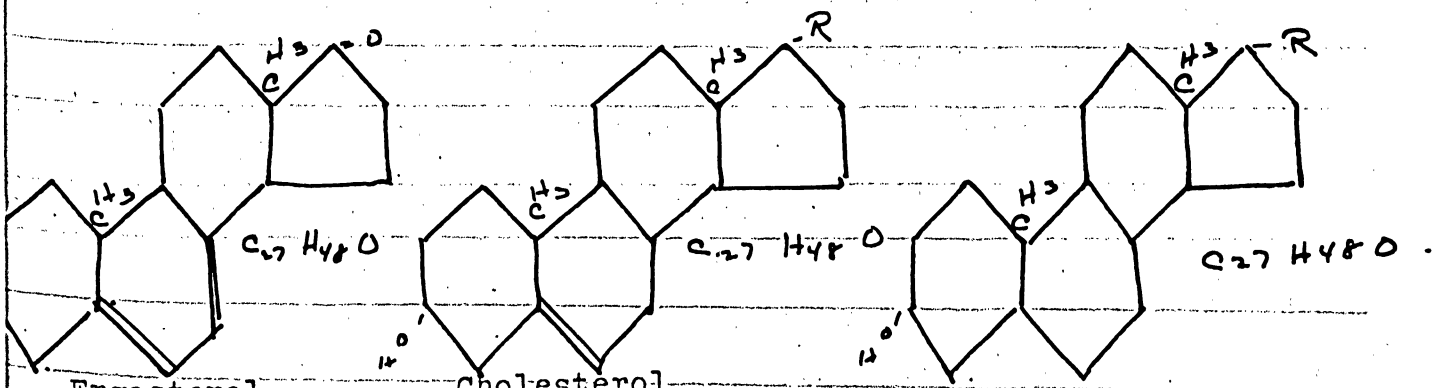
THE STRUCTURAL FORMULAE OF THE ESTROGENIC HORMONE AND RELATED COMPOUNDS



Estrogenic hormone
Theelin

Estrogenic hydrate
Theelol

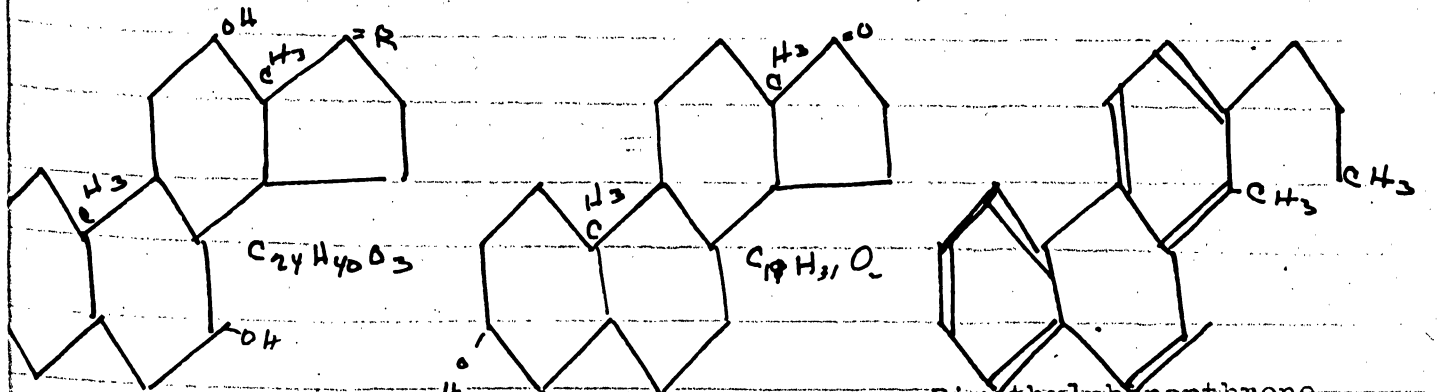
Corpus luteum hormone
Progestin



Ergosterol

Cholesterol

Coprosterol



Cholic Acid

Androsteron
Male sex hormone

Dimethylphenanthrene

Auxin plant growth hormone C 18 H 22 O 5

Strophanthin cardiac glucoside

Toad poison

develop aggressive natures after the operation and will fight other cats where previously they had been timid. All these observations appear to indicate a relation between the estrogenic hormone and mental state. It is hoped this study will contribute to establishing this relationship.

The Chemistry of the Estrogenic Hormone.

The study of the excretion of the estrogenic hormone necessitates a familiarity with its chemical properties. Extracted with butyl alcohol, ethyl acetate, acetone, chloroform, ether, alcohol, olive oil and propylene glycol,(in small amounts by petroleum ether and dilute Sod. Hydroxide) from the urine the hormone displays an oily nature. It can be saponified and extracted later without changing, as this material is very stable to heat, acids and other materials altho less so to alkalies. Recrystallized from butyl alcohol or ethyl alcohol the material appears as flat colorless plates. Due to the painstaking work of Doisy, Marrian, Butenandt, Allen and Dingemanse the structural formula is probably known, as are also its relations to many other materials of great physiological importance.

In the accompanying chart are seen the formulas of some of these materials. Estrogenic hormone is a tetracyclic ketohydroxide with a formula of C18 H22 O2. It has a melting point of 256° according to Butenandt. The hydroxide prepared by adding ~~two~~ one molecule of water to the above substance is called "theelol" , by Doisy. It is less active, more inert than "theelin". It has also been crystalized from urine. Esters of these substances are active physiologically and are

not inactivated, as are the original hormones, when injected into the body.

Addition of a methyl group with a loss of the double bonds in the estrogenic hormone gives "androsteron" the male hormone which is very like the cholane nucleus of which bile acids, cholesterol, vitamin D and other substances are made. Butenandt believes that these substances are likely built up from each other in the body since intermediate forms are found and cholesterol and bile salts as well as theelin are known to be made as well as destroyed in the living organism. Incidently it is interesting that cholesterol as well as some of the related carcinogenic phenanthrenes have estrogenic activity. Zondek noted that even massive doses of estrogenic hormone disappeared within 24 hours after injection while the ester did not. He could regain some of this material by hydrolizing the liver. It is possible that some inert stable ester is formed when the pure hormone is injected and therefore estrogenic activity is lost. It is of interest to note that the estrogenic hormone has been recovered not only from the follicular fluid, from the urine of pregnant women, from placentas but has also been crystallized after extraction from palm leaves. The similarity to the plant hormone auxin, having a formula of $C_{18}H_{28}O_4$, to the estrogenic hormone both in structure and activity has been noted by Schoeller who observed the rapid growth and blossoming of lilies and hyacinths fed small amounts of the female sex hormone which was inactivated as it was used.

11 PROBLEM

The purpose of this study was to determine more definitely whether the estrogenic hormone has any bearing on behavior in mental disease. If such a relationship were found we desire to inquire as to how the hormone could influence the psychic and physiologic behavior. In order to accomplish this we proposed to determine the deviations from the normal level of the excretion of the estrogenic hormone, and compare it with the behavior and physiology of the patient.

111 EXPERIMENTAL DATA

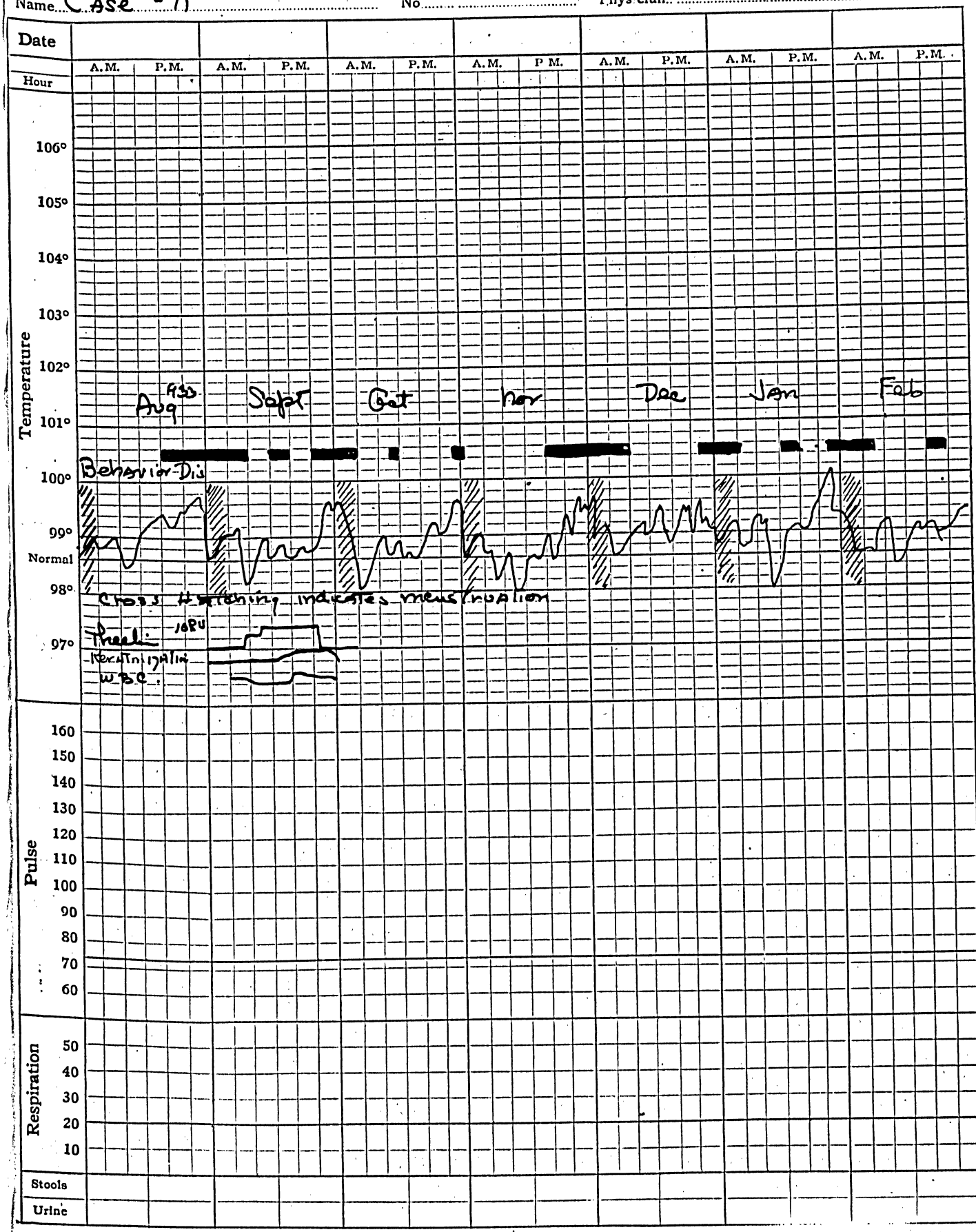
A Correlation of the behavior with ovarian activity.

It was necessary at the beginning of this study to decide which of the 450 women available would be examined for the estrogenic excretion. We therefore decided to determine which of the patients fitted in the groups normally showing a high percentage of coincidence with the generative processes. We sought, therefore, for those with violent periodic behavior synchronous with the menstrual cycle, a few amenorrhoeic patients, several involutional cases, a few post partem psychoses, several adolescent schizophrenias and a few castrates. In order to locate these patients prolonged behavior studies had to be made and a record kept of the menstrual periods in all of the menstruating women. By excluding the patients with organic disease such as syphillis, tuberculosis and diabetes we eventually selected fifty likely subjects. Correlation of the behavior as it varied from day to day was noted and twelve cases showed a definite cycle. Many of the patients supposed to show some correlation between disturbance and menstruation showed no regularity in this matter upon close check. In the cases observed to maintain this relation, it was noted that all showed the disturbance for a few days before the period and appeared to quiet down by a week post-menstrually. There were additional minor fluries at the 12-16th. day in three patients. The amenorrhoea patients were either consistently depressed or disinterested and inaccessible as well as incoordinated.

Form - T

LONGVIEW HOSPITAL
MEDICAL DEPARTMENT
GRAPHIC CHART

Name Case #11 No Physician M. D.



EXPERIMENTAL DATA cont

B Determination of estrogenic activity level

1 Rectal Temperature of patients

In order to further sort the patients so as to separate those likely to show increases in the hormone level, we studied the daily temperature charts with the hope that since the hormone causes increases in vascularization and activity in the reproductive tract, that it might raise the rectal temperature. Temperatures on more than 50 patients were plotted therefore with the menstrual periods recorded when possible.

Of these patients we were able to obtain records for more than a year on some and we did not consider charts for less than four months of much significance. Outstanding in these records were the very slight temperature fluctuations in the schizophrenics of the real dementia precox type who nevertheless showed the menstrual temperature drop expected in normals. In direct contrast to these patients were the non menstruating schizophrenic reaction types with unaccountable and unbelievably wide temperature deflections. Of the involutional cases we found two types, the group with an unaccountable steady elevation of temperature and the others who regardless of manic activities never reached even the normal limits.

Four manics of the chronic type showed a definite rise in the temperature to 100 or more from the 10th. day of the cycle and a few others showed a later rise but one equally high. The inclosed table gives the temperature of Case 20 on each day of ten successive periods with the average

Form - T

LONGVIEW HOSPITAL

MEDICAL DEPARTMENT

GRAPHIC CHART

Name Case #11 No. Physician M. D.

Date

Hour — A.M. P.M. A.M. P.M. A.M. P.M. A.M. P.M. A.M. P.M. A.M. P.M. A.M. P.M.

Temperature

106° 105° 104° 103° 102° 101° 100° 99° Normal 98° 97°

Summation of 10 menstrual periods = Average Temperatures

Day of cycle

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

Pulse

160 150 140 130 120 110 100 90 80 70 60

Respiration

50 40 30 20 10

Stools

Urine

temperature for that day below. Altho this patient has a not very marked temperature rise when so figured yet she presents an unfailing disturbed mental period before and during each menstrual cycle.

B Determination of Estrogenic Level

2 Reactions in Rabbit after injection of urine.

Specimens of urine were injected in virginal and castrate rabbits with the object of assaying the amount of sex hormone present, by the degree to which uterine contractions could be stimulated. As will be seen below the method was discarded because it was uncertain altho large amounts of the hormone could activate a quiescent uterus. Even the castrate uteri responded to the stimulation of drying and the air however so that it appeared to offer too many chances for error.

Moreover, we observed a return of uterine sensitivity in several castrates who had shown no activity shortly after loss of the sex glands. Therefore we felt that the use of this test would be too uncertain to give us an accurate idea of the state of the endocrine secretions in our patients.

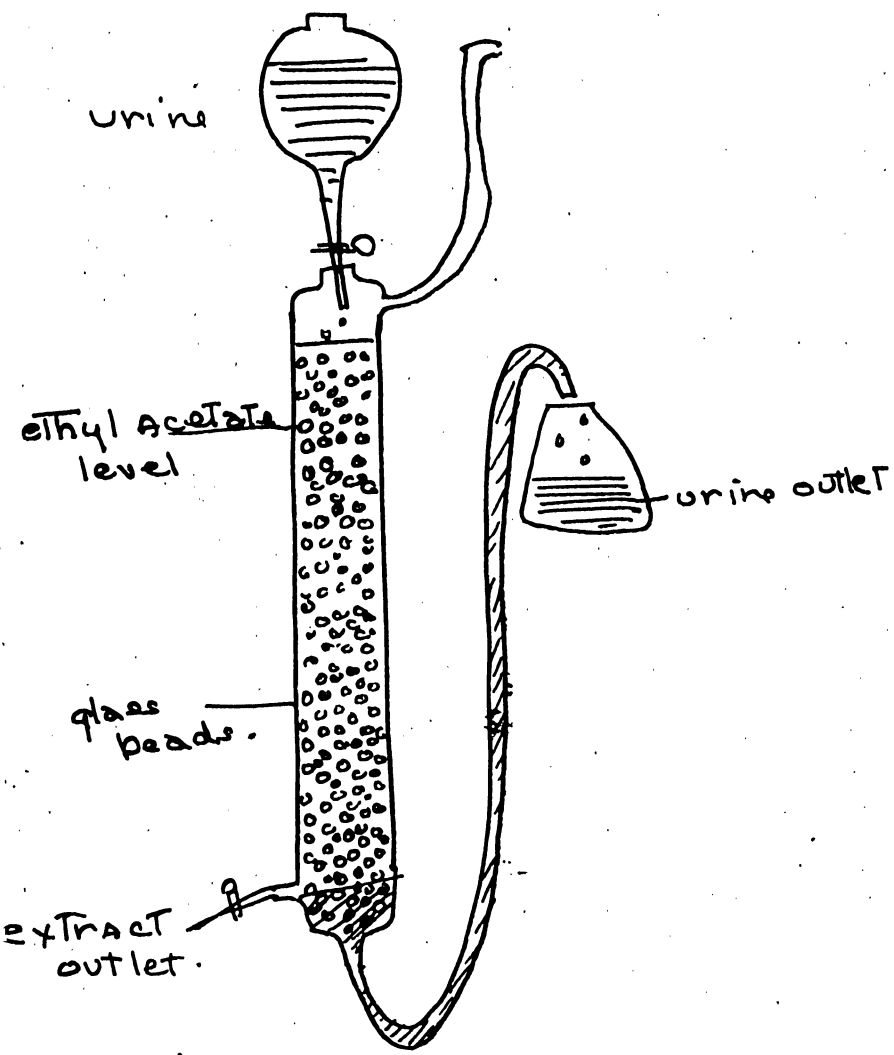
111 EXPERIMENTAL

B Determination of Estrogenic hormone.

Millon Reaction

Because of this great unreliability in the biologic assay an attempt was made to use chemical tests to estimate the content of oestrogenic hormone in our cases.

As demonstrated by Butenandt the molecule of oestrogenic hormone contains a phenol group so that it gives a pink color with Millons reagent used to test for this Nucelus. Preparations of crystalline theelin (Park-Davis Co.) were tested and we found that it was easily possible to estimate the presence of 10 rat units by the careful regulation of heat so as not to destroy the color. When our specimens were run however the color was very intense since the unpurified extracts contained many other Millon positive substance such as tyrosine and other phenol compounds. The separation of the substances from oestrin is rather complex, necessitating a repeated extraction after saponifying solutions and we did not attempt to prepare such purified preparations as we needed to continue the solution as started in propylene glycol for the biologic assays. It appears, however, that some careful studies might result in the development of a reliable chemical method of assay.



Apparatus for the rapid extraction of the estrogenic hormone from the urine.

111 EXPERIMENTAL WORK

B Determination of the Estrogenic hormone level.

3 Rat reaction or urinary extracts.

In order to determine the level of estrogenic hormone in the body we made use of the biologic assay of urinary specimens collecting 24 hour specimens weekly from our patients. The extraction of the oestrogenic hormone from the urine was facilitated by hydrolysis with acetic acid after saturation with sodium chloide to prevent the absorption of the solvent. The urine was then placed in a large separating funnel from which it was allowed to drop into a tube containing ethyl acetate and full of glass beads. The beads broke the urine droplets into fine particles allowing for a vast surface of contact between the two fluids. The extracted urine then fell to the bottom of the tube because of its greater specific gravity and was allowed to drop from the outlet tube at the same rate as the fresh urine fell from the separating funnel. The ethyl acetate was drawn off at the top, fresh solvent placed in the tube and a re-extraction of the urine accomplished to add to the yield. In this manner the entire extraction could be accomplished in less than one hour without the use of heat and since we have repeatedly obtained 1000 units from the 24 hour specimen of urine from a pregnant woman, it appears to be sufficiently efficient. The extracted urine was discarded and the solvent distilled off under vacuum according to the technic used by Kurzurok. The residue was dissolved while still hot by adding olive oil originally and then by the use of propylene glycol upon suggestion of Dr. Kurzurok's coworkers who felt it led to

more even absorption. Although the bulk of our experiments were carried out using this material for injection I believe it is more toxic, at any rate the sample we obtained was and I prefer to use the olive oil as it caused no irritation of the animals.

Using 5cc of the solvent we injected ½ cc in each of two adult castrate female rats in three equally divided doses 4 hour apart thus using the original Allen Doisy Unit. The animals were examined in 48 and 52 hours.

The test animals were occasionally retested with a standard oestrogenic preparation to ensure reactive membranes. The presence of an oestrus smear (absent white cells with squamous and round epithelial cells as observed in the hanging drop prepared by injecting a drop of water in the vagina of the rat) was considered positive.

If a positive reaction were obtained we considered that 1/10 of the 24 hour output contained 1 rat unit and therefore there were more than 10 rat units in a 24 hour specimen. If so the dose was divided and rerun to determine if there were as much as 20 rat units. Since the presence of more than 20 rat units was considered abnormally high (Kurzurok states the normal runs between 10 and 20 rat units) we did not attempt to assay the specimen further.

The assay of the sex hormone is subject to many sources of error. In the first place to obtain 24 hour specimens on mental patients is a difficult task since only rarely is cooperation obtained. Sometimes a resistant patient will

will refuse to void for 24 hours if forced to use a bed pan or some other method of collection.

Numerous experiments have demonstrated that hydrolysis with acid greatly increases the yield so that the temperature at which the urine stays while exposed to the acid, the concentration of the urine and the presence of absorptive materials in the urine all lessen the accuracy. Furthermore, the estrogenic hormone in slightly different form as Theelol may give much less affect on the membranes of the rat, as is recognized by the fact that the new international unit has been standardized on the basis of weight of the crystals rather than on the biologic affect, In the test animal. It is not necessary to state moreover that the use of the test animal for standardization is apt to give quite different results with different animals when slight variations in hormone assay are attempted. For this reason it was felt that the attempt to make any fine estimate of the hormone content was foolish. When it is considered that it takes hundreds of rat units daily to affect the mucous membranes of a woman to a point where the change could be detected by present methods(Papanicopou) it appears foolish to believe that the difference between 28 and 35 R.U. in the urinary excretions as reported by some examiners could have any reliable meaning.

cont III EXPERIMENTAL

B Determination of the estrogenic hormone level

5 Direct vaginal smear of patients

In the meantime however, a study of the articles of Papanicolaou led us to make slides of our patients. This author demonstrated that the vaginal secretion loses leucocytes and contains only epithelium until ovulation when the leucocytes reappeared and keratization of the squamous cells began to develop. It appeared to us desirable to study whether the oestrogenic hormone present in a particular case was sufficient to produce a copulative smear in the vagina, when the rat assay demonstrated that the amount excreted in the urine amounted to less than 10 R.U. per day. If a patient had a lowered or increased kidney threshold or if the susceptibility of the hormone were lessened or if there was an increased neutralization of the hormone, the results obtained by these two vaginal smears would not check with our estimates as to whether the patient had an abnormally high or unusually low sex hormone excretion.

A study of several hundred patients of all ages was made to see whether the absence of leurocytes and presence of keratinized cells in the vaginal smear was really indicative of active ovaries. A modified technic was necessary. We used direct examination of eosine-tinted saline suspensions, prepared by touching the vaginal ovall with an uncovered applicator without the use of a speculum and transferring the material so obtained to the drop of saline placed on a slide. This led to an extreme simplification of the technic with a minimum

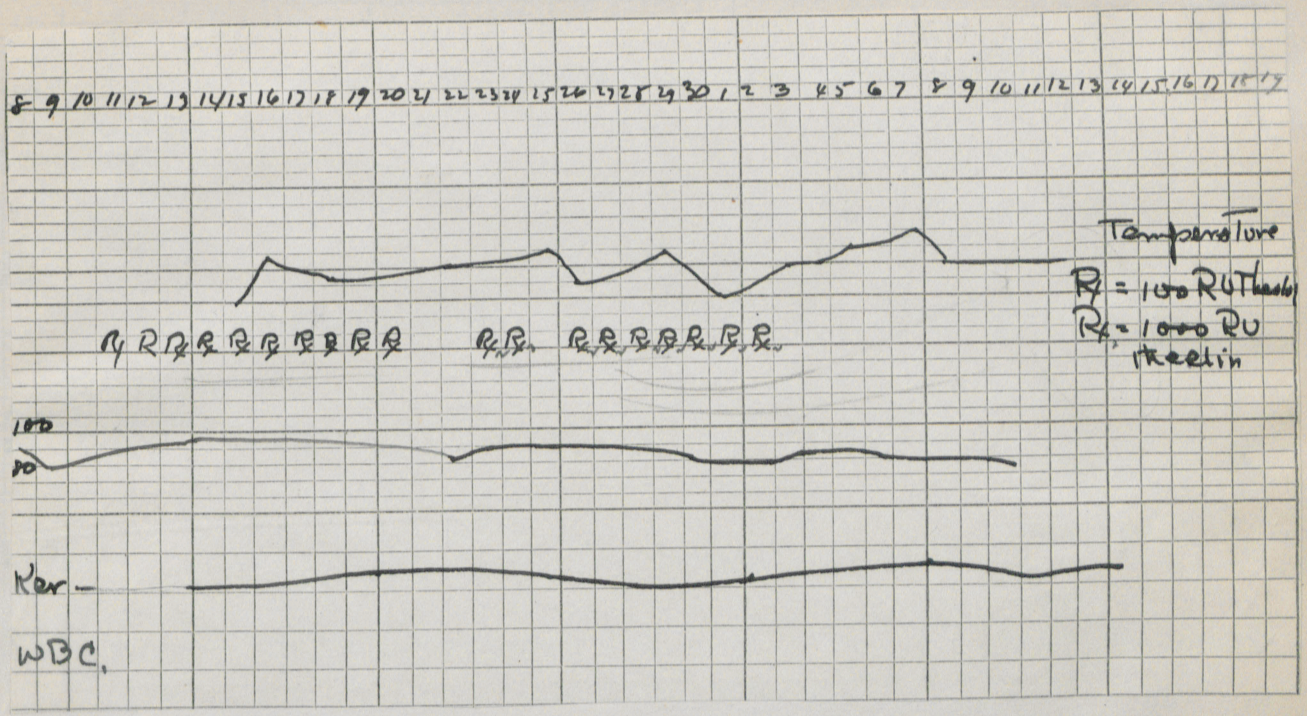
of instrumentation and time. As many as 50 patients were examined in an hour. The cells were allowed to stay a minute before being examined and the coloration of the cells + presence of leucocytes. noted

111 EXPERIMENTAL

Estrogenic assay, vaginal smear, temperature and blood pressure in a heterogenous group of selected patients.

A group of 22 women patients including schizophrenics, manics, involutional melancholias, and several patients showing disturbances synchronous with the menstrual cycle were studied for three months. The daily behavior was charted and significant changes noted. At 8 A.M. on five mornings a week the temperature, blood pressure and vaginal smear were examined under as uniform conditions as possible. The same operator made all the observations and only one thermometer was used. Twenty-four hour specimens of urine were collected weekly, when possible, for estrogenic hormone assay. The vaginal secretions were collected on a bare applicator and suspended in an eosin tinted saline solution. After a few moments they were examined for white cells and keratinized squamous cells. Only rectal temperatures were taken. Several glucose tolerance tests were made. The following cases were chosen to illustrate the observations made.

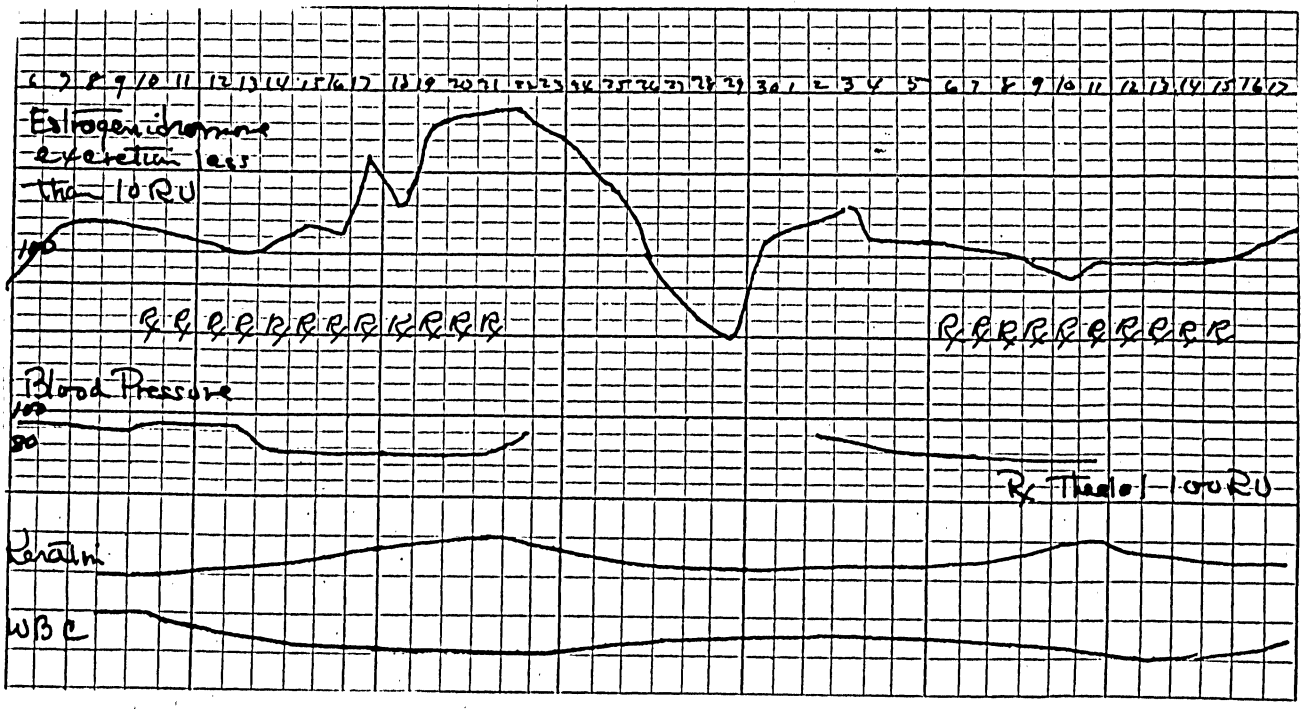
Case No. 2 Age 26 Adm. March 1933 as a hebephrenic schizo. Single. Onset 4 years previously. Patient was sterilized by salpingectomy.



On admission BP 98/62. Dilated pupils showing a mixed vegetative disturbance. Amenorrhea. Had responded by menstruating after theelin and after antuitrin S on previous tests. Preliminary examinations showed temperature varied from 98.8 to 99.2. Blood pressure between 84 to 94 systolic. Vaginal smears showed WBC and non-keratinized squamous cells. Estrogenic hormone below 10 R.U. per day. Administration of 9000 I.U. theelin caused no change in temperature, drop in B.P. to below 80, diminution of white cells and appearance of keratinized cells. This patient always shows marked improvement with theelin. She becomes brighter, stops wetting her clothes altho she complains of the weakness when the blood pressure becomes low. Since she had previously had several courses of this medication we also observed that when the medication stopped the patient not only became untidy but had episodes when she would suddenly attack some one near her.

This patient demonstrates a hypohormonal amenorrhea with probable deficiency of gonadal stimulation since Antuitrin S was able to induce the cycle.

Case No. 3 Female age 21 Admitted Diag. Schizophrenia
Amenorrhea preceded onset. General withdrawal and incondination



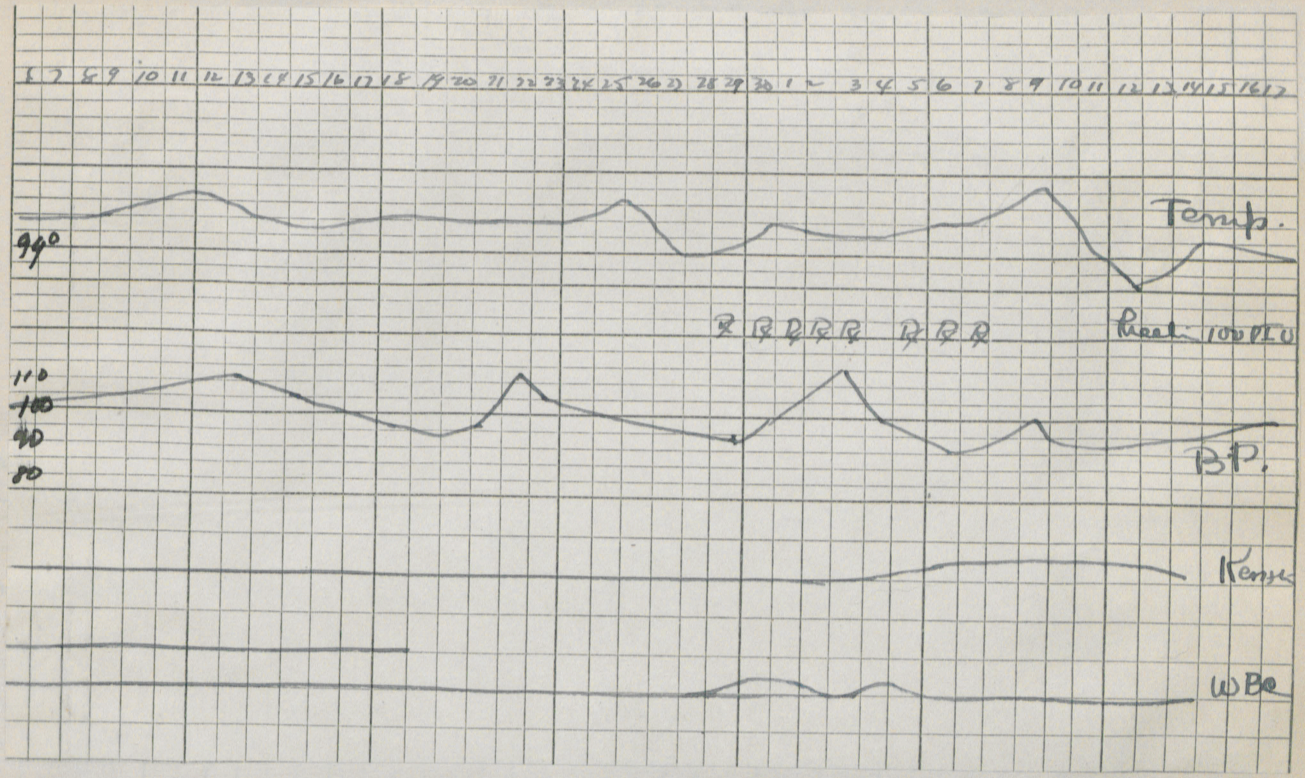
Preliminary period. Temp. 98.6-99.2. Blood pressure systolic 90-100. Vaginal smear shows leucocytes and non-keratinized squamous cells. Urinary estrogenic hormone less than 5 R.U. per 24 hour specimen. No menstruation.

Administration of theelol 100 R.U. daily by mouth was followed by temperature rise after 7th dose, persisting until 4 days after last dose. On the 6th day off medication blood pressure dropped. Vaginal smear showed less leucocytes with appearance of keratinized cells by the 4th day of treatment. No complete leucopenia or keratinization. No menstruation following withdrawal of medication.

Later administration of theelol 100 ~~international units~~ RU daily ~~by hypos~~ for 10 days causes another blood pressure drop but no temperature change. Smear changes similar to first experiment. No menstruation.

This patient demonstrates amenorrhea with low estrogenic secretion. The large doses that produced only slight change in the vaginal secretion indicates very poor ovarian activity or rapid destruction of the hormone. Stimulation by pituitary extract must be attempted to determine if the ovarian failure is primary or secondary. Theelin produced no change in mental state. Patient very resistant and inaccessible.

Case No. 4 Age 23½ Ad Dec. 1933 Schizophrenia
Single Onset 2 mo. previously Paranoid and suicidal



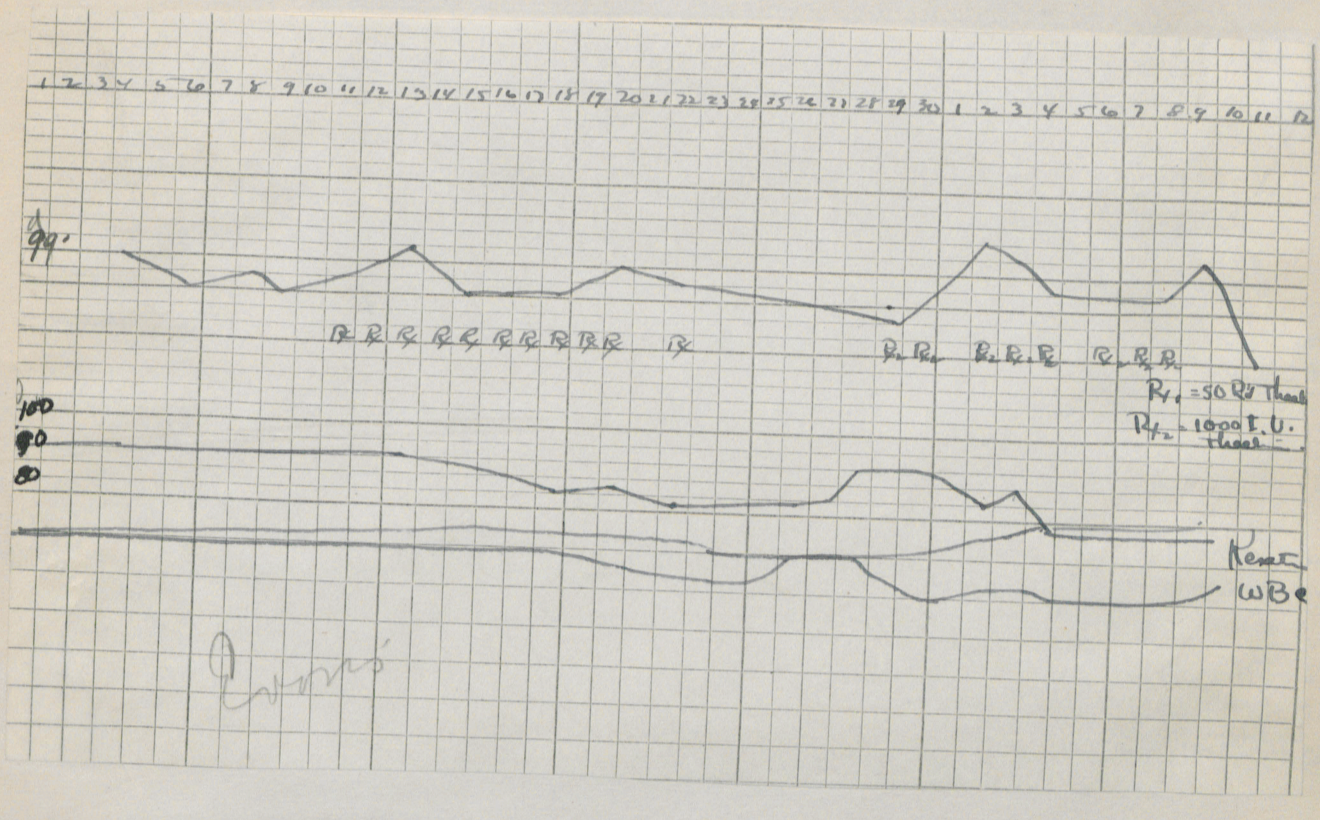
On admission pupils were dilated. Blood pressure 114/78 incontinent
No menstruation since admission
Preliminary period Rectal temperature 98.6-99.4
Systolic blood pressure 90-110 Smear showed non keratinized epithelial cells of squamous variety. few leucocytes.
Urinary estrogenic hormone less than 10 rat units per 24 hour specimen

Administration of 8000 international units of theelin on 8 successive days resulted in slight temperature rise with fall secondarily. Slight keratinization. No menstruation followed withdrawal.

This patient demonstrates an amenorrhea with low estrogenic excretion.

8000 international units of theelin was without affect either indicating high tolerance with a previous low ovarian activity or rapid destruction. To determine whether the ovarian inactivity is primary the results of administration of gonadotropic hormone must be attempted.

Case No. 5 Female age 32 Admitted 1932 with a diagnosis of Catatonic Schizophrenia. She had a baby 2 months old. No menstruation since birth of child and not since admission.



On admission the pupils were dilated and the patient had a dilated bladder. Blood pressure 130/80.

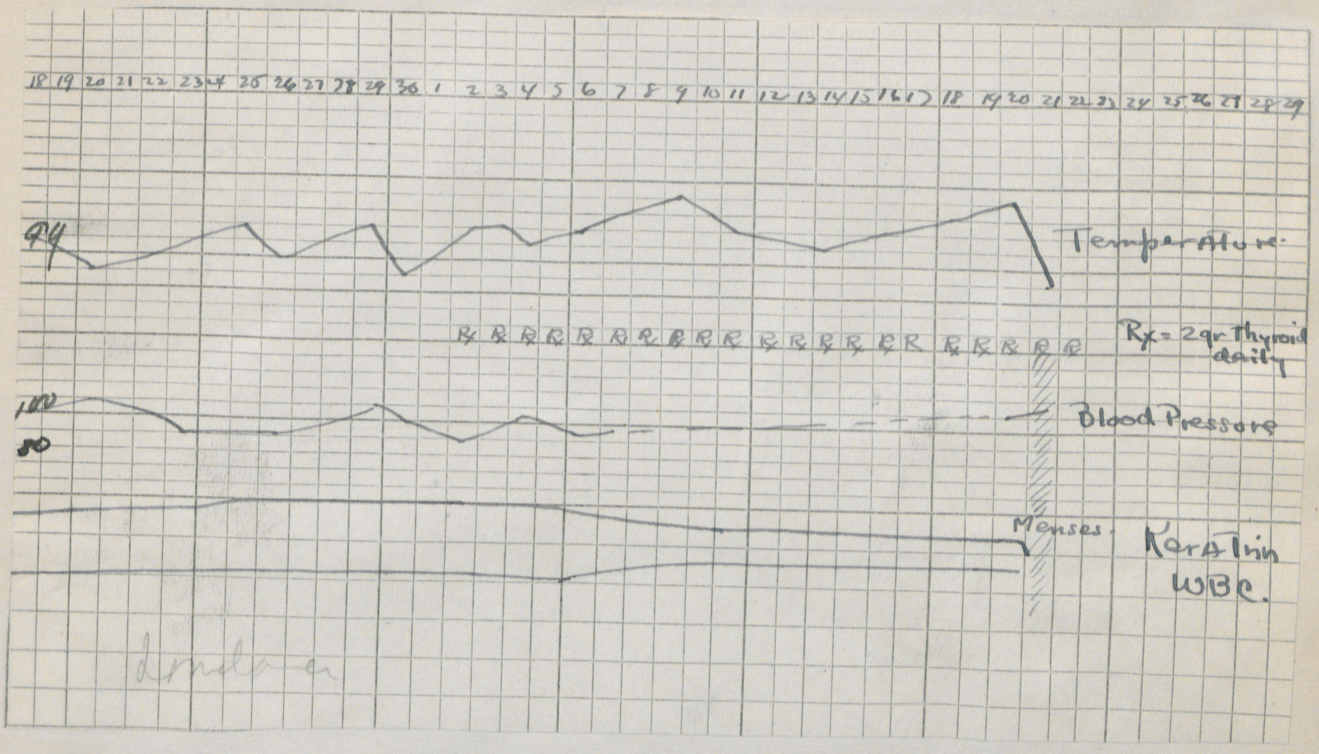
Preliminary observation showed rectal temperature varied between 98.8 and 99. The blood pressure was 90 ~~or above~~ systolic. The patient was extremely atonic. Vaginal smear showed a menopausal appearance of large amounts of white cells. and non-keratinized squamous cells. Estrogenic hormone in 24 hour specimen of urine less than 10 R U per day.

Administration of 1200 R.U. theelol by mouth slightly diminished the number of white cells and caused some keratinization but not menstruation. It caused a drop after the 5th day in the blood pressure to 76 systolic which rose to 90 in one week. There was no marked change. A week later eight injections of 1000 I.U. of theelin on as many days caused a drop in the blood pressure to 68 on the 7th to the 12th days with great weakness. The white cells almost entirely disappeared and keratinization was complete. The patient was less interested than ever after treatment.

This patient showed an acute blood pressure drop with fair mucous membrane response to the hormone. She had a hypohormonal amenorrhea with possible good ovaries which will be tested for activity with gonadotropic hormone.

During treatment with estrogenic hormone there was some increased glucose tolerance.

Case No. 6 Female age 40 Adm. June 1934 as suicidal depressive manic. Onset 1929 5 days following confinement; previous attacks also after childbirth. Patient has had 12 pregnancies. Amenorrhoic.

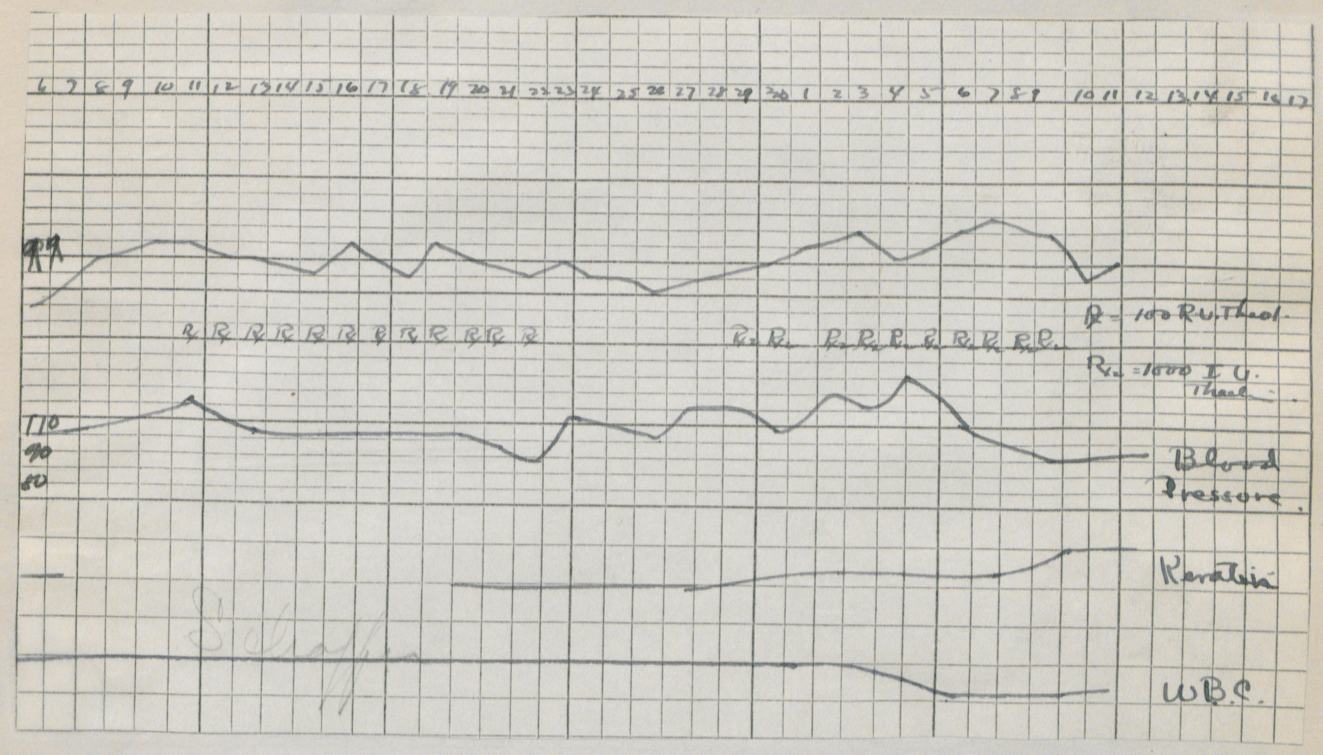


On admission blood pressure was 130/78. Preliminary period showed this patient had a temperature as volatile as her moods, the former fluctuated from 98.6 to 99.6. The blood pressure likewise fluctuated but was between 100 and 90 systolic for the most part. The vaginal smear showed no or rare leucocytes and keratinized squamous cells in abundance. The patient was quite erotic. Estrogenic hormone assay revealed more than 20 rat units in two different 24 hour specimens of urine. The patient was given thyroid extract 2 grains daily. A few white cells and non-keratinized cells appeared but on the 18th day the patient began to menstruate. The temperature which had raised slightly to 99.8 fell to 98.6 with the onset of menstruation. The patient was noticeably more stable. The blood pressure rose to 118/78.

This patient appeared to have a hyperhormonal amenoorhea and treatment with thyroid not only caused menstruation but apparently caused mental improvement.

Glucose tolerance normal but there was a relative leucocytosia.

Case No. 7. Female aged 36. Admitted Nov. 1934 with a diagnosis of depressive-manic with suicidal tendencies. The patient was married and had 3 children. There was a history of cessation of menses 4 years before.



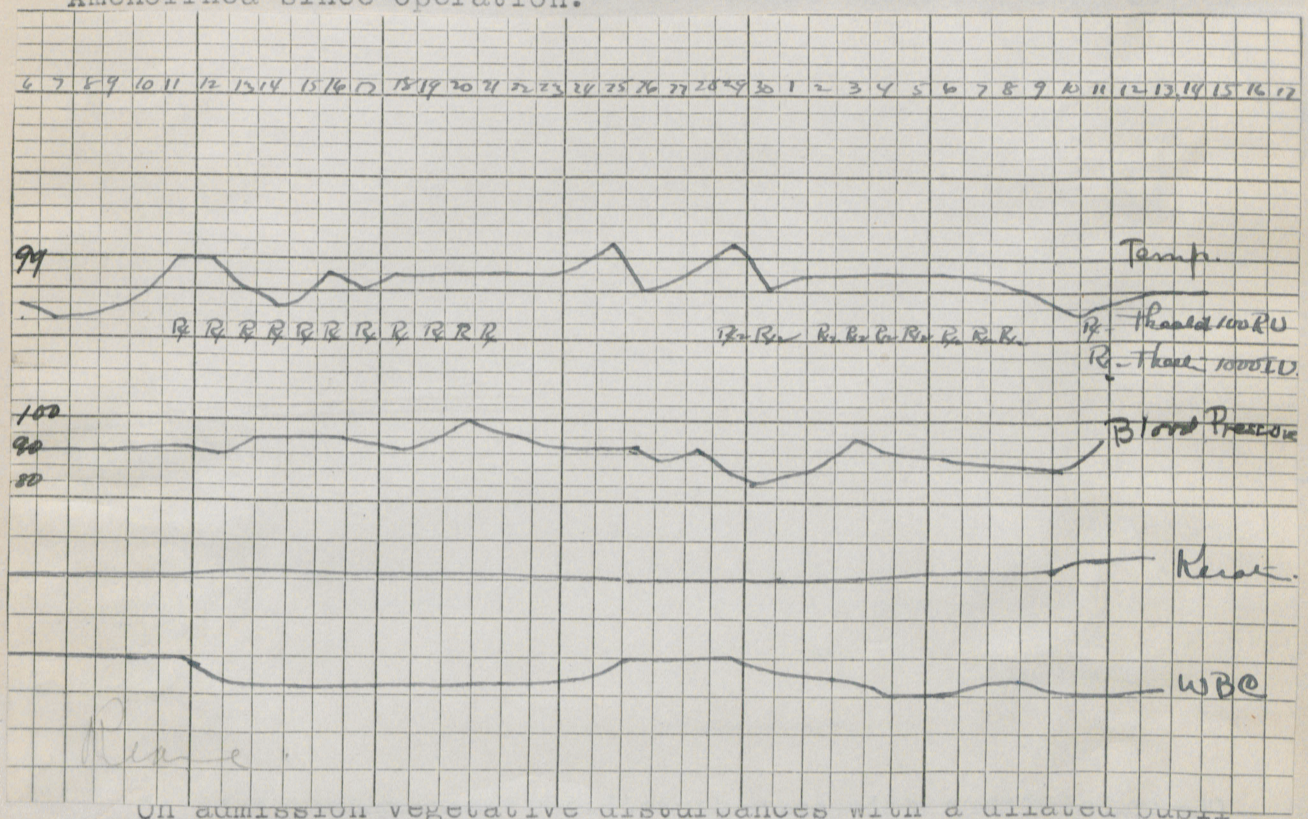
On admission the pupils were dilated, the patient was incontinent and the blood pressure was 105/65.

Preliminary observation of the case showed a temperature varying only a few tenths from 99 in either direction. The blood pressure was between 94 and 104 systolic. Vaginal smears revealed many leucocytes and non-keratinized squamous cells. Estrogenic hormone in the urine was less than 10 R U 24 hour specimens.

Administration of large quantities of theelol 1400 R.U. over 2 weeks time apparently made no affect on any of the observed phenomena in this patient except that she stated that she felt livelier. Eight theelin hypos given 1000 I.U. daily for as many days (a total of 8000 I.U.) caused the temperature to rise to 100 while the hormone was administered. On the 5th day however the blood pressure was found to be dropping and on the 8th. day it was below 90 systolic. Altho the vaginal smear showed keratinization of the squamous cells on the second day the white cells were only partly reduced. This patient demonstrated a hypohormonal amenorrhea which was resistant to 8000 I.U. of Theelin. Probably showing an early menopause. She likewise should be tested for secretion of prolan and at least given the benefit of sufficient injections of anterior pituitary substance to test the possibility of stimulating her ovaries.

During administration of the Theelin this patient showed an increased glucose tolerance, and relative lumphocytosis.

Case No. 8 Female aged 28, admitted Sept. 1934 as suicidal depressed manic. There was a history of a pelvic operation in 1929 involving the removal of one ovary and part of the other after which ovarian replacement therapy was used for a time. Amenorrhea since operation.

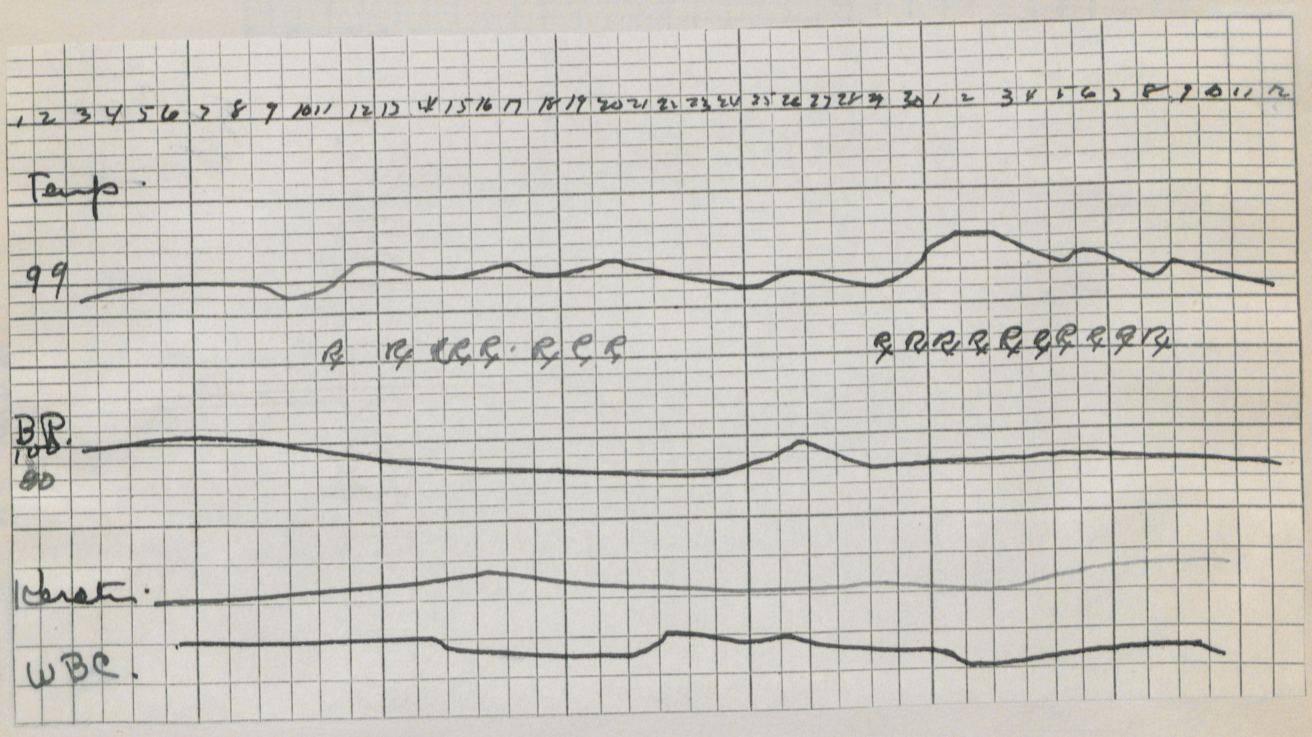


On admission vegetative disturbances with a dilated pupil were observed.

Preliminary observations of this patient showed a persistantly low temperature (rectal between 98 and 99). The blood pressure fluctuated between 90 and 100 systolic. Vaginal smears showed presence of white cells and non-keratinized squamous cells consistantly. Estrogenic hormone below 10 R.U. for 24 hours. Hypodermic theelin (Parke Davis 50 R.U.) for 10 days and one week later 9 injections of theelin 1000 I.U. were given. Following the first course a slight drop in blood pressure was noted and this remained until the second course at which time it remained slightly lowered. The temperature remained unchanged. The patient's mental condition appeared somewhat improved and following cessation there was a recurrence of crying spells. The vaginal smear showed some keratinization but only partial disappearance of the white cells and that only after the 2nd course.

It appeared that the administration of 9000 I.U. in this case was too small a dosage and failure to menstruate on cessation bears this out. The patient appears to present a castration amenorrhea and later treatment with the gonadal stimulating preparation will have to be attempted. Glucose tolerance test after administration of 5000 I.U. was found to be very much increased. There was also a relative lymphocytosis.

Case No. 9 Female age 28 Adm. 1926 Diag. Schizophrenia
Married. Castrated in 1929 since onset followed childbirth.

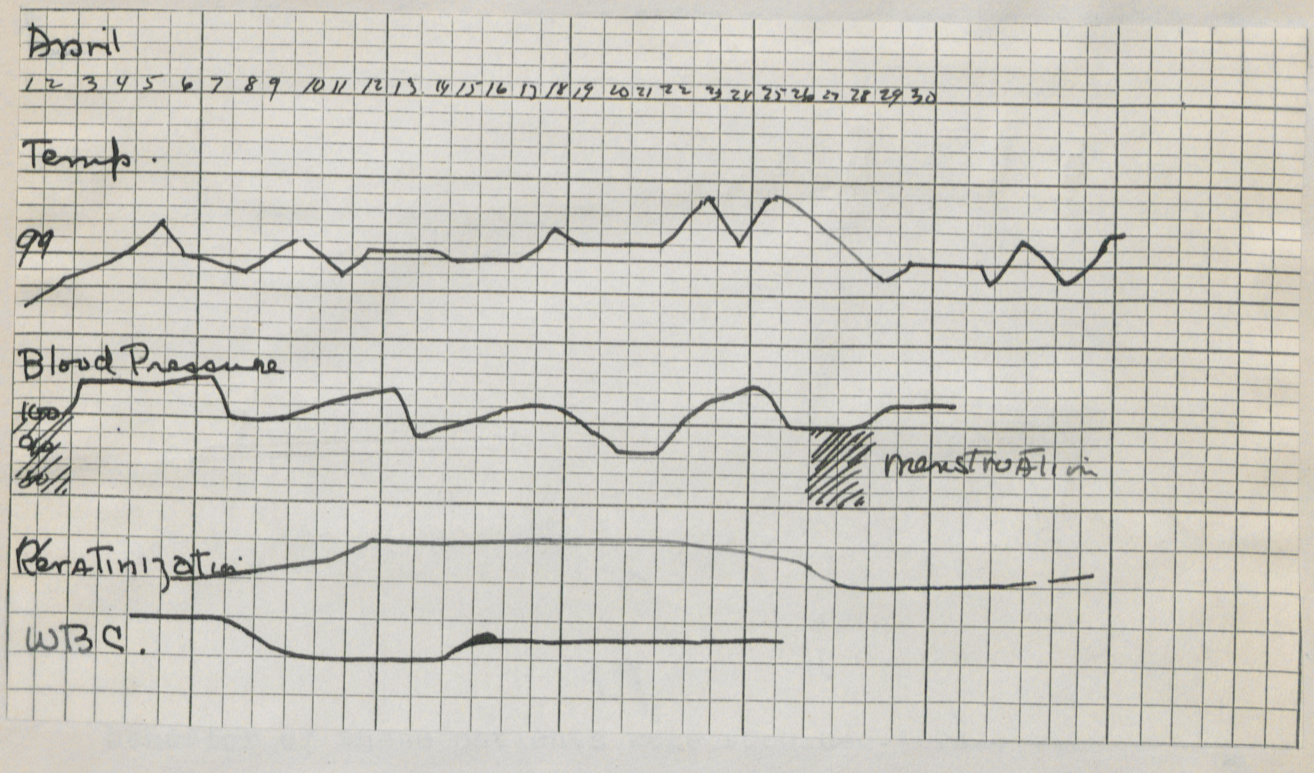


Preliminary observations showed a temperature 98.8-99.2. Blood pressure 90-100 systolic. Smear - WBC and non-keratinized squamous. Estimates of estrogenic hormone could not be run because patient would not cooperate. After 8 injections of 50 R.U. theelin in as many days blood pressure 80 systolic, temperature unchanged; smear shows no decrease of white cells, some keratinization.

After 8 injections of 1000 I.U. theelin the blood pressure went down to 78 systolic, patient disturbed. Smear shows few white cells, many keratinized. No menses.

Apparently dosage used was too small.

Case No. 10. Female aged 42 admitted 1933. Manic depressed phase. Complained of dizziness and pain at periods. Menstruated every 2 to 3 weeks. Single.



Patient becomes destructive just before periods, which are monthly and remains that way for two weeks. She tears clothes. Estrogenic hormone above 10 but below 20 at time of ovulation. Patient appeared better at that time. Blood pressure up during period shows drop at end of intermenstrual period.

Apparently the hormonal activity of this patient is normal as the rat assay and vaginal smear indicate. But she shows definite destructive and disturbed episodes at time of low estrogenic periods. Seven days after menstruation estrogenic excretion is less than 10 R.U. daily but it rises to that level at the time of 3rd day of ovulation. Specimens taken just before menstruation were again low. . This chart is essentially normal as it follows in general outline the observations on normal women by other investigators.

IV DISCUSSION

Of 450 psychotic women fifty were chosen as possibly demonstrating some relation between disturbance of the reproductive cycle and the mental abberations they displayed. The reasons for selecting this group were: (a) the presence of an amenorrhea in a patient less than 40 years old. (b) a history of onset after childbirth (c) an apparent increase in the mental disturbance synchronous with the menstrual cycle (d) a few miscellaneous cases showing excessive eroticism, one case of nymphomania following hysterectomy followed by irradiation castration, and eight young regularily menstruating dementia precox cases.

The daily rectal temperatures, menstrual cycles and behavior of these patients were recorded. This led to the following observations:

(a) The amenorrhoeic patients showed widely fluctuating temperatures and unpredictable behavior.

(b) The three post partum cases were each entirely different in behavior and temperature reactions. Case #6 was consistantly inactive and apathetic, amenorrhoeic, and with a rather steady low temperature. Case #5 was agitated and depressed amenorrhoeic and with a temperature as volatile as her moods and the last case whose record is not included in this record because she was not followed thruout the period, was manic but had an apparently constant temperature with the slight premenstrual rise before her regular menstrual periods.

(c) The cases showing periodic disturbances synchronous with the menstrual cycle demonstrated an increased premenstrual rise (up to 100 rectally) and showed the exacerbations a few

days before and continuing as long as a week after menstruation. The disturbances were crying, fighting, noisyness and destructiveness for the most part. A few of these cases showed a rise beginning at about the tenth day and remaining elevated for the last half of the period but due to their removal to another service could not be furether studies by this examiner.

(d) The patient who had had the hysterestomy exhibited periodic occurrence of fever, with excitement, congested conjunctive and occasional hallucinatory experiences. Due to this fact the idea that a portion of ovary was still active and not being utilized by the ovary was responsible for the disturbance led to the inclusion of this patient in our series.

(e) The chronic dementia precox patients exhibited no true cycle character to their behavior and no relation of their unexpected outbreaks to the menstrual period which was quite regular in this group. The temperatures were uniformly remarkable steady with only a slight premenstrual rise and menstrual drop (Usually the maximum variation of this drop in these cases did not exceed .6 of a degree. Due to the poor co-operation afforded by these patients I was unable to make further studies of these cases.

2 Correlation of the excretion of the estrogenic hormone in the urine with the menstrual cycle.

(a) In an attempt to estimate the excretion of the estrogenic hormone in the urine we injected specimens into rabbits in the attempt to obtain differences in degree of activity of the uterine horn musculature with different amounts of the hormone.

With both normal virgin and castrate rabbits, however, our results were so inconstant that the method was abandoned.

(b) Utilization of the Millon reaction in urinary extracts was likewise found useless because of the large number of Millon positive impurities in the urinary extracts.

(c) The estimation of the excretion of the estrogenic substance in urine was then attacked by using the reaction of a twenty four hour specimen of urine (extracted) in a castrate female rat.

Since we had no facilities for following the technic of extraction used by Kurzrok and necessitating constant redistilling of the solvent for at least 24 hours, it was necessary that we adopt some more rapid and simple technic. As was shown we developed an extraction apparatus which enables us to complete the extraction without the use of heat within an hour with a fair degree of completion. We can also use the entire 24 hour specimen regardless of the volume as it seems to us that in view of the vast differences of amount in a 24 hour specimen which in our cases ran from 250 cubic centimeter to 3000, we would make assays in terms of letters of urine of small value.

According to the procedure shown the acidified, salted urine is allowed to drop into a long column of ethyl acetate, which is filled with glass beads. The fluids are slip into fine droplets by the beads and the heavier urine drops to the bottom for removal. A reextraction with fresh solvent completes the first step. The solvent is removed by a vacuum distilling process as is done in the Kurzok method and the dark syrupy extract dissolves in propylene glycol (5cc) ready for injection.

If 1/10th of this material is then injected into a castrate female rat in three divided doses four hours apart, and if after 48 hours the rat shows the typical estrus smear the sample is considered to contain 10 rat units per day. Only complete absence of leucocytes with presence of squamous keratinized cells was considered estrus in these tests.

In essentially normal cases it is usually observed that for about 4 days before and for the week following the onset on menstruation less than 10 rat units of estrogenic material are found in a twenty four hour specimen but in the interval about 10 rat units are excreted. In our cases therefore we were surprised to find that very few of the cases in our series showed even this much excretion. Only two cases (#6) and one other showed increases above 20 rat units daily and both of these were amenorrhoeic.

That the test for estrogenic activity in the urine as indicated by the rat reaction is not a true indication of the activity of an estrogenic patient would be feared due to the unreliability of biologic tests as well as the possibility for individual variations in renal threshhold as suggested by Frank and the possibility that the material may exist in some form such as theelol showing a less active estrogenic activity in the rat.

(d) We therefore attempted to follow the suggestion of Papanicolaou in studying the estrogenic cycle as demonstrated by the changes in the vaginal fluid of the patient herself. We were not able to follow his careful histologic technic but contented ourselves with direct examinations of the secretions suspended in eosin tinted saline solutions.

After making daily or almost daily smears on 30 patients for at least two and in most cases over three months we found the following:

1 The regularily menstruating woman even with our rather crude technic exhibited a regular cycle starting with white cells and epithelial cells in the first week, with the disappearance of the leucocytes in the second week, and a reappearance of the leucocyte at the tenth to the fifteenth day or about the time when one would expect ovulation to occur. Curiously enough one of our patients showing periodic disturbances always could tell when this (i.e. Ovulation) occurred as she said she feld blood vessels burst in her. This was volunteered of course with no indication given her of the examiners findings. At the time of the reappearance of the white cells keratinialso began to appear in the epithelial cells now becoming squamous in type.

2 All of our cases in whom we demonstrated a low estrogenic excretion contained white cells and unkeratinized epithelial cells in the vaginal smear, except case 12(with the history of castration) and case 4. The first of these showed a persistent leucopenia and keratined cells during a disturbed spell in which repeated rat assays indicated a daily extretion of less than 10 rat units, of the estrogenic material. The second case did not have keratinized cells but she did present a leucopenia with a rat assay of less than 10 rat units per day. Both of our cases with urinary secretion of over 10 rat units of estrogenic material per

day demonstrated estrus smears i.e. lack of leucocytes with presence of keratinized squamous cells.

3. The regularily menstruating cases with periodic disturbance exhibited the same cycle as is described for the normal above.

3 The artificial increase of estrogenic hormone either by administration of theelin or injection of Antuitrin (new prepared from the ant pituitary) resulted in the ddimunition of the number of white cells and the appearance of keratinized cells. The fact that this change was obtained in cases 1 and 2 with antuitrin as well as the estrogenic material indicates that in these two schizophrenics at least the ovarian inactivity is not primary but results from a failure of gonadotropic stimulation.

That there is a quantitative difference in cases with low and high theelin sensitivity is shown by the early disappearance of the leucocytes in cases, 1 2 and three with early presence of considerable keratinization while in case 9 even much larger quantities of the estrogenic hormone only slightly reduced the number of leucocytes. The fact that the last case is a castrate probably accounts for this fact as in rats it is necessary to give larger doses to animals that have been without estrogenic stimulation for some time to get the same affect that can be gained with a small dose in a recent castrate.

Attempting to lower the high estrogenic hormone content of case 6 by means of thyroid extract as suggested

by Dr. Weichert resulted in the appearance of leucocytes in the smear and the onset of menstruation in two weeks.

4 In the course of the medications with estrogenic hormone it was noted that a few days after the treatment was started the patient demonstrated a fall in systolic blood pressure (Cases 3,5,7 and 9). Comparison of the blood pressures and the menstrual cycle in normal records likewise showed a slightly lower pressure at the time when the estrogenic hormone had been elevated for some days e.g. in the third week. Since the drop followed the administration of the hormone in our cases, it appears that in some was the estrogenic hormone must be responsible for the lowering of the blood pressure. Glucose tolerance tests on five of our patients also demonstrated a rise of tolerance in three patients showing a definite drop of pressure with theelin treatment.

5 Comparing these observations with the findings of other investigators cited in the introduction, we note that the metabolic constants show changes during the menstrual cycle of sympathetic activity(see chart on page 6) These changes include a rising blood pressure, an increase in blood sugar, increased basal metabolic rate, increased pulse rate and calcium while water and nitrogen elimination are decreased.

During the third week of the cycle the results of the chemical and physical investigations however, show a definite correlation to vagus stimulation. There

is a drop in blood pressure, drop in blood sugar increase in nitrogen elimination and water excretion and decreased calcium. The fact that recent observers note the vaso dilatation occurring in transplanted uterine endothelium during the intermenstrual period with a constriction of the vessels immediately before menstruation likewise fits nicely into this grouping. Since the menstrual period is the time of low estrogenic activity, and since the third week is the time of actice estrogenic function it appears likely that this change in the preponderance of one of these autonomic influences might have some relation to the estrogenic level. When moreover we have described that soethe same drop in the systolic pressure that we see with vagus stimulation appears some days after the administration of theelin it appears that the hormone must act as a means of rendering the body more susceptible to vagus stimulation.

6 The relation of this interesting mechanism to the state of the mental disturbances is at once apparent. If at the dimunition of the estrogenic hormone the sympathetic stimulation is released we could well expect the signs of aggression and attack to appear since the sympathetic system is a special mechanism for defense and attack. Certainly Bards hypothalamic cats with their peaceful estrus cycles interspersed with the non estrus sham rage periods demonstrate this relationship. It therefore appears that since the destructive fighting tendencies in our patients was associated with periods of low estrogenic activity that they might well be due to increased sympathetic stimulation.

7 The fact that this overstimulation does not occur in every woman with the change in the estrogenic level is easily understood when we refer to Pendes discussion of the constitutionally inadequate individuals whose sensitive autonomic systems show wide fluctuations from relatively mild stimulants. We believe therefore that the reason some women show violent disturbances of mind and activity at the time of decreased estrogenic activity may well be accounted for by the affect of the normal hormonal rhythm on a constitutionally inadequate autonomic nervous system.

V THE ESTROGENIC HORMONE IN MENTAL DISEASE

by Esther Bogen Tietz

CONCLUSIONS:

1. The literature concerning the role of the reproductive system in women in mental instability is reviewed with a consideration of the possible mechanisms for their interrelationship.
2. The determination of the levels of the estrogenic activity in mental patients was studied to elucidate any relationship between them and the disturbance in the behavior and emotional response.
3. Experiments are reported to demonstrate the relation of temperature and behavior to the ovarian activities.
4. Correlation of the excretion of the estrogenic hormone in the urine with the menstrual cycle are made by means of rabbit reaction, Millon test, assay by means of injecting the extracted urine into castrate female rats to observe induction of estrus and the direct vaginal smear of the patient.
5. A rapid method for extracting the urine for estrogenic hormone is described.

6. The correlation between the rat assay and the repeated vaginal smear of the patient is compared in the different cases described and are both shown to be of value and necessary.
7. The low estrogenic level of the patients with amenorrhea in the course of schizophrenia is shown with experiments to indicate that in at least two of these cases the ovarian failure is secondary to a lack of pituitary stimulation.
8. The essentially normal estrogenic activity of the periodically disturbed patients is described.
9. A drop in the systolic blood pressure several days after the administration of theelin was observed.
10. The autonomic rhythm including an intermenstrual vagotonia and menstrual sympathicotonia in some of these cases and in the literature is discussed.
11. This rhythm is shown to be dependent upon the level of the estrogenic activity.
12. The mechanism that results in the development of aggressiveness and destructibility at periods of low estrogenic activity is accounted for by the increase in sympathetic irritability in its absence in individuals with constitutionally inadequate autonomic nervous systems.

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Esther Boyd Tuttle M.D.