HORMONE EXCRETION DURING

THE NORMAL MENSTRUAL CYCLE.

being

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by

ROBERT HORSBURGH TAIT,

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C O N T E N T S.

Cases/

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INTRODUCTION.

There are many subjects in modern medicine concerning which much remains still to be discovered, and few of these present a greater and more interesting scope for investigation and speculation than do the organs of the endocrine system and their internal secretions, and of these, perhaps the most intriguing are the female sex hormones.

In this last decade, a vast amount of research work has been done on this subject of the female sex hormones and the literature relating to it has become immensely complex.

Although there are still many matters for conjecture in female sex physiology, yet, as the result of brilliant investigations in Britain, America, and on the Continent, a rapid advance in knowledge has been made, and more definite views concerning the source and function of the ovarian and pituitary hormones have been established.

In consequence of this, many phenomena of menstruation, conception, pregnancy and parturition have been

more/

more definitely elucidated and beneficial results obtained with hormone therapy in the treatment of obstetrical and gynaecological disorders.

Even better results could be obtained, however, with hormone therapy, if it were not for the divergent views still held on the phenomena of menstruation and the relation to it of the autocoids of the ovary and pituitary; and if unequivocal threshold values of the hormonic content of the blood and urine could be computed in the normal menstrual cycle, and a similar, almost routine, hormone analysis could be conducted in abnormal cases, the rationale of treatment in these cases could be placed on a more stable and scientific basis and would do much to confound the "blind empiricism" and "hit or miss" principles ascribed to much of the present hormone therapy.

Such were the principles in mind, when it was decided to take as the subject of this thesis the study of the phenomenon of the normal human menstrual cycle and the relation to it of the sex hormones excreted into the urine. As an essential basis of this study, at least a brief description of the normal menstrual cycle is required.

CHANGES IN THE ENDOMETRIUM

DURING THE NORMAL MENSTRUAL CYCLE.

The normal menstrual cycle is regarded as being of twenty-eight days' duration, the first day of bleeding being taken as the first day of the cycle.

It is commonly divided into four phases which are:-

(1) Menstrual or destructive phase, which lasts about five days.

(2) Post-mentrual or reparative phase, which occupies about five days.

(3) Quiescent or resting phase, lasting about five days.

(4) Pre-menstrual, progestational or constructive phase, which lasts about fourteen days.

The endometrium, throughout the cycle, is in a constant state of transition. According to the views of Schröder,[1] the endometrium is divisible into basal and functional layers. The basal layer takes no part in the monthly cyclical changes and serves only as a source for the regeneration of the mucous membrane after it has disintegrated during menstruation.

In the post-menstrual phase, immediately after the/

the period, a process of repair occurs in the endometrium, when the epithelium grows and replaces the cast-off epithelium, and the glands are simple straight tubes.

After the repair process is completed, the endometrial activity diminishes for about five days during the quiescent phase, and then about the middle of the cycle the endometrium resumes its activity and the glands become lengthened and tortuous, and dilated with secretion, and have a characteristic cork-screw appearance. The endometrium is increased in thickness and is very similar to the decidua of early pregnancy. This is the premenstrual or progestational stage and continues for about fourteen days until the onset of menstruation, when the superficial hypertrophied layers of the endometrium degenerate and are expelled together with blood which is simultaneously effused and constitutes the menstrual discharge.

THE OVARIAN HORMONES.

On the researches of Crew and Wiesner,[2] and others working at the University of Edinburgh, are based many of the modern views concerning the menstrual mechanism, which will be more fully discussed later.

That menstruation depends on the active functioning of the ovary stimulating the uterus, has for long been accepted. For, it begins at puberty, when ovulation and maturation first occur in the ovaries, and ends at the menopause, when this ovarian activity ceases.

Furthermore, after the operation of bilateral total oöphorectomy and after X-ray and radium therapy to the ovaries, an artificial menopause is induced. This is usually accompanied, as at the normal change of life, by atrophy of the genital tract and the breasts, by symptoms of increased excitability of the sympathetic nervous system and by lowered basal metabolism with frequently the development of secondary masculine characteristics. The above mentioned facts and the results obtained by castration, ablation and transplantation experiments, and by those of injecting ovarian, placental and urinary extracts, indicate that blood-borne ovarian stimuli or internal secretions induce/

induce the menstrual uterine changes, and that these changes are without any direct control from the central nervous system. Indirectly, however, through the action of the sympathetic nervous system causing a hypersecretion of adrenaline, when over-stimulated, a disharmony of ovarian function and upset of menstruation probably results. This temporary upset of control of menstruation is shown as is well known in "newly married women, ... by the fear of pregnancy in unmarried women who have exposed themselves to the risk of it, and by the excessive desire for children in married women who are both neurotic and barren". (Johnstone[3])

Analogous to this inhibitory action of adrenaline on ovarian activity, Robson,[4] working on the lower rodents, found that the administration of adrenaline besides luteal extracts inhibited oestrus.

This exemplifies the fact, that the functions of the glands of the endocrine system, as the thyroid, suprarenals, pituitary and ovaries, etc., are all intimately linked up with each other, and that hyperstimulation of one of them often upsets the harmonious working of the others or, to put it briefly, the products of the endocrine system are either synergistic

or/

or antagonistic in action to each other, and in this connection, as will later be discussed, the internal secretions of the ovary and pituitary are closely related.

The results of modern treatment for diabetes mellitus and myxoedema with extracts from animal pancreas and thyroid (insulin and thyroxin respectively) prove that the hormones from the endocrine glands are not specific for species, race or sex and that the sex hormones are interchangeable in action from species to species and produce similar effects, e.g. extracts of the anterior pituitary lobe and of its hormone secretion obtained from the urine of the adult human female, and implants of the gland from an animal such as the ox, cause maturation of the ovaries of immature rats and mice.

The view that the hormones act as catalytic agents, which initiate chemical changes and themselves remain unaffected thereby, is supported by the work of Frank,(5) who found a large concentration of ovarian hormone in the menstrual fluid, compared with that in the blood and thus, it would appear that the endometrium concentrates, but does not metabolise the ovarian hormone. Before proceeding further, a brief account/

account of the ovary and the changes occurring in it throughout the menstrual cycle, will be described, as a knowledge of this is essential for understanding the hormonal control of the genital tract.

CHANGES IN THE OVARY DURING THE NORMAL MENSTRUAL CYCLE.

The ovary is divided into two regions - (1) the Medulla and (2) the Cortex.

(1) The Medulla consists of unstriped muscular and connective tissue, blood vessels, nerves and lymphatics and is partly surrounded by the cortex.

(2) The Cortex is the important functioning part of the ovary and has three main constituents:-

(a) Germinal Epithelium, which is a layer of cuboidal epithelium covering the surface of the ovary.

(b) Stroma, consisting of connective tissue cells, throughout which are scattered immature Graafian Follicles.

(c) The Graafian Follicles each contain a potential ovum and are derived from the germinal epithelium. It is reckoned that, at birth, each ovary contains about 100,000 ova of which at puberty nearly 30,000 remain.

At/

At puberty, an immature Graafian Follicle or Primordial Follicle containing the ovum, develops and ripens, forming a mature Graafian Follicle which consists of an outer connective tissue covering - tunica externa and tunica interna, over several layers of cuboidal epithelial cells, called the membrana granulosa, which forms a cavity containing the liquor folliculi, and embedded in the membrana granulosa at a point where its cells are heaped up into many layers, called the discus proligerus, is the ovum.

The Graafian Follicle rapidly increases in size and comes to the surface of the ovary and ruptures, expelling the ovum surrounded by membrana granulosa cells, called the corona radiata, and liquor folliculi into the peritoneal cavity, where it reaches the mouth of the uterine tube and, if not fertilized in the uterine tube, it rapidly degenerates. One ovum, rarely two ova, is extruded from the ovary at regular, usually monthly, intervals from puberty to the menopause.

The general opinion of modern observers is that ovulation occurs from twelve to sixteen days after the onset of the last menstrual period, and is usually reckoned as occurring about the fourteenth day (Corner[6]).

After/

After rupture of the Graafian Follicle, the cells of the theca interna and membrana granulosa lining the walls of the follicle, assume a new structure, by proliferation and vascularisation and a convoluted yellow body, known as the corpeus luteum, is produced, which fills up the cavity.

The corpus luteum, by this process, develops to full maturity and increases in size for about a fortnight, when, if pregnancy has not supervened, retrogressive changes take place, commencing about two days before the onset of the next menstrual period. These retrogressive changes result in the corpus luteum becoming converted into a structureless hyaline body known as the corpus albicans. A new Graafian Follicle again assumes control and so the cycle continues. This process results in the minimum of scar tissue being formed in the ovary.

If pregnancy does occur, the mature corpus luteum persists in that state for many weeks. It is then called the "corpus luteum of pregnancy" in contradistinction to the "corpus luteum of menstruation" above described. The ripening of the next Graafian Follicle is arrested until some time after the termination of pregnancy, when it is still possible to see/

see the corpus luteum, but it is diminished in size owing to similar degenerative changes as before having occurred in it.

A double cycle of changes thus occurs in the ovary - ripening of the Graafian Follicle terminating in rupture, followed in turn by the formation of the corpus luteum.

From the foregoing description of changes in the ovary, one may surmise that it has two main functioning parts - (1) the Graafian Follicle, and (2) the Corpus Luteum - which are hormone secreting, and that they are intimately concerned with the menstrual cycle.

The pioneer work on the study of the ovarian hormones was done by Iscovesco[7] in 1912, when he found, by injecting immature female rabbits, that aqueous glandular ovarian extracts were inactive, while alcoholic or ethereal extracts caused hyperplastic changes in the genital tract and breast tissue to result, and he found the same changes to occur when he injected placental extracts into the animals. The usage of immature rabbits excluded the possibility of corpus luteum tissue affecting the action of the extracts, for ovulation in the rabbit is only evoked by coitus or "jumping" and does not occur spontaneously.

Iscovesco/

Iscovesco thus discovered the solvent of one of the ovarian hormones or "female sex hormone" as it was called.

In 1915, Frank and Rosenbloom[8] similarly observed hyperplastic effects in the immature animal treated with lipoid extracts of the placenta and corpus luteum.

The above mentioned investigations on the immature animal, in which extracts of the Graafian Follicle, corpeus luteum and placenta were used, imply that a similar hormone is secreted by these three structures, which is capable of producing hyperplastic changes in the genital tract, typical of oestrus; and this hormone has received the name of "oestrin" from Parkes and Bellerby,[9] which is more appropriate than that of the female sex hormone or the various other names given it as Alpha hormone, Theelin, Menformon, Folliculin, etc. (The name "oestrone" will be used for "oestrin" hereafter, in the text.)

To recapitulate, the hormone oestrone is secreted by the ovary from the Graafian follicle, corpus luteum and tissue stroma, and when injected into the immature or oöphorectomised animal, produces specific changes typical/

typical of oestrus, as, hypertrophy of the uterus including the endometrium and cornification of the vagina. In addition, the placenta manufactures oestrone of which large amounts are found in the urine of pregnant women.

Using the immature monkey Macacus Rhesus, Courrier, Kehl and Raynaud[10] were able by injections of oestrone to produce cornification of the vagina and hypertrophy of the uterus involving both the muscle and the endometrium; but, it was found, that the full endometrial development, characteristic of the late premenstrual stage, was never obtainable.

This ability of oestrone to produce nidatory changes was demonstrated by Zondek,[11] who curetted castrated women after prolonged oestrone medication, when it was found that the endometrium, although showing signs of proliferative activity, yet did not conform to that of the later premenstrual phase.

Werner and Collier[12] curetted castrated women, to whom had been administered large doses of oestrone, and they found that the endometrium was only characteristic of the proliferative phase. They also found, during treatment and after its cessation, that vaginal bleeding occurred. This latter effect is most/

most probably due to the absence of the controlling influence of the hormone progesterone from the corpus luteum, which is to be discussed later.

Thus, oestrone is responsible for the production of the proliferative phase of the endometrium in the menstrual cycle, but it is unable to incite the occurrence of the premenstrual or secretory phase.

Oestrone has several other important actions. Allen[13] showed that the injection of large doses of oestrone into immature monkeys produced harmful effects on the gonads and inhibited ovulation.

Dahlberg[14] was able to inhibit the gonadotropic action of the urine of pregnant women by simultaneously injecting the test animals with follicular fluid, and he concluded that oestrone prevents maturation of the ovum and ovulation, by antagonising the effect of the anterior pituitary lobe hormone.

Zondek and Aschheim[15] noted that large doses of oestrone produced abortion regularly in mice during the second half of pregnancy, but, when attempts were made in the human female to induce abortion and premature labour with large doses of oestrone, as was done by Robinson, Datnow and Jeffcoate,[16] their efforts were met with almost complete failure. A further important action/

action of oestrone is that it is almost specific in relieving the symptoms of upset which frequently occur at the menopause or after bilateral oöphorectomy.

CHEMICAL CONSTITUTION OF OESTRONE.

Until now oestrone has been referred to as a single substance, but recent work has shown that several oestrone-like substances are obtainable from the urine.

Doisy[17] and Butenandt[18] each were able to isolate a crystalline oestrus-producing substance from the urine, which has been named theelin and is stated to be a triple unsaturated oxyketone probably of the stearin series with the formula of $C_{18}H_{22}O_8$ (Ketohydroxyoestrin),

More recently Doisy[19] and others isolated another oestrus inducing substance similar to theelin, with the formula of $C_{18}H_{24}O_3$ (Trihydroxy oestrin) and called theelol or oestriol.

These workers have shown that the pure substance is lipoidal in character, being very soluble in methylated ether, alcohol, chloroform, acetone and benzol and only slightly soluble in water. It is thermostable for it is not destroyed by boiling.

THE CORPUS LUTEUM HORMONE.

Perhaps the first person to direct attention to the corpus luteum as being a gland of internal secretion was Fraenkel[20] who, as early as 1903, working with rabbits, found that removal of the corpus luteum during early pregnancy, was followed by abortion or absorption of the foetus.

Thus, he established that the corpus luteum hormone was necessary for the continuance of early pregnancy. If, however, the corpus luteum is removed late in pregnancy, it has been found that abortion does not occur and this is explained by the assumption that the placenta later takes over the functions of the corpus luteum.

Fraenkel further showed that menstruation occurred prematurely after removal of the ovary containing the corpus luteum and also after excision of the corpus luteum itself. This gave foundation to the conception that menstruation is associated with the retrogression of the corpus luteum at the end of the menstrual cycle, and with the removal from the circulation of its hormone.

Corner and Allen,[21] who gave this luteal hormone the name of Progestin (hereafter referred to as progesterone)/

progesterone), observed in 1929, while working with the immature rabbit, that the injection of a luteal extract promoted, but not consistently, the development of the endometrium to the late premenstrual or progestational state.

Hisaw and Leonard[22] found, if injected with oestrone for some time before the administration of progesterone, that the immature animal developed the progestational type of endometrium.

Allen,[23] working on the mature oöphorectomised animal observed, that the injection of luteal extracts had no effect on the endometrium, unless sufficient oestrone had been administered.

Thus, by itself, progesterone has no stimulating effect on the endometrium, but when it is previously sensitised by oestrone, progesterone carries on the proliferative phenomena to those of the pregravid state.

That this relationship applies to the human subject, has been recently demonstrated by Kaufmann.[24] He had as a patient a twenty-one year old girl who had been castrated five years previously on account of dermoid cysts in both ovaries, and whose uterus was in an atrophic condition. He administered to her for about/

about a month, a million international units of oestrone and thirty-five rabbit units of progesterone. The endometrium developed enormously, to the pre-menstrual stage, as confirmed by examining a portion of the endometrium curetted. Menstruation did not occur. He repeated the experiment and found that menstruation commenced two days after the last injection of corpus luteum extract.

He was thus able for the first time to reproduce exactly the normal menstrual cycle in the castrated woman.

This experiment demonstrates conclusively that oestrone and progesterone are the only hormones required to produce full functional development of the endometrium.

The luteal hormone, besides its influence on the endometrium, also exerts control over the uterine musculature. Hisaw[25] was the first to demonstrate the inhibitory effect of progesterone on uterine contractions, and Knaus[26] further showed that injection of the hormone into the extirpated rabbit uterus in vitro, caused inhibition of the reaction of the muscle to pituitrin.

It is well known in the human subject, that the uterine/

uterine muscle is relatively insensitive to the action of pituitrin during pregnancy and until a short time before parturition.

Knaus's work therefore suggests that the onset of labour at the end of pregnancy may be due to the uterine muscle recovering its power of response to the posterior pituitary hormone, which till then had been dominated by the corpus luteum hormone.

Progesterone has other important activities. It has been found in the lower rodents, that large and continued dosage of progesterone inhibits maturation of the ovarian follicles and ovulation, and leads to the suppression of oestrus. This was demonstrated by Parkes and Bellerby[27] and later by Patel,[28] when they injected luteal preparations into the lower rodents.

Another power, which has been attributed to the corpus luteum, is that of relaxation of the pelvic ligaments of the guinea-pig, which usually occurs later in pregnancy and at parturition (Fevold, Hisaw & Mayer[29]).

CHEMICAL CONSTITUTION OF PROGESTERONE.

Unlike oestrone, progesterone has not yet been obtained in pure form and is thus not readily available/

available commercially. It is soluble in water, but insoluble in lipoid solvents. It is destroyed at a temperature of 50°C.

THE PITUITARY HORMONES.

In brief, the pituitary gland is a small oval-shaped gland, attached to the floor of the third ventricle of the brain and situated in the sella turcica of the sphenoid bone. It consists of three distinct parts:

(1) Anterior Lobe,

(2) Posterior Lobe,

(3) Pars Intermedia.

The anterior lobe consists mainly of epithelial cells, which are of three types:

(a) The Chromophobe or chief cells, which remain clear when the tissue is stained.

(b) The Chromophil cells, which stain deeply and comprise (1) Basophilic and (2) Acidophilic cells.

Evidence favours the view that each of these three varieties of cells has different functions.

The chromophobe cells of the anterior pituitary increase/

increase in size and number during pregnancy and, occasionally, to such an extent that due to pressure on the optic tract hemianopia is caused. This has been shown by Erdheim and Stumme[30] in the human and by Cushing[31] in the pregnant animal.

Cushing and Davidoff,[32] on the other hand, have shown that the acidophilic cells are chiefly concerned with growth and the production of the hormone connected therewith. They found at autopsy in cases of acromegaly in which invariably genital hypoplasia is present, that adenomatous or hyperplastic changes of the acidophilic cells of the pituitary existed, and was usually accompanied by changes of an adenomatous nature in the other endocrine glands.

It would appear, therefore, that derangement of the anterior pituitary upsets the function of the endocrine system more generally than does the malfunction of one of its other glands. Thus it might be said that the pituitary gland exerts a controlling force on the endocrine mechanism.

It has been shown that periodicity and rhythm prevail in the uterus throughout the normal sexual cycle, and the question arises - Is there a mechanism controlling this rhythm, and if so, where is it to be found?

In/

In 1901, Fröhlich[33] drew attention to there being a relationship between the pituitary gland and the ovary, when he described his famous syndrome of hypophyseal deficiency, later called Dystrophia Adiposo-genitalis. This condition is characterised by abnormal deposition of fat on the lower abdomen, hips, upper thighs and breasts and sometimes hirsutism of an almost masculine nature and amenorrhoea. The disorder is thought to be due to atrophy of the chromophobe or chief cells of the anterior lobe of the pituitary, for autopsy sometimes reveals an adenoma affecting these cells.

In addition to clinical observations as above mentioned, the present knowledge of the anterior pituitary lobe function was obtained by removal and implantation experiments on animals, and also by feeding and injecting them with extracts of the gland.

In 1916 Goetsch[34] showed that immature rats fed on anterior pituitary substance grew more rapidly, and more quickly attained complete sexual development. He also found that posterior pituitary feeding failed to produce similar phenomena.

Numerous unsuccessful attempts at experimental hypophysectomy were made before an efficient technique was/

was evolved. Smith[35] demonstrated that removal of the pituitary from rats was followed by ovarian atrophy and total suppression of oestrus, but that these phenomena could be prevented and the oestrus cycle re-established by grafts of the anterior pituitary lobe tissue into the hypophysectomised animals.

It was also found that pituitary implants and extract injections into the oöphorectomised animal produced no effect whatsoever on the genital tract.

Thus Smith[35] was the first to establish the fact that the follicular maturation process in the ovary and the secretion of the hormone responsible for oestrus are both dependent on the presence of the pituitary gland, and its hormone secretion.

Smith and Engle[36] further showed that implantation of anterior pituitary tissue into immature female mice was, in addition to follicular maturation and secretion of oestrone, also followed by ovulation.

Another investigator, Evans,[37] working with alkaline extracts of the pituitary, obtained by grinding up pituitary glands with $\frac{N}{10}$ caustic soda, and which were administered by injection into mature rats for several days, demonstrated an inhibition of oestrus. On examining the ovaries of these animals when/

when killed, he found an extensive luteinization of the ovaries had occurred, without the formation of mature Graafian follicles, which had been apparently converted into corpora lutea. Evans had thus been able, by the use of an alkaline extract of the pituitary, to cause luteinization of the Graafian follicles and secretion of the corpus luteum hormone, progesterone.

Wiesner and Crew,[38] working with the alkaline extract of the pituitary, removed from it the growth factor by precipitating the protein with 20% sulphosalicylic acid and subsequent neutralization with sodium bicarbonate, and they thus obtained a luteinizing principle, free from the growth hormone, whose presence in the alkaline extract previously used had been causing some confusion in interpreting the results of other workers. With this extract Wiesner and Crew demonstrated luteinization of the mouse ovaries with a transformation of the vaginal epithelium into a state analogous to the progestational or nidatory phase of the endometrium, due to the influence of the corpus luteum hormone Progesterone, and they term this state that of "mucification". They maintain that this extract is purely kyogenic, i.e. capable of producing progesterone alone through its action on the ovary, and/

and furthermore that this mucification reaction represents a test for the luteinizing hormone. This has not been substantiated and is not accepted as previous work but Hisaw[39] has shown that the progestational effect in the animal thus treated is incomplete without the administration of a balanced quantity of oestrone in addition to progesterone. The contention is, therefore, that the product employed by Wiesner and Crew incites the production of oestrone as well as progesterone and that it has not a specific action as was claimed in stimulating the production of progesterone from the ovary, for the latter has no effect without the preparatory action of the former.

From the foregoing experimental evidence, one may conclude that the pituitary is capable of bringing about two main reactions in the ovary:

(1) Follicular maturation, ovulation and the secretion of oestrone, i.e., oestrogenic reaction.

(2) Luteal tissue formation and the secretion of progesterone, i.e., luteinizing or kyo-genic reaction.

These two main reactions in the ovary have been brought about by two kinds of pituitary preparation, viz., anterior lobe grafting and alkaline extracts respectively.

Bellerby/

Bellerby,[40] by a series of experiments, claimed that the oestrogenic factor could be obtained in acid media, and that the luteinizing factor could be isolated from alkaline media, but no such conclusion is justifiable in view of subsequent work.

These two different effects produced gave rise to the theory, formulated by Wiesner and Crew, that two gonadotropic hormones are produced by the anterior pituitary lobe controlling ovarian function, and they described these hormones as the Rho factors - Rho I being responsible for the oestrogenic reaction and secretion of the Alpha hormone or oestrone, whereas Rho II was assumed to incite the luteinizing reaction and secretion of the Beta hormone or progesterone. Because of the divergent nature of the results occurring after injection with various extracts, they assume that these hormones occur in varying proportions and that both their relative concentration and absolute quantity determine the effect produced.

On different grounds, Zondek and Ascheim[41] also postulated the existence of two gonadotropic hormones. They discovered that during pregnancy in the human subject an excess of anterior pituitary hormone is excreted in the urine as the result of the hypertrophy of/

of the pituitary gland, which normally occurs at that time. This discovery is the basis of the famous Aschheim-Zondek pregnancy diagnosis test. These workers, using immature mice which they injected with urinary gonadotropic preparations, observed certain characteristic reactions in the ovaries which they divided into three main groups:

(1) The ovary showed follicular maturation, ovulation and oestrone was secreted.

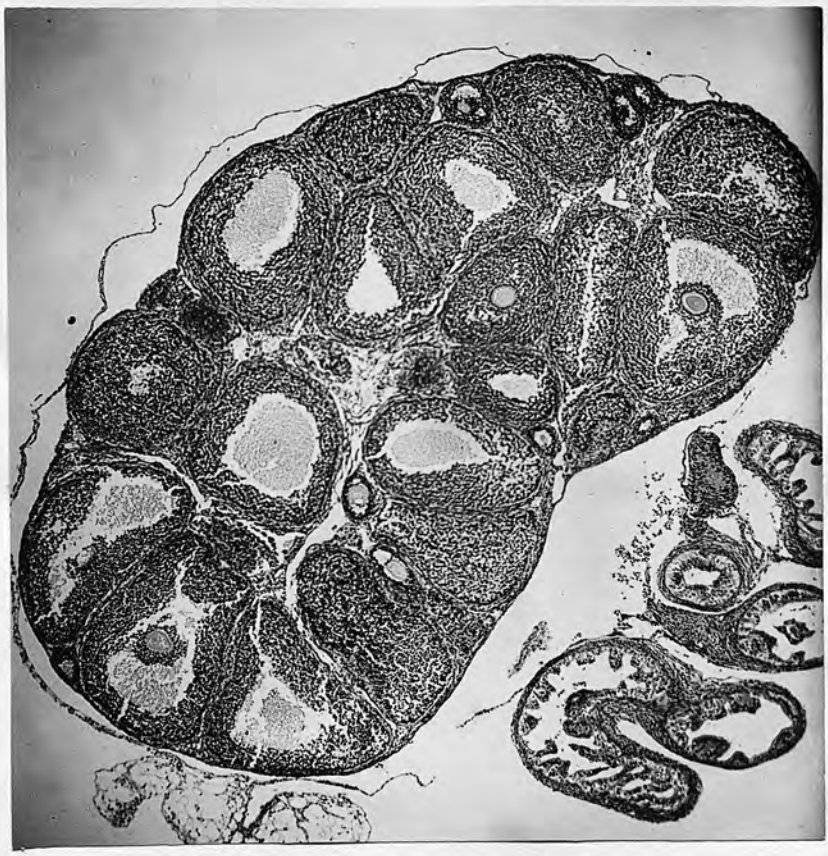
(2) In addition to the above effects, the ovary was hyperaemic and showed blood spots.

(3) Luteinization of the ovary and formation of corpora lutea atretica.

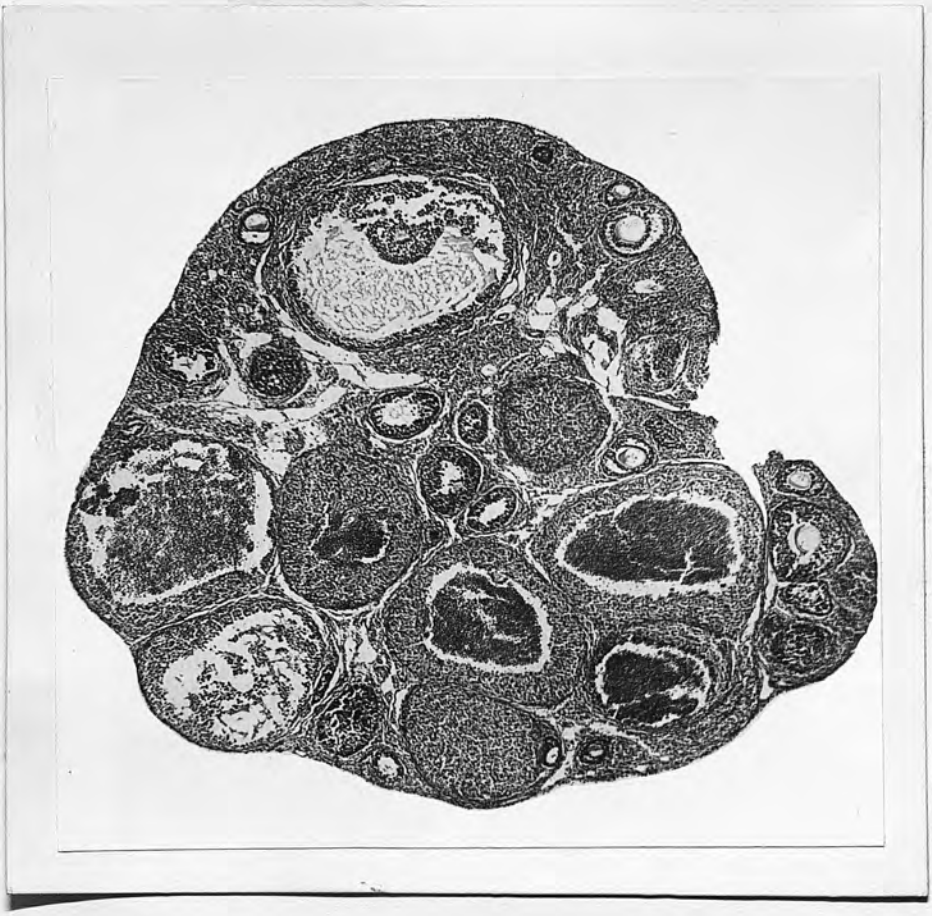
They applied the term, Prolan, to the gonadotropic factors present in and recoverable from the urine, and from the duality of the results obtained, i.e., (1) and (3) more particularly, they also presumed the existence of two gonadotropic hormones, of which the first, Prolan A, caused follicular maturation with secretion of oestrone, and the second, Prolan B, incited luteinization followed by the secretion of the corpus luteum hormone.

Prolan A and Prolan B are thus similar to the Rho I and Rho II factors respectively of Wiesner and Crew.

These/



Micro-photograph of section of Mouse Ovary showing maturing Graafian follicles.



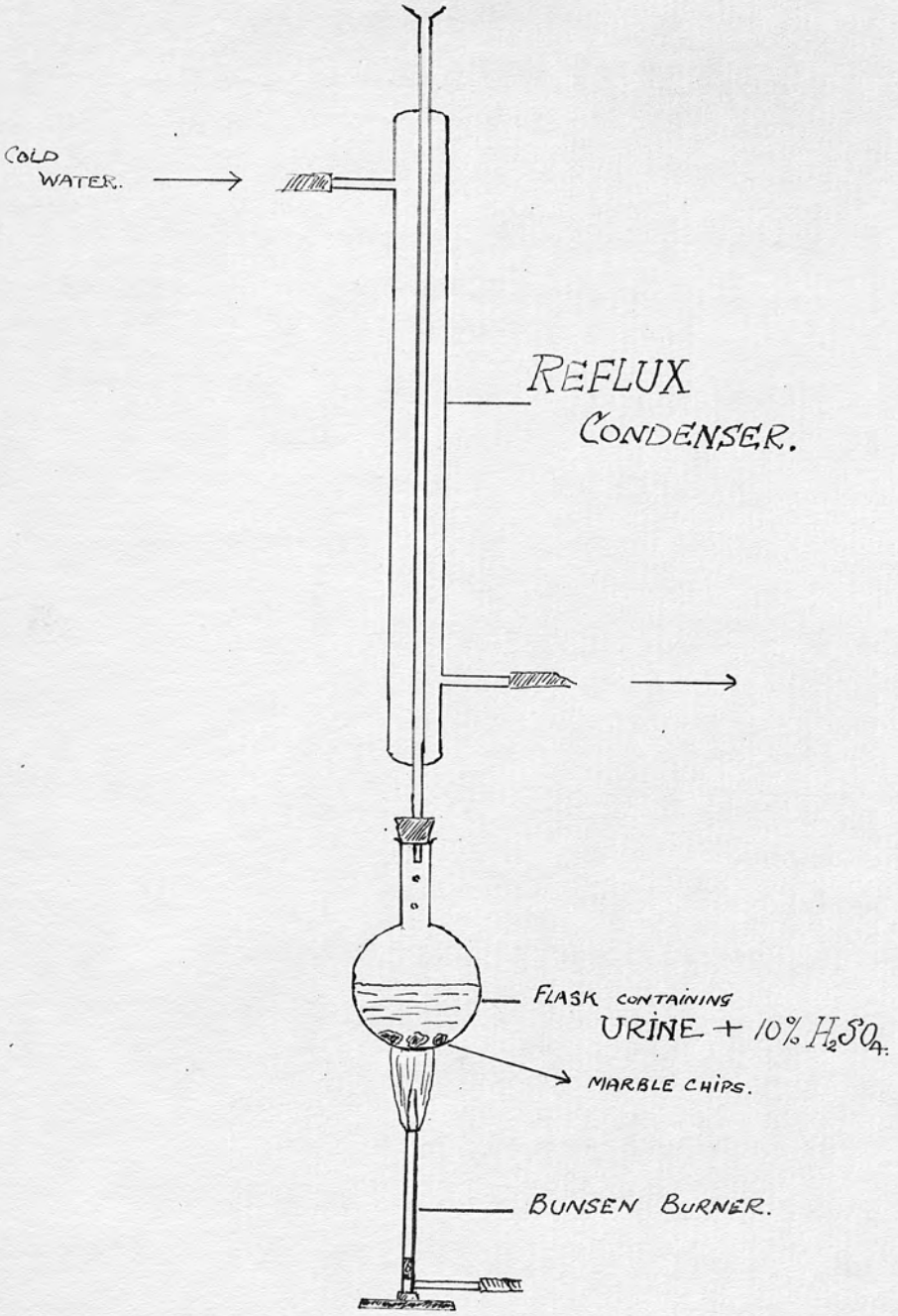
Microphotograph of section of ovary of a mouse which had previously been treated with a gonadotropic hormone urine preparation, showing marked luteinization.

These gonadotropic hormones are said to be excreted together in the urine in varying proportions and that in certain conditions the urine may contain a preponderance of the one hormone, e.g. Zondek states that the urine of gravid women yields only the luteinizing principle (Prolan B or Rho II) because the oestrogenic principle (Prolan A, Rho I) becomes inert very rapidly, even on standing.

From the findings that small doses of urine preparations prove oestrogenic, whilst large doses induce the second ovarian phase or luteinizing reaction, a simple hypothesis suggests itself, that there is only one gonadotropic hormone manufactured by the anterior pituitary and that the reactions produced by it depend on its degree of potency, and this theory receives some support from other workers, e.g. Mazer and Goldstein.(42) Much of the evidence, however, used to support the respective views as to whether the pituitary has one or two gonadotropic hormones is inadequate, but on the whole, at present the weight of evidence inclines towards the existence of two gonadotropic hormones. The nature of these hormones is still unknown and even their separate identity is by no means definitely established.

CHEMICAL/

Hydrolysis of Urine.



CHEMICAL PROPERTIES OF PROLAN.

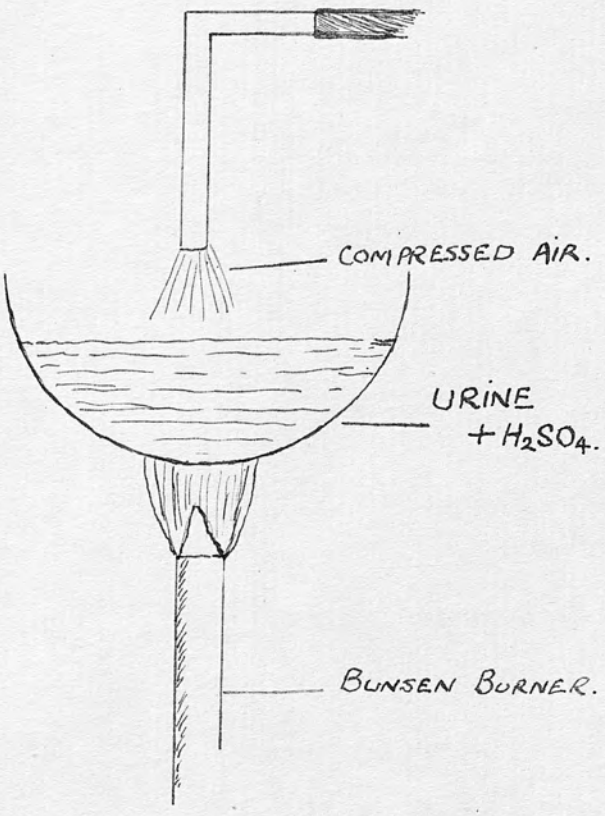
1. Soluble in water.
2. Insoluble in ether and other lipoid solvents.
3. Precipitated by ethyl alcohol but not precipitated by sulphosalicylic acid.
4. Not heat stable, destroyed at temperature above 70°C.
5. Destroyed by strong acids and alkalis.
6. Molecule at least as big as that of Congo red.

METHOD ADOPTED FOR URINARY OESTRONE EXTRACTION.

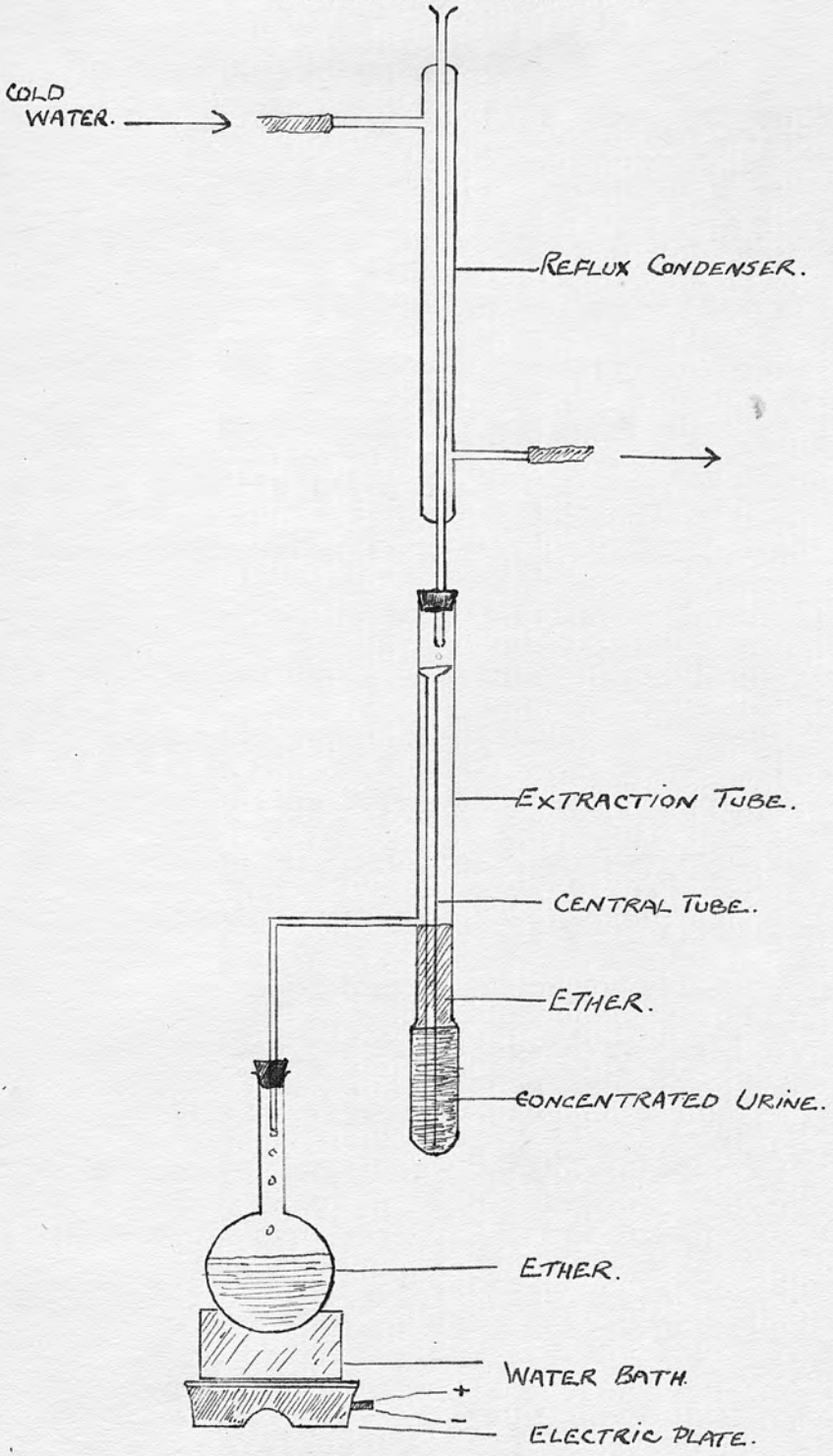
1. The total 24-hours output of urine, less 60 ccs. for the gonadotropic hormone estimation, to which has been added 30 ccs. of 10% H_2SO_4 per 270 ccs. of urine, is hydrolysed for eight hours under a reflux condenser. This liberates the oestrone from its organic combination in the urine. Oestrone is thermostable and survives unimpaired boiling-point temperature, as is produced in this process of hydrolysis.

2. The hydrolysed urine is then concentrated to/

Concentration of Urine.



Oestrone Extraction.



to 250-300 ccs. by heating it in a fume chamber, in a porcelain basin under a constant stream of compressed air which reduces the boiling point of the evaporating urine. The concentrated urine is thereafter placed in the extraction tube under a reflux condenser.

Its level is brought to just below that of the branch tube with distilled water washings from porcelain basin, which leads to a flask containing methylated ether, in which oestrone is soluble, and set on a water bath heated by an electric plate, because of the inflammable nature of the flask's contents. Vaporised ether passes from the flask into the extraction tube, reaches the condenser, where it is condensed and drops down on to the concentrated urine and then flows back to the flask, thus maintaining a constant circulation of the ether through the urine.

This process is allowed to continue for 24 hours, after which the flask with its contents and any supernatant ether from the extractor is removed.

N.B. After cleansing the extraction tube before further use, the extraction tube is thoroughly rinsed with absolute alcohol (an oestrone solvent) to prevent contamination of subsequent extractions.

The next procedure is to drive off the ether from the extract. The ether is allowed to partly evaporate/

evaporate off from the flask, which is placed on the water-bath until the volume of the ethereal extract is sufficiently small to allow of its transference to a small wide-necked bottle, when the extract is brought to a state of dryness by further evaporating off the ether on the water-bath or by blowing it off under a current of compressed air.

More methylated ether is added to the dry extract, and the whole is agitated with a glass rod, and the supernatant ether poured into another bottle through a filter paper to prevent any of the dark coloured débris from the extract being carried into the other bottle, as it was found that these proved highly toxic to the mice after injection.

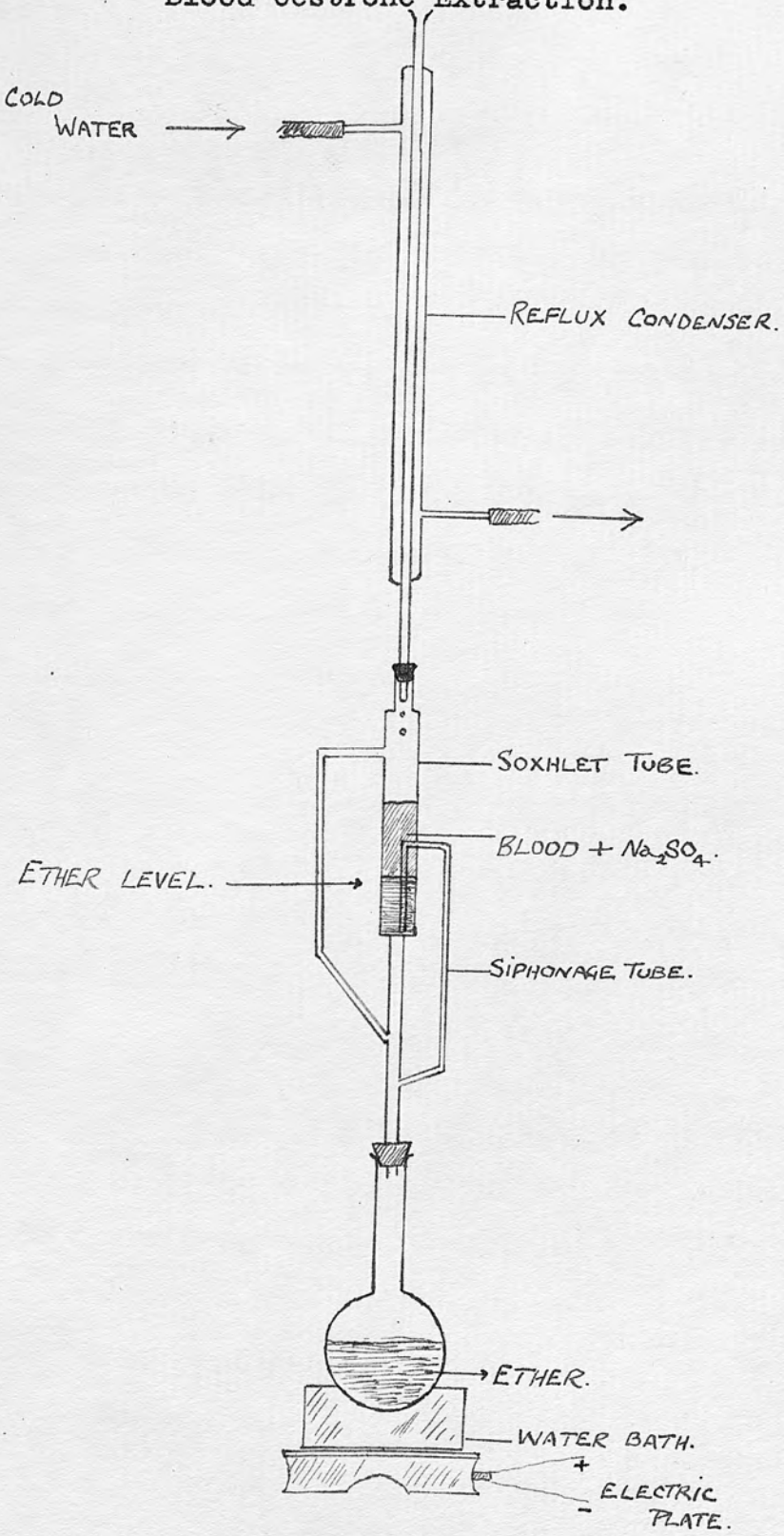
To the other bottle, 8 ccs. of olive oil are added and the ether is removed as before, when the final oestrone extract has been prepared.

METHOD ADOPTED FOR BLOOD OESTRONE EXTRACTION.

To 45 ccs. of blood in a mortar, anhydrous sodium sulphate is added in small amounts and the mixture pounded with a pestle, until a fine dry powder is obtained. The powder is then enclosed in fine dry filter paper, into the form of a cylinder and placed in the cylindrical container of a Soxhlet Tube apparatus, which is connected above to a reflux condenser and below to a flask, almost filled with methylated ether, and set on an electric water-bath and extraction allowed to take place for 24-36 hours.

Vaporized ether passes from the flask up an external tube to the main Soxhlet Tube, where it impinges on the reflux condenser, is condensed and drops down on to the blood and sodium sulphate mixture enclosed in the filter paper, and percolates through it to gradually accumulate till its level rises to the upper limit of the syphon pipe of the apparatus, when the whole ethereal content of the Soxhlet is syphoned back into the flask and the process resumed. After extraction has occurred for 24 to 36 hours, all the ether in the apparatus is collected in the lower flask and/

Blood Oestrone Extraction.



and almost all evaporated off, and the remainder transferred to a small wide-necked bottle, in which it is then evaporated off to dryness.

The deposit is stirred up with a little more ether, 1 cc. of olive oil added to it, and the residual ether evaporated off when the blood oestrone extract, ready for injection, has been prepared. This technique is that described by Frank[43] and has been modified for our own use.

METHOD ADOPTED FOR GONADOTROPIC HORMONE PREPARATION.

Sixty ccs. of urine are filtered, and to it are added 240 ccs. of absolute alcohol, and the whole amount is centrifuged in an electric centrifuge at the rate of 3,000 revolutions per minute for ten minutes. The supernatant fluid is discarded and the precipitate is washed well with ether, to remove the oestrone which is soluble in ether but in which the gonadotropic hormone is insoluble, and the ether is discarded.

To/

To this residue is added 7 ccs. of distilled water and the solution is neutralised by sodium bicarbonate if acid, or by sulphosalicylic acid if alkaline. Neutrality of the solution is indicated by the appearance of a deep buff colour with "Universal Indicator". This aqueous solution, in which the gonadotropic hormone is soluble, is now ready for use. (This technique is that of Zondek's Precipitation Method modified for our own use.[44])

THE SEX CYCLE OF THE MOUSE.

The sex cycle of the mouse will now be considered, as this was the only experimental animal used in the course of this investigation, because it has proved such a useful medium in the assay of the sex hormones and furthermore, because an understanding of its sexual cycle has greatly elucidated that of the human cycle.

Stockard and Papanicolaou[45] in 1917, first described the specific oestrous changes occurring in the uterine and vaginal epithelium of the guinea pig. They/

They were able to determine the state of the vaginal epithelium at various stages in the oestrous cycle by their method of taking vaginal smears from the animal. This they did by introducing a wire loop into the vagina and removing from it some of its desquamated cellular content, which was spread on a glass slide, stained and examined microscopically.

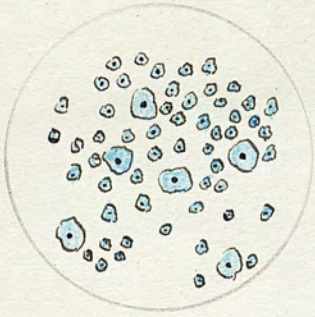
Allen[46] later described the changes taking place during the oestrous cycle in the mouse.

In this species the female will only mate at certain periods, namely, when in heat or during oestrus. Oestrus usually occurs every four to six days and lasts for one or two days. In the interval between oestrus, termed di-oestrus, the animal does not mate. Oestrus is accompanied by typical changes in the uterus, vagina and ovaries. The oestrous cycle, like the human menstrual cycle, may be divided into four phases:

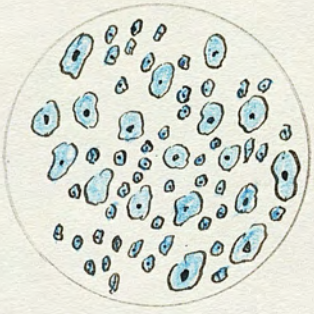
(1) Resting Phase or Di-oestrus.

The ovaries and uterus are small and the vaginal mucosa consists of only several layers of nucleated epithelial cells. The vaginal smear shows an abundance of leucocytes, mucus and a few small epithelial cells.

(2)/



Vaginal smear from mouse in Resting or Di-oestrus phase, showing numerous leucocytes and a few nucleated epithelial cells.
(Stained methylene blue.)



Vaginal smear from mouse in Constructive phase or Pro-oestrus, showing many nucleated epithelial cells and some leucocytes.
(Stained methylene blue.)

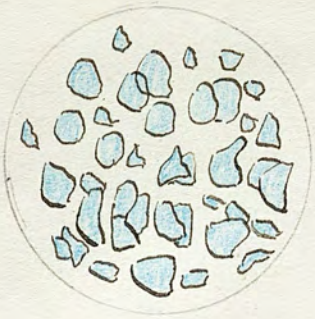
(2) Pro-oestrus or Constructive Phase.

The ovaries are somewhat increased in size and contain maturing Graafian follicles. The uterus becomes vascular, enlarged and distended with secretion. The vaginal mucosa shows a marked multiplication of its epithelial cell layers and the vaginal smear consists of nucleated epithelial cells and only a few leucocytes (see Diagram).

(3) Oestrus or Heat is the stage of ovulation and mating. The ovaries are increased in size and show matured Graafian follicles. The uterus is markedly increased in size, and is distended with secretion. The vaginal epithelial cells are very numerous and the surface layers become cornified and shed large non-nucleated epithelial cells which are demonstrable in the vaginal smear (see Diagram).

(4) Post-oestrus.

At this stage corpora lutea replace the ruptured Graafian follicles, but the endometrial changes do not proceed to the progestational or mucification stage, unless progesterone or the anterior pituitary luteinizing hormone is administered, or unless mating takes place. In the absence of mating, retrogressive changes/



Vaginal smear from mouse during Oestrus or heat, showing cells which are all large non-nucleated squamous cells and represents full cornification, e.g. C $\frac{4}{4}$.

Microscopic section during Oestrus of mouse uterus which is greatly increased in size, showing endometrial cells distended with secretion and the surface layers becoming cornified.

changes occur in the uterus and the vagina and the cellular composition of the vaginal smear returns to that of the di-oestrous phase.

Long and Evans[47] and Allen[48] showed that the changes above outlined in the mouse in the oestrous cycle, cease to occur after bilateral oöphorectomy, but in 1923 Allen and Doisy[49] succeeded in reproducing artificially the phenomena of the oestrous cycle in spayed mice by the injection of a lipoid extract of follicular fluid.

This discovery has become the basis of the test for the ovarian hormone, oestrone, and as such has been used for that purpose in this work.

This test, then, of Allen and Doisy, is based on the fact that the injection of immature or castrated female mice with an active oestrone preparation brings about in 72 to 96 hours the typical vaginal changes, indicative of oestrus and demonstrable by vaginal smearing.

PREPARATION OF MICE FOR ASSAY OF OESTRONE.

For the assay of oestrone, on any moderate scale, a large supply of castrated female mice is essential.

Mature, young, healthy, adult mice were selected for bilateral oöphorectomy. This operation was performed under ether anaesthesia. The animal selected for castration is placed on its abdomen, head-first into the mouth of a tube of appropriate diameter and at the bottom of which is a piece of cotton wool saturated with ether.

When fully anaesthetised, as is indicated by the rump and hind legs of the animal sagging, a transverse incision is made through the skin on the dorsum, which has been previously cleansed with alcohol. The incision is made about a centimetre's distance superior to the pelvic girdle and the skin flaps retracted.

Each ovary, and as much of the peri-ovular fatty tissue as possible, is removed through a small incision made in the overlying muscles, using the peritoneal fold running from the ovary to the kidney region as a guide. The ovary is found in an antero-inferior position to the lower pole of the kidney. The muscle wounds are left unstitched and the skin edges are brought/

brought into apposition with a few stitches of linen thread. This operation can be completed in five minutes and with a mortality of about two per cent., when the operator becomes proficient.

After a few days, the oöphorectomized mice are smeared daily for eight days to exclude the possibility of a remnant of ovarian tissue making unreliable, subsequent oestrone assays. A persistent negative smear indicated by the presence of leucocytes and occasional nucleated epithelial cells substantiates that all the ovarian tissue has been removed. About three weeks after an oestrone assay with a batch of mice, it was the practice to take smears daily from each mouse for about eight days, in order to be absolutely certain that no regeneration of ovarian tissue had occurred to upset the results, as in a few rare instances this was recognized from the vaginal smear showing a positive result of cornified non-nucleated epithelial cells.

STANDARDIZATION OF THE HORMONES.

This is most conveniently done in relation to the mouse.

The Standardization of Oestrone is performed on mature oöphorectomized mice and is estimated in mouse units.

In all cases thus investigated, standardization was performed by the injection of four doses at twelve-hourly intervals, in the course of thirty-six hours and the mouse unit was taken as that amount of oestrone which would produce full vaginal cornification in fifty per cent. of the animals injected.

According to Deanesley and Parkes,(50) the absorption of oestrone from an oily basis is said to be very slow, as appreciable amounts of it remain unabsorbed several days after injection, and divided injections in an aqueous medium are suggested as being more suitable, for their effects can be compared with those given by an international standard.

The Gonadotropic Hormone Standardization is performed on immature mice and is estimated in mouse units.

Each mouse receives one injection at twelve-hourly intervals/

intervals, until six such doses have been administered. About one hundred hours after the first injection, the animal is killed and the state of the ovaries assessed. The minimum amount which brings about a definite effect in at least one ovary is termed the unit of the substance.

This test has to be performed on several animals as there is often a slight variation in the reaction, from ovary to ovary in individual animals.

TECHNIQUE OF ASSAY OF OESTRONE.

As the twenty-four hours urine oestrone extract contains an unknown quantity of oestrone, its estimation is done by one of assay or trial and error.

As a preliminary, to indicate the potency of the oestrone extract being estimated, three mice are used, and each is injected with .2 cc. of the extract twice daily for two days, and smeared thereafter twice daily on the third, fourth and fifth days. If the smears, on examination microscopically on a slide stained with methylene/

methylene blue, are positive, as is shown by full cornification in more than half the number of mice, five mice are taken and injected with .1 cc. of the extract, twice daily for two days, and smeared on the subsequent three days, twice daily.

If these smears prove positive, five more mice are employed and injected with .05 cc. of the extract as before. If these smears prove negative the result lies between the doses of .1 cc. and .05 cc.

If, however, the injection of .1 cc. as before had given negative smears, a further five mice with the dosage of .15 cc. would have been used and if this gave positive smears the result would lie between the doses of .15 cc. and .1 cc., e.g. -

(1) 3 mice with .2 cc. gave positive smears.
(2) 5 mice " .1 cc. " positive "
(3) 5 mice " .05 cc. " negative "

∴ result lies between .1 cc. and .05 cc.

If, however, (2) 5 mice with .1 cc. gave negative smears

(4) 5 mice with .15 cc. gave positive smears

∴ result lies between .15 cc. and .1 cc.

The estimation of the potency of the urine oestrone extract in mouse units can be performed quite simply.

As/

As will be remembered, the total twenty-four hour oestrone content of the urine was contained in a basis of 8 ccs. of olive oil.

∴ in (1) where .2 cc. was the dose, each mouse received .8 cc. of the extract, which gave a positive result

∴ each mouse received $\frac{8 \text{ ccs.}}{.8 \text{ cc.}}$ = more than 10 mouse units of oestrone.

In (2), where .1 cc. was the dose, each mouse received .4 cc. of the extract, which gave a positive result

∴ each mouse received $\frac{8 \text{ ccs.}}{.4 \text{ cc.}}$ = more than 20 mouse units of oestrone.

In (3) where .05 cc. was the dose, each mouse received .2 cc. of the extract, which gave a negative result

∴ each mouse received $\frac{8 \text{ ccs.}}{.2}$ = less than 40 mouse units of oestrone.

∴ The potency of the extract is between more than 20 and less than 40 mouse units.

In (4) similarly, where .15 cc. was the dose, each mouse received .6 cc. of the extract, which gave a positive result

∴ each mouse received $\frac{8 \text{ ccs.}}{.6}$ = more than 13 mouse units of oestrone.

∴ The potency of the extract would lie between more/

more than 13 mouse units and less than 20 mouse units.

By a process of dilution of the extract and gradation of the dosage, still more accurate results are obtainable but sometimes minor catastrophes occurred, as the extract proving toxic to the mice, upset complete estimation.

The number of oöphorectomized mice used for the assay of each extract varied in amount up to about twenty mice and this too, at first, varied according to the stock of unused mice, and mice previously used and when ready for injection.

One had to regularly prepare fresh mice by oöphorectomy, as some of the test animals succumbed owing to the extracts proving toxic, or to kill off many of the animals owing to their having developed sloughing sores on the back, at the site of injection.

For the purpose of injecting the extracts, tuberculin syringes were used which had long needles (1½ inches long).

The animal was gripped by the nape of the neck and tail and the extract injected through the loose skin at the back of the neck into the tissue immediately underlying the skin.

Blood/

Blood oestrone assay was carried out as for urinary oestrone estimation, except that only one mouse was used for each assay, as the oestrone content of 40 ccs. of blood in the cases examined was small.

TECHNIQUE OF ESTIMATION OF GONADOTROPIC HORMONE.

Healthy, immature, female mice about three weeks old were used for this purpose. For each extract, three mice are employed. Each mouse receives by injection .3 cc. twice daily for three days, and, about one hundred hours after the first injection, are killed and the genital tract examined for macroscopic changes, and the ovaries removed, preserved in 5 per cent. formalin to be later serially sectioned and examined under the microscope for signs of follicular activity. This was, however, very seldom found to be the case.

HORMONE EXCRETION.

It is now an accepted fact that oestrone is excreted in the urine and faeces by the human female throughout the period of sexual activity.

The excretion of oestrone in the urine begins at puberty, when menstruation is first initiated, for oestrone is not demonstrable in the urine before then, and is continued until some time after the menopause. There are varying amounts of the hormone excreted, corresponding to the hormone content of the blood and the various phases of the menstrual cycle.

If pregnancy supervenes there is a marked increase in the hormone excretion as was shown by Zondek and Aschheim.

There are several factors which affect hormone excretion.

It has already been mentioned that the hormones function as catalysts, which are unaffected by any action that they initiate, but more recent work by Robson, MacGregor *et alia*,(52) indicates that the metabolism of the body can destroy or render inactive an appreciable amount of the oestrous hormone.

Siebke and Shuschania(53) have shown that the total/

total daily excretion of oestrone is more or less equally divided between the urine and faeces, i.e. fifty per cent. eliminated by each route.

Thus, appreciably less than fifty per cent. of the total oestrone produced, is excreted in the urine and faeces.

During the menstrual cycle, oestrone is continuously excreted in the urine. Frank[54] has shown that there is an increase in the amount excreted occurring about the middle of the cycle, about the time of ovulation, and towards the end of the cycle, a few days before the onset of menstruation.

Siebke[55] found that the maximum oestrone excretion occurred about nine days before the onset of the next period. Zondek[56] found that the oestrone in the urine was about 10 mouse units per litre during the intermenstrual period and about 20-30 mouse units per litre during the premenstrual phase.

The presence of the corpus luteum hormone is not demonstrable in the urine.

The gonadotropic hormones are excreted throughout the greater part of the life cycle and are present in the urine before puberty. Zondek states that during the menstrual cycle only the follicular maturation hormone/

hormone, Prolan A, is excreted and that its excretion is increased during the premenstrual phase.

During pregnancy, however, he showed that there is a very marked increase in the excretion of the gonadotropic hormone.

HORMONE ANALYSIS OF CASES INVESTIGATED.

This investigation was undertaken in order to establish normal values for the amounts of oestrone and gonadotropic hormone excreted at the various phases of the menstrual cycle.

The urines examined were obtained from healthy women, of whom five were nulliparous and one parous, who menstruated normally and who had regular twenty-eight day menstrual cycles.

A twenty-four hours output of urine was obtained from each woman at weekly intervals, so as to coincide with the main phases of the menstrual cycle.

The estimation of the total hormone excretion, each/

each day throughout the menstrual cycle, was found impracticable, partly owing to the enormous amount of work entailed and to the fact that the women were engaged in active work daily which would have upset their convenience, and it was considered that reliable specimens at weekly intervals would be of more value than quantitative estimations of doubtful daily urinary output.

The age period of cases investigated was between twenty-four and forty-three years, and is fairly comprehensive of the average duration of sexual activity.

Miss A.

Age - 43 years.

Menarché - 14½ years.

Menstruation - $\frac{4}{27}$ regular.

L. M. P. - 19:8:35.

Number of Specimen	Date	Day of Cycle	Total 24 Hrs. Urine Output.	Oestrone in Mouse Units per 24 Hrs.	Gonadotropic Hormone
A_1	25:8:35	8	1640 ccs.	27-40	0
A_2	1:9:35	15	1000 ccs.	< 10	0
A_3	8:9:35	23	1260 ccs.	10-13	+
A_4	12:9:35	28	290 ccs.[x]	< 40	-

A 1.

25.8.35 24-hour Specimen of Urine = 1640 ccs.

Urine Oestrone 24-hour Output.

(1) Total Extract 8 cc. 3 mice. Dose .2 cc.

	M.	E.
6.10.35		.2
7.10.35	.2	.2
8.10.35	.2	

10.10.35 Smears Read: C $\frac{4}{4}$ (3).

Result: More than 10 m.u. per 24 hours.

(2) Total Extract 8 cc. 5 mice. Dose .1 cc.

	M.	E.
10.10.35		.1
11.10.35	.1	.1
12.10.35	.1	

14.10.35 Smears Read: C $\frac{4}{4}$ (5).

Result: More than 20 m.u. per 24 hours.

(3) Total Extract 8 cc. 5 mice. Dose .05 cc.

	M.	E.
19.10.35		.05
20.10.35	.05	.05
21.10.35	.05	

24.10.35 Smears Read: C $\frac{4}{4}$ (2); C $\frac{1}{2}$ (3).

Result: Less than 40 m.u. per 24 hours.

(4) Total Extract 8 cc. 5 mice. Total Dose .15 cc.

	M.	E.
31.10.35		.1
1.11.35	.05	.1
2.11.35	.05	

4.11.35 Smears read: C $\frac{4}{4}$ (4); C $\frac{1}{2}$ (1).

Result: Less than 27 m.u. per 24 hours.

URINE OESTRONE: 27-40 m.u. per 24 hours.

A 1.

Urine Gonadotropic Hormone, 25.8.35 (8th day).

60 ccs. Urine.	3 mice.	Dose .3 cc.
	M.	E.
31.8.35		.3
1.9.35	.3	.3
2.9.35	.3	.3
3.9.35	.3	

4.9.35 All dead, but no effect uteri or ovaries.

Microscopic Examination of Ovaries showed no signs of follicular activity (0).

A 2.

1.9.35 24-hour Output of Urine = 1000 ccs.

Urine Oestrone 24-hour Output.

(1)

Total Extract 8 ccs.	3 mice.	Dose .2 cc.
	M.	E.
6.10.35		.2
7.10.35	.2	.2
8.10.35	.2	

10.10.35 Smears Read: C $\frac{0}{4}$ (3).

Result: Less than 10 m.u. per 24 hours.

(2)

Total Extract 8 ccs.	5 mice.	Dose .2 cc.
	M.	E.
10.10.35		.2
11.10.35	.2	.2
12.10.35	.2	

15.10.35 Smears read: Leucocytes mainly - few nucleated cells.

Result: Less than 10 m.u. per 24 hours.

URINE OESTRONE: Less than 10 m.u. per 24 hours.

A.2.

Urine Gonadotropic Hormone, 1.9.35 (15th day).

60 ccs. Urine.	3 mice. M.	Dose .3 cc. E.
10.9.35		.3
11.9.35	.3	.3
12.9.35	.3	.3
13.9.35	.3	

15.9.35 Mice killed.
Very slight enlargement uterus.
Nil ovaries. Only one survived.
Microscopic Examination of Ovaries showed no signs of follicular activity (0).

A. 3.

8.9.35 24-hour Output of Urine = 1,260 ccs.

Urine Oestrone 24-hour Output.

(1) Total Extract 8 ccs. 3 mice. Dose .2 cc.

	M.	E.
6.10.35		.2
7.10.35	.2	.2
8.10.35	.2	

10.10.35 Smears read: 6 $\frac{4}{4}$ (2).

Result: More than 10 m.u. per 24 hours.

(2) Total Extract 8 ccs. 5 mice. Dose .1 cc.

	M.	E.
10.10.35		.1
11.10.35	.1	.1
12.10.35	.1	

15.10.35 Smears read: Leucocytes mainly.

Result: Less than 20 m.u. per 24 hours.

(3)/

(3) Total Extract 8 ccs. 5 mice. Dose .15 cc.

	M.	E.
19.10.35		.15
20.10.35	.15	.15
21.10.35	.15	

24.10.35 Smears read: Leucocytes mainly - few cornified cells.

Result: Less than 13 m.u. per 24 hours.

URINE OESTRONE: 10-13 m.u. per 24 hours.

A 3.

Urine Gonadotropic Hormone 8.9.35 (23rd day).

60 ccs. Urine. 3 mice. Dose .3 cc.

	M.	E.
10.9.35		.3
11.9.35	.3	.3
12.9.35	.3	.3
13.9.35	.3	

15.9.35 Mice killed.
Slight enlargement of uterus.
Nil ovaries.
Two dead.
Microscopic Examination of Ovaries showed slight signs of follicular activity (+).

A 4.

12.9.35 Urine collected 24 hours before onset of period which came on unexpectedly and so is not the full 24-hour Output - 290 ccs.

Urine Oestrone.

Total Extract 4 ccs. 3 mice. Dose .1 cc.

	M.	E.
10.10.35		.1
11.10.35	.1	.1
12.10.35	.1	

14.10.35/

14.10.35 Smears read: Negative, leucocytes mainly.

Result: Less than 10 m.u. per 24 hours in 290 ccs.

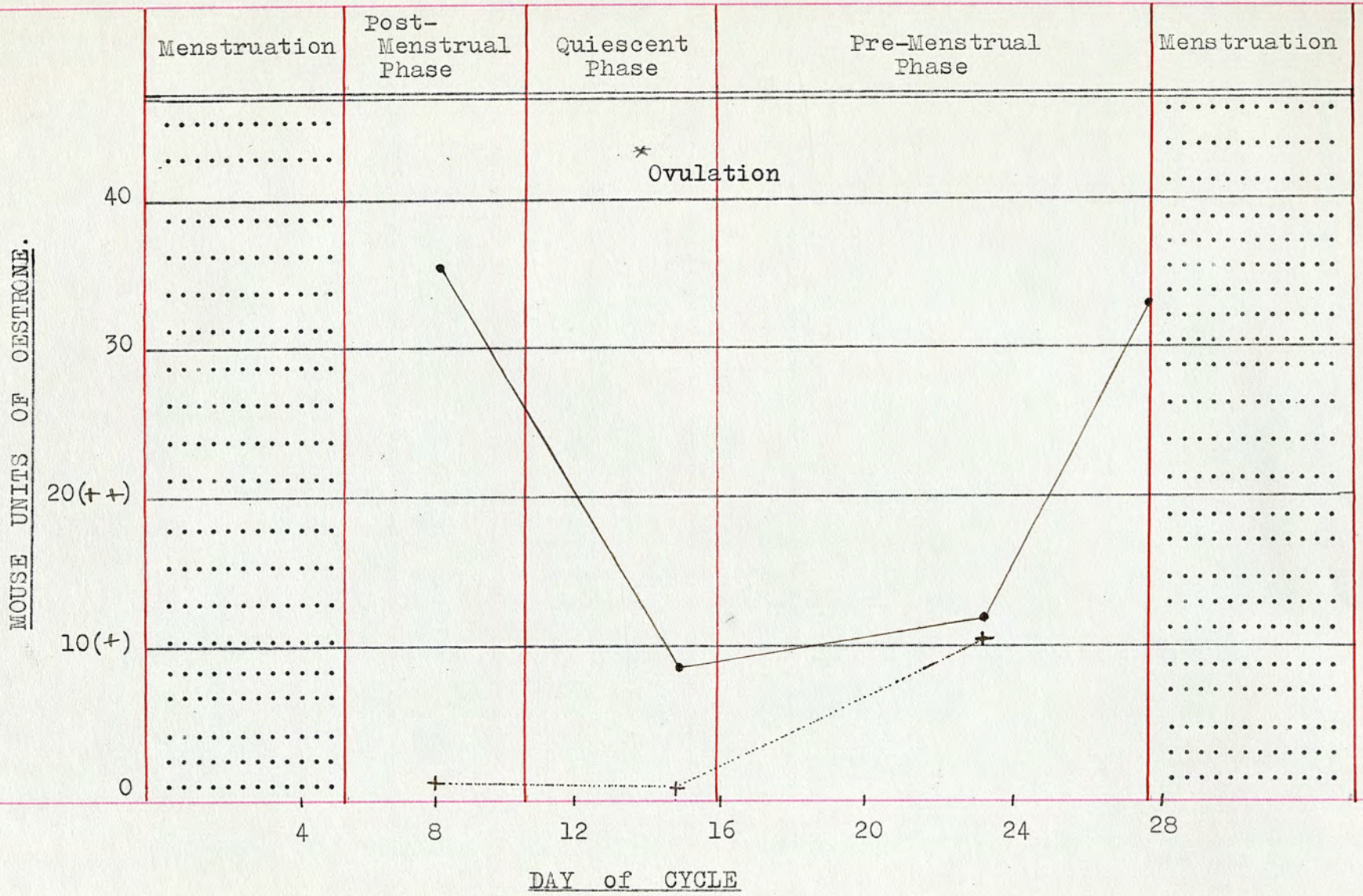
Assuming that this is only a fourth of the total average output, the oestrone content of 24-hours specimen would be less than 40 m.u. per 24 hours.

A 4.

Urine Gonadotropic Hormone 12.9.35 (28th day).

60 ccs. Urine.	3 mice.	Dose .3 cc.
	M.	E.
16.9.35		.3
17.9.35	.3	.3
18.9.35	.3	.3
19.9.35	.3	

19.9.35 Mice died.
Uteri and ovaries negative.
Microscopic Examination of Ovaries:
Ovaries not sectioned as Test not complete.



Gon. Horm. - - - - - (0)(+)(++)
Oestrone in Mouse Units - - - - 10, 20, 30, 40.

Miss B.

Age - 33 years.

Menarché - 18 years.

Menstruation - $\frac{6}{27}$ regular.

L. M. P. - 4:9:35.

Number of Specimen	Date	Day of Cycle	Total 24 Hrs. Urine Output	Oestrone in Mouse Units per 24 Hrs.	Gonadotropic Hormone
B_1	11:9:35	7	1700 ccs.	< 13	0
B_2	17:9:35	13	1350 ccs.	< 13	0
B_3	24:9:35	20	1560 ccs.	13-16	0
B_4	1:10:35	27	1660 ccs.	< 13	0

B 1.

11.9.35 24-hour Output of Urine = 1,700ccs.

Urine Oestrone 24-hour Output.

Total Extract 8 ccs.	5 mice.	Dose .15 cc.
	M.	E.
27.10.35	.15	.15
28.10.35	.15	.15

31.10.35 Smears read: C $\frac{3}{4}$ (2); C $\frac{0}{4}$ (3).

Result: Less than 13 m.u. per 24 hours.

B 1.

Urine Gonadotropic Hormone 11.9.35.

60 ccs. Urine.	3 mice.	Dose .3 cc.
	M.	E.
16.9.35		.3
17.9.35	.3	.3
18.9.35	.3	.3
19.9.35	.3	

20.9.35 Mice killed.
No effect uteri or ovaries.
Microscopic Examination of Ovaries showed no signs of follicular activity (0).

B 2.

17.9.35 24-hour Output of Urine = 1,350 ccs.

Urine Oestrone 24-hour Output.

Total Extract 8 ccs.	5 mice.	Dose .15 cc.
	M.	E.
27.10.35	.15	.15
28.10.35	.15	.15

30.10.35 Smears read: C $\frac{0}{4}$ (5).

Result: Less than 13 m.u. per 24 hours.

B 2.

Urine Gonadotropic Hormone 17.9.35.

60 ccs. Urine.	3 mice. M.	Dose .3 cc. E.
18.9.35		.3
19.9.35	.3	.3
20.9.35	.3	.3
21.9.35	.3	

22.9.35 Mice killed.
No effect uteri or ovaries.
Microscopic Examination of Ovaries showed no signs of follicular activity (0).

B 3.

24.9.35 24-hour Output of Urine = 1,560 ccs.

Urine Oestrone 24-hour Output.

(1)

Total Extract 8 ccs.	5 mice. M.	Dose .15 cc. E.
27.10.35	.15	.15
28.10.35	.15	.15

31.10.35 Smears read: C $\frac{4}{4}$ (3); C $\frac{3}{4}$ (2).

Result: More than 13 m.u. per 24 hours.

(2)

Total Extract 8 ccs.	2 mice. M.	Total Dose .5 cc. E.
3.11.35		.1
4.11.35	.15	.1
5.11.35	.15	

8.11.35 Smears read: C $\frac{4}{4}$ (1); C $\frac{0}{4}$ (1).

Result: Less than 16 m.u. per 24 hours.

(3)/

(3) Total Extract 8 ccs. 5 mice. Total .4 cc.

	M.	E.
3.11.35		.1
4.11.35	.1	.1
5.11.35	.1	

8.11.35 Smears read: C $\frac{4}{4}$ (1); C $\frac{0}{4}$ (2); C $\frac{1}{4}$ (2)

Result: Less than 20 m.u. per 24 hours.

(4) Total Extract 8 ccs. 3 mice. Total Dose .5 cc.

	M.	E.
8.11.35	.1	.15
9.11.35	.1	.15

12.11.35 Smears read: C $\frac{3}{4}$ (1).
(2) Leucocytes mainly.

Result: Less than 16 m.u. per 24 hours.

URINE OESTRONE: 13-16 m.u. per 24 hours.

B 3.

Urine Gonadotropic Hormone 24.9.35.

60 ccs. Urine. 3 mice. Dose .3 cc.

	M.	E.
25.9.35		.3
26.9.35	.3	.3
27.9.35	.3	.3
28.9.35	.3	

30.9.35 Mice killed.
No effect uteri or ovaries.
Microscopic Examination of Ovaries showed no signs of follicular activity (0).

B 4./

B 4.

1.10.35 24-hour Output of Urine = 1,660 ccs.

Urine Oestrone 24-hour Output.

(1) Total Extract 8 ccs. 5 mice. Dose .15 cc.

	M.	E.
27.10.35	.15	.15
28.10.35	.15	.15

31.10.35 Smears read: C $\frac{4}{4}$ (2); C $\frac{1}{2}$ (1); C $\frac{1}{4}$ (2).

Result: Less than 13 m.u. per 24 hours.

(2) Total Extract 8 ccs. 5 mice. Total Dose .4 cc.

	M.	E.
31.10.35		.15
1.11.35	.1	
2.11.35	.15	

4.11.35 Smears read: C $\frac{0}{4}$ (5).

Result: Less than 20 m.u. per 24 hours.

(3) Total Extract 8 ccs. 5 mice. Total Dose .35 cc.

	M.	E.
31.10.35		.2
1.11.35	.15	

3.11.35 Smears read: C $\frac{0}{4}$(5).

Result: Less than 22 m.u. per 24 hours.

URINE OESTRONE: Less than 13 m.u. per 24 hours.

B 4.

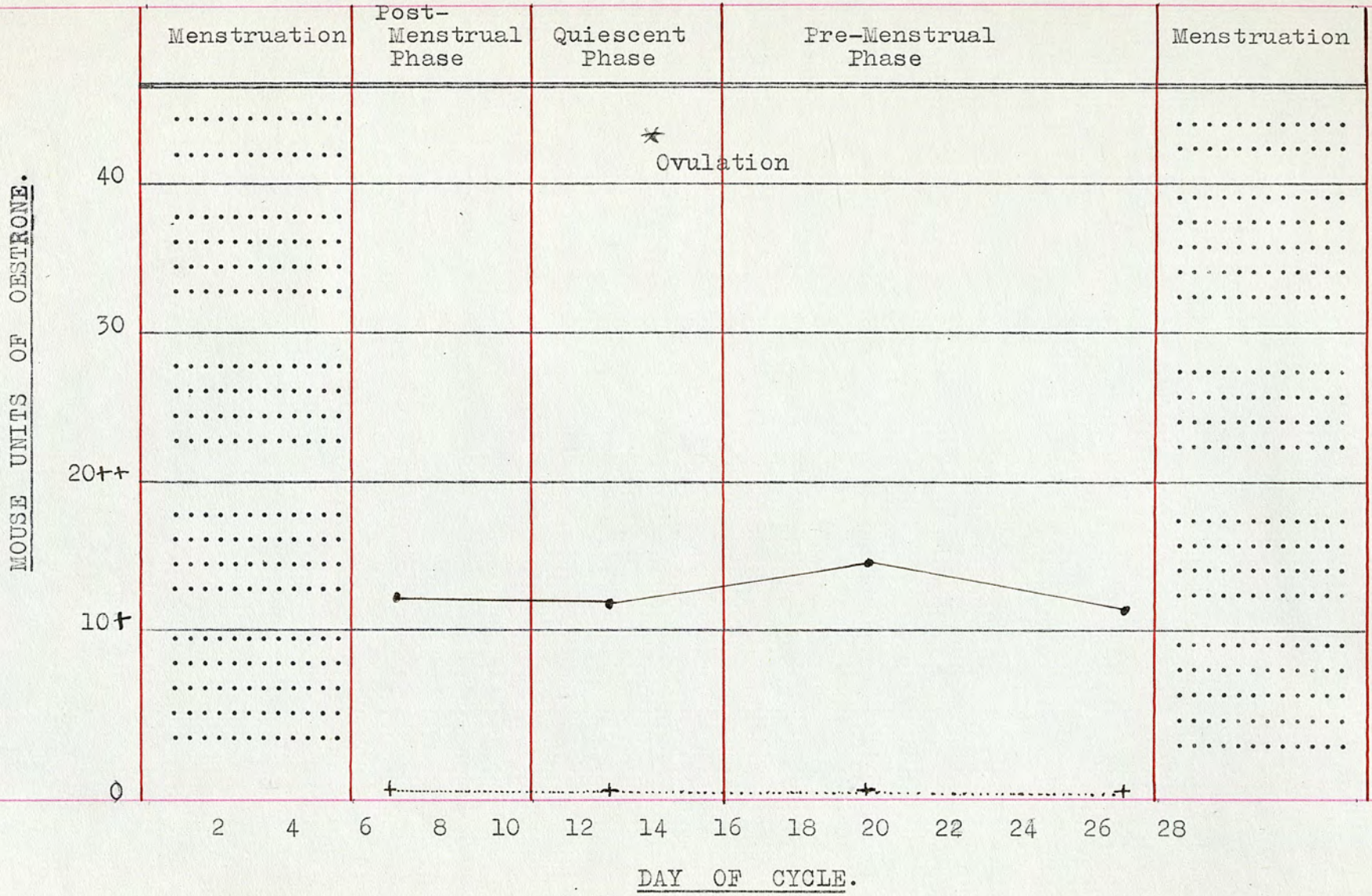
Urine Gonadotropic Hormone 1.10.35.

60 ccs. Urine. 3 mice. Dose .3 cc.

	M.	E.
11.10.35		.3
12.10.35	.3	.3
13.10.35	.3	.3
14.10.35	.3	

15.10.35/

15.10.35 Mice killed.
No effect uteri or ovaries.
Microscopic Examination of Ovaries showed no signs of follicular activity (0).



Gon. Horm. - - - - - - (0(+)(++)

Oestrone in Mouse Units - - - - 10, 20, 30, 40.

Miss C.

Age - 24 years.

Menarche' - 14 years.

Menstruation - $\frac{3\text{-}4}{28}$

L. M. P. - 25:11:35.

Number of Specimen	Date	Day of Cycle	Total 24 Hrs. Urine Output	Oestrone in Mouse Units per 24 Hrs.	Gonadotropic Hormone
C_1	3:12:35	8	1610 ccs.	10-20	0
C_2	7:12:35	12	990 ccs.	< 8	0
C_3	15:12:35	19	1265 ccs.	< 10	+
C_4	23:12:35	26	1290 ccs.	> 40	+

C 1.

3.12.35 24-hour Output of Urine = 1,610 ccs.

Urine Oestrone 24-hour Output.

(1) Total Extract 8 ccs. 5 mice. Dose .1 cc.

	M.	E.
20.1.36		.1
21.1.36	.1	.1
22.1.36	.1	

25.1.36 Smears read: Leucocytes mainly.

Result: Less than 20 m.u. per 24 hours.

(2) Total Extract 8 ccs. 3 mice. Dose .2 cc.

	M.	E.
22.3.36	.2	.2
23.3.36	.2	.2

26.3.36 Smears read: C $\frac{4}{4}$ (2).

Result: More than 10 m.u. per 24 hours.

(3) Total Extract 8 ccs. 5 mice. Dose .1 cc.

	M.	E.
26.3.36	.1	.1
27.3.36	.1	.1

30.3.36 Smears read: C $\frac{4}{4}$ (1); C $\frac{2}{4}$ (2); C $\frac{1}{4}$ (2).

Result: Less than 20 m.u. per 24 hours.

URINE OESTRONE: 10-20 m.u. per 24 hours.

C 1.

Urine Gonadotropic Hormone 3.12.35 (8th day).

ccs. Urine/

60 ccs. Urine.	3 mice.	Dose .3 cc.
	M.	E.
12.12.35	.3	.3
13.12.35	.3	.3
14.12.35	.3	.3

16.12.35 Mice killed.
No effect uteri or ovaries.
Microscopic Examination of Ovaries showed no signs of follicular activity (0).

C 2.

7.12.35 24-hour Output of Urine = 990 ccs.

Urine Oestrone 24-hour Output.

(1)

Total Extract 8 ccs.	5 mice.	Dose .2 cc.
	M.	E.
25.1.36		.2
26.1.36	.2	.2
27.1.36	.2	

30.1.36 Smears read: C $\frac{4}{4}$ (2); C $\frac{0}{4}$ (2); 1 dead.

Result: Less than 10 m.u. per 24 hours.

(2)

Total Extract 8 ccs.	3 mice.	Dose .2 cc.
	M.	E.
22.3.36	.2	.2
23.3.36	.2	.2

25.3.36 Smears read: C $\frac{3}{4}$ (1); C $\frac{0}{4}$ (2).

Result: Less than 10 m.u. per 24 hours.

(3)

Total Extract 8 ccs.	4 mice.	Dose .25 cc.
	M.	E.
26.3.36	.25	.25
27.3.36	.25	.25

30.3.36 Smears read: C $\frac{1}{4}$ (4).

Result: Less than 8 m.u. per 24 hours.

URINE OESTRONE: Less than 8 m.u. per 24 hours.

C 2.

Urine Gonadotropic Hormone 7.12.35 (12th day).

60 ccs. Urine.	3 mice.	Dose .3 cc.
	M.	E.
12.12.35	.3	.3
13.12.35	.3	
14.12.35	.3	.3
15.12.35	.3	

16.12.35 Mice killed.
No effect uteri or ovaries.
Microscopic Examination of Ovaries showed no signs of follicular activity (0).

C 3.

15.12.35 24-hour Output of Urine = 1,265 ccs.

Urine Oestrone 24-hour Output.

(1)

Total Extract 8 ccs.	3 mice.	Dose .2 cc.
	M.	E.
7.4.36		.2
8.4.36	.2	.2
9.4.36	.2	

12.4.36 Smears read: C $\frac{1}{4}$ (2).

Result: Less than 10 m.u. per 24 hours.

(2)

Total Extract 8 ccs.	5 mice.	Dose .2 cc.
	M.	E.
15.4.36		.2
16.4.36	.2	.2
17.4.36	.2	

20.4.36 Smears read: C $\frac{4}{4}$ (1); C $\frac{2}{4}$ (2); C $\frac{1}{4}$ (1).

Result: Less than 10 m.u. per 24 hours.

URINE OESTRONE: Less than 10 m.u. per 24 hours.

C 3.

Urine Gonadotropic Hormone 15.12.35 (19th day).

60 ccs. Urine.	3 mice.	Dose .3 cc.
	M.	E.
14.1.36		.3
15.1.36	.3	.3
16.1.36	.3	.3
17.1.36	.3	

Two mice died 17.1.36 (uteri and ovaries unaffected).

18.1.36 One mouse killed.
No effect uterus or ovaries.
Microscopic Examination of Ovaries showed slight signs of follicular activity (+).

C 4.

23.12.35 24-hour Output of Urine = 1,290 ccs.

Urine Oestrone 24-hour Output.

(1)

Total Extract 8 ccs.	3 mice.	Dose .2 cc.
	M.	E.
18.3.36		.2
19.3.36	.2	.2
20.3.36	.2	

23.3.36 Smears read: C $\frac{4}{4}$ (3).

Result: More than 10 m.u. per 24 hours.

(2)

Total Extract 8 ccs.	3 mice.	Dose .2 cc.
	M.	E.
22.3.36	.2	.2
23.3.36	.2	.2

25.3.36 Smears read: C $\frac{4}{4}$ (3).

Result: More than 10 m.u. per 24 hours.

(3)/

(3) Total Extract 8 ccs. 5 mice. Dose .1 cc.

	M.	E.
23.3.36	.1	.1
24.3.36	.1	.1

27.3.36 Smears read: C $\frac{4}{4}$ (4); C $\frac{1}{2}$ (1).

Result: More than 20 m.u. per 24 hours.

(4) Total Extract 8 ccs. 5 mice. Dose .05 cc.

	M.	E.
29.3.36	.05	.05
30.3.36	.05	.05

2.4.36 Smears read: C $\frac{4}{4}$ (5).

Result: More than 40 m.u. per 24 hours.

(5) Total Extract 3 ccs. of 25% sol. 5 mice, Dose .1 cc.

	M.	E.
4.4.36		.1
5.4.36	.1	.1
6.4.36	.1	

9.4.36 Smears read: C $\frac{4}{4}$ (5)

Result: More than 7.5 m.u.
= more than 80 m.u. in 24 hours
in 8 ccs. 100% solution.

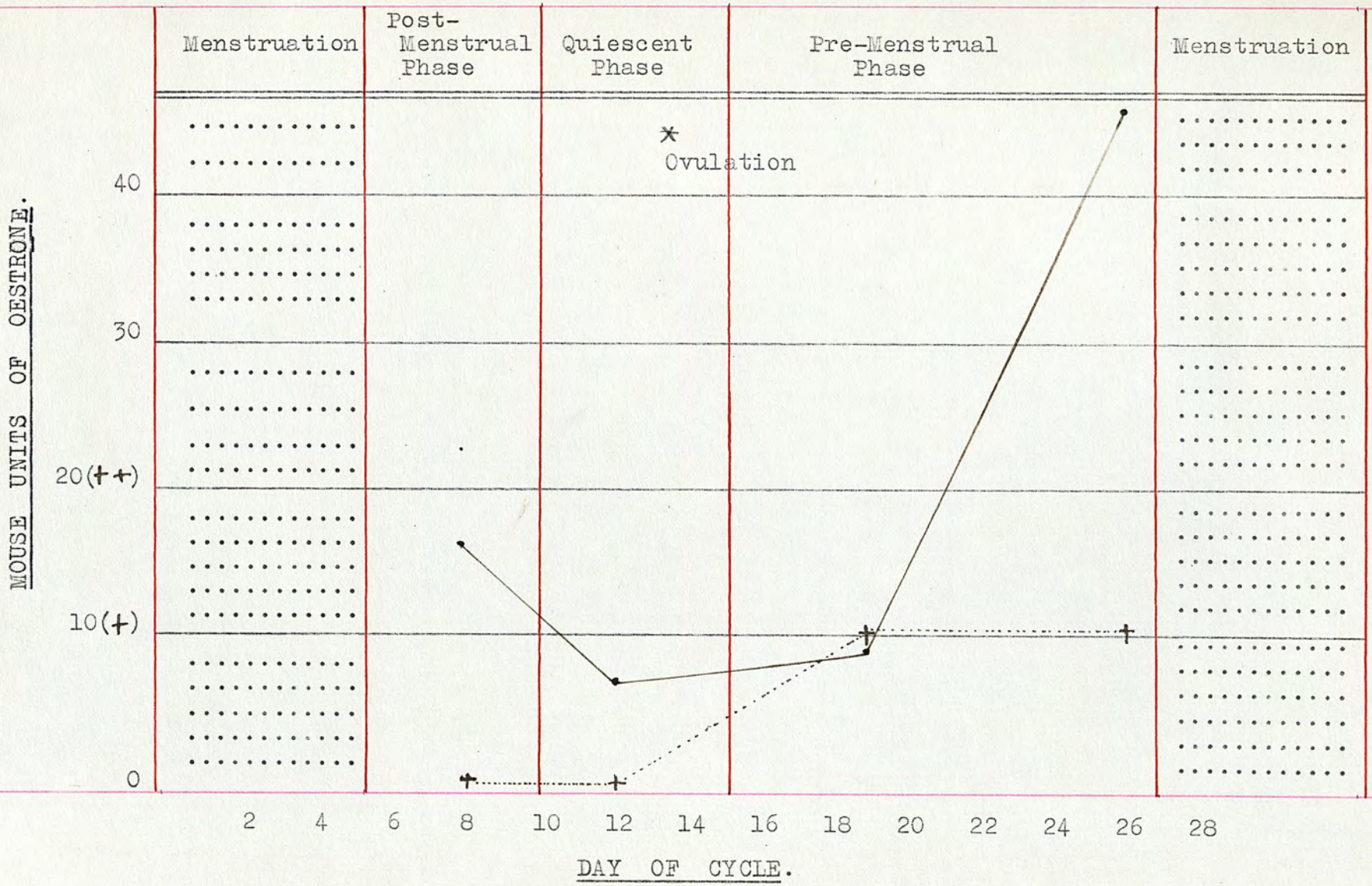
URINE OESTRONE: More than 80 m.u. per 24 hours.

C 4.

Urine Gonadotropic Hormone 23.12.35 (26th day).

60 ccs. Urine.	3 mice.	Dose .3 cc.
	M.	E.
24.11.35	.3	.3
25.11.35	.3	.3
26.11.35	.3	

27.11.35 Mice killed.
No effect uteri or ovaries.
Microscopic Examination of Ovaries showed slight signs of follicular activity (+).



Gon. Horm. - - - - - (o(+)(++) etc.)
Oestrone in Mouse Units - - - - - 10, 20, 30, 40, etc.

Miss D.

Age - 35 years.

Menarché - 13 years.

Menstruation - slightly irregular.

L. M. P. - 7:11:35.

Number of Specimen	Date	Day of Cycle	Total 24 Hrs. Urine Output	Oestrone in Mouse Units per 24 Hrs.	Gonadotropic Hormone
D_1	6:11:35	28	620 ccs.	27-40	
D_2	14:11:35	7	730 ccs.	20-40	0
D_3	21:11:35	14	1000 ccs.	< 8	+
D_4	29:11:35	21	1060 ccs.	10-13	

D 1.

6.11.35 24-hour Output of Urine = 620 ccs.
(24 hours before period.)

Urine Oestrone 24-hour Output.

(1) Total Extract 8 ccs. 3 mice. Dose .2 cc.

	M.	E.
1.1.36		.2
2.1.36	.2	.2
3.1.36	.2	

6.1.36 Smears read: C $\frac{4}{4}$ (3).

Result: More than 10 m.u. per 24 hours.

(2) Total Extract 8 ccs. 4 mice. Total Dose .4 cc.

	M.	E.
5.1.36		.2
6.1.36	.1	.1

9.1.36 Smears read: C $\frac{4}{4}$ (4).

Result: More than 20 m.u. per 24 hours.

(3) Total Extract 8 ccs. 5 mice. Dose .05 cc.

	M.	E.
12.1.36	.1	
13.1.36	.05	.05

18.1.36 Smears read: C $\frac{4}{4}$ (1);
Leucocytes mainly (4).

Result: Less than 40 m.u. per 24 hours.

(4) Total Extract 8 ccs. 5 mice. Total Dose .3 cc.

	M.	E.
20.1.36		.05
21.1.36	.1	.05
22.1.36	.1	

25.1.36 Smears read: C $\frac{4}{4}$ (4); C $\frac{0}{4}$ (1).

Result: More than 27 m.u. per 24 hours.

(5)/

(5) Total Extract 8 ccs. 5 mice. Dose .05 cc.

	M.	E.
25.1.36		.05
26.1.36	.05	.05
27.1.36	.05	

28.1.36 Smears read: C $\frac{4}{4}$ (1); C $\frac{0}{4}$ (4).

Result: Less than 40 m.u. per 24 hours

URINE OESTRONE: 27-40 m.u. per 24-hour Output.

D 2.

14.11.35 24-hour Output of Urine - 730 ccs.

Urine Oestrone 24-hour Output.

(1) Total Extract 8 ccs. 3 mice. Dose .2 cc.

	M.	E.
22.3.36	.2	.2
23.3.36	.2	.2

25.3.36 Smears read: C $\frac{4}{4}$ (3)

Result: More than 10 m.u. per 24 hours.

(2) Total Extract 8 ccs. 5 mice. Dose .1 cc.

	M.	E.
26.3.36	.1	.1
27.3.36	.1	.1

30.3.36 Smears read: C $\frac{4}{4}$ (4).

Result: More than 20 m.u. per 24 hours.

(3) Total Extract 8 ccs. 5 mice. Dose .05 cc.

	M.	E.
17.4.36		.05
18.4.36	.05	.05
19.4.36	.05	

22.4.36/

22.4.36 Smears read: C $\frac{3}{4}$ (1); C $\frac{1}{4}$ (2); C $\frac{0}{4}$ (2).

Result: Less than 40 m.u. per 24 hours.

URINE OESTRONE: 20-40 m.u. per 24-hour Output.

D 2.

Urine Gonadotropic Hormone 14.11.35 (7th day).

60 ccs. Urine.	3 mice. M.	Dose .3 cc. E.
27.11.35		.3
28.11.35	.3	.3
29.11.35	.3	.3
30.11.35	.3	

30.11.35 Two mice dead, one dying.
Slight enlargement of uterus.
Microscopic Examination of Ovaries showed no signs of follicular activity (0).

D 3.

21.11.35 24-hour Output of Urine = 1,000 ccs.

Urine Oestrone 24-hour Output.

(1)

Total Extract 8 ccs.	3 mice. M.	Dose .2 cc. E.
22.3.36	.2	.2
23.3.36	.2	.2

25.3.36 Smears read: C $\frac{1}{4}$, Leucocytes mainly (3)

Result: Less than 10 m.u. per 24 hours.

(2)/

(2) Total Extract 8 ccs. 4 mice. Dose .25 cc.

	M.	E.
26.3.36	.25	.25
27.3.36	.25	.25

30.3.36 Smears read: C $\frac{0}{4}$ (3); C $\frac{2}{4}$ (1).

Result: Less than 8 m.u. per 24 hours.

URINE OESTRONE: Less than 8 m.u. per 24 hours.

D 3.

Urine Gonadotropic Hormone 21.11.35 (14th day).

60 ccs. Urine. 3 mice. Dose .3 cc.

	M.	E.
7.12.35	.3	.3
8.12.35	.3	.3
9.12.35	.3	.3

10.12.35 Mice killed.
No effect uteri or ovaries.
Microscopic Examination of Ovaries showed slight signs of follicular activity (+).

D 4.

29.11.35 24-hour Output of Urine = 1,060 ccs.

Urine Oestrone 24-hour Output.

(1) Total Extract 8 ccs. 3 mice. Dose .2 cc.

	M.	E.
22.3.36	.2	.2
23.3.36	.2	.2

25.3.36 Smears read: C $\frac{4}{4}$ (2); C $\frac{3}{4}$ (1).

Result: More than 10 m.u. per 24 hours.

(2)/

(2) Total Extract 8 ccs. 5 mice. Dose .1 cc.

	M.	E.
26.3.36	.1	.1
27.3.36	.1	.1

30.3.36 Smears read: C $\frac{0}{4}$ (4); C $\frac{2}{4}$ (1)

Result: Less than 20 m.u. per 24 hours.

(3) Total Extract 8 ccs. 4 mice. Dose .15 cc.

	M.	E.
17.4.36		.15
18.4.36	.15	.15
19.4.36	.15	

22.4.36 Smears read: C $\frac{4}{4}$ (1); C $\frac{3}{4}$ (1); C $\frac{0}{4}$ (1).

Result: Less than 13 m.u. per 24 hours.

URINE OESTRONE: 10-13 m.u. per 24-hour Output.

D 4.

Urine Gonadotropic Hormone 29.11.35 (21st day).

60 ccs. Urine. 3 mice. Dose .3 cc.

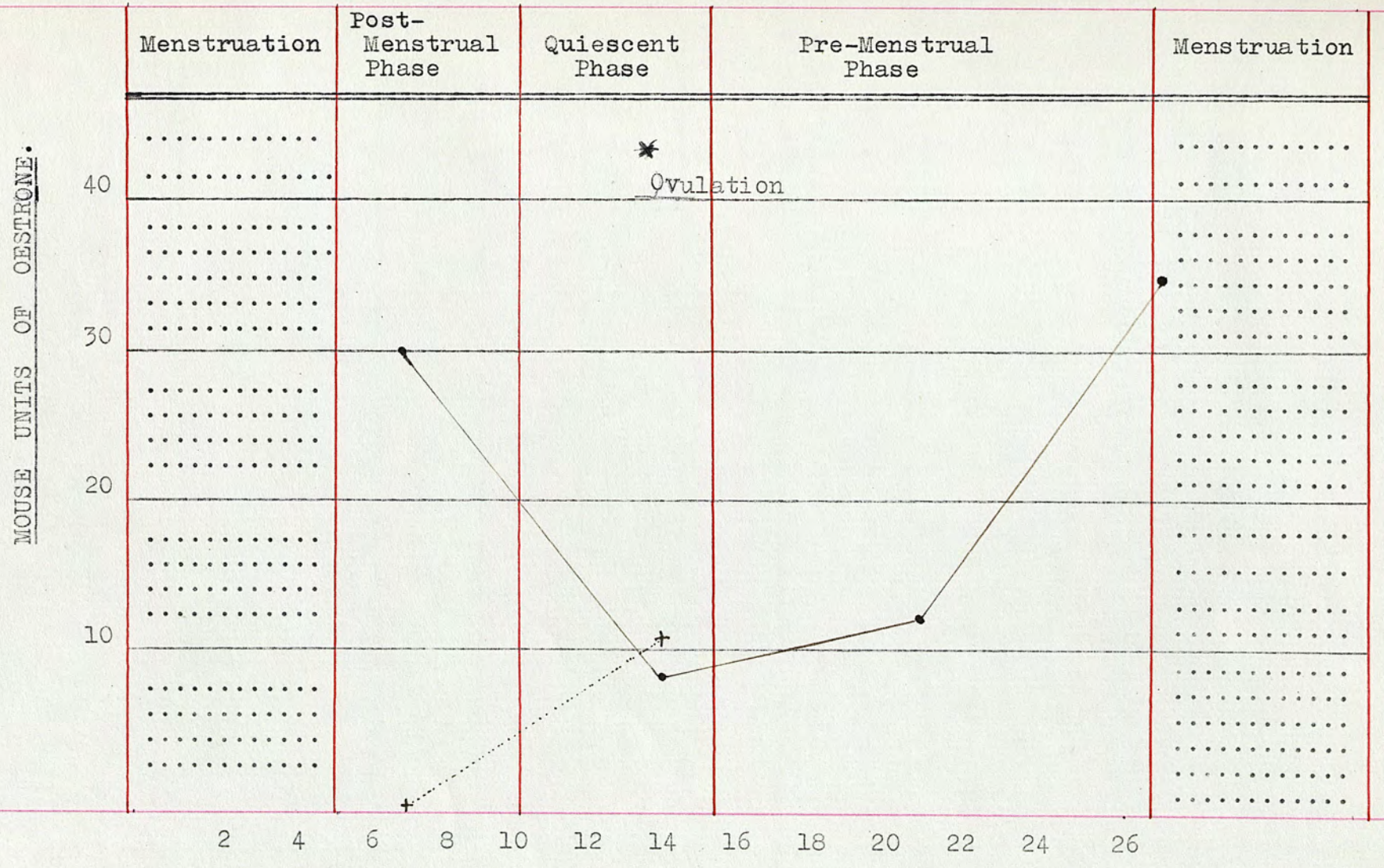
	M.	E.
12.12.35	.3	.3
13.12.35	.3	.3
14.12.35	.3	.3

14.12.35 All mice found dead.

60 ccs. Urine. 3 mice. Dose .3 cc.

	M.	E.
16.12.35		.3
17.12.35	.3	.3
18.12.35	.3	.2
19.12.35	Extract finished.	

20.12.35 Mice killed.
Uteri slightly enlarged (3).
Ovaries unaffected.
Vaginae patent.



Gon. Horm. - - - - - (0) (+)(++)
Oestrone in Mouse Units - - - - 10, 20, 30, 40.

Miss E.

Age - 25 years.

Menarche - 12 years.

Menstruation - $\frac{6}{28}$ regular.

L. M. P. - 5:11:35.

Number of Specimen	Date	Day of Cycle	Total 24 Hrs. Urine Output	Oestrone in Mouse Units per 24 Hrs.	Gonadotropic Hormone
E_1	6:11:35	2	1350 ccs.	10-20	0
E_2	14:11:35	10	1320 ccs.	$<$ 10	+
E_3	20:11:35	16	1065 ccs.	$>$ 20	+
E_4	26:11:35	22	1400 ccs.	20-40	

E 1.

6.11.35 24-hour Output of Urine = 1,350 ccs.

Urine Oestrone 24-hour Output.

(1) Total Extract 8 ccs. 5 mice. Dose .2 cc.

	M.	E.
25.1.35		.2
26.1.36	.2	.2
27.1.36	.2	

30.1.36 Smears read: C $\frac{4}{4}$ (5)

Result: More than 10 m.u. per 24 hours.

(2) Total Extract 8 ccs. 5 mice. Dose .1 cc.

	M.	E.
20.1.36		.1
21.1.36	.1	.1
22.1.36	.1	

25.1.36 Smears read: Leucocytes mainly (5)

Result: Less than 20 m.u. per 24 hours.

URINE OESTRONE: 10-20 m.u. per 24 hours.

E 1.

Urine Gonadotropic Hormone 6.11.35 (2nd day).

60 ccs. Urine. 3 mice. Dose .3 cc.

	M.	E.
24.11.35	.3	.3
25.11.35	.3	.3
26.11.35	.3	.3

All mice died after second injection (0).

E 2.

14.11.35 24-hour Output of Urine = 1,320 ccs.

Urine Oestrone 24-hour Output.

(1) Total Extract 8 ccs. 3 mice. Dose .2 cc.

	M.	E.
18.3.36		.2
19.3.36	.2	.2
20.3.36	.2	

23.3.36 Smears read: C $\frac{4}{4}$ (1); leucocytes mainly (2).

Result: Less than 10 m.u. per 24 hours.

(2) Total Extract 8 ccs. 5 mice. Dose .2 cc.

	M.	E.
17.4.36		.2
18.4.36	.2	.2
19.4.36	.2	

22.4.36 Smears read: C $\frac{4}{4}$ (1); C $\frac{3}{4}$ (1); leucocytes mainly (3)

Result: Less than 10 m.u. per 24 hours.

URINE OESTRONE: Less than 10 m.u. per 24 hours.

E 2.

Urine Gonadotropic Hormone 14.11.35 (10th day).

60 ccs. Urine. 3 mice. Dose .3 cc.

	M.	E.
27.11.35		.3
28.11.35	.3	.3
29.11.35	.3	.3
30.11.35	.3	

1.12.35 Mice killed.
No effect uteri or ovaries.

Microscopic Examination of Ovaries showed slight signs of follicular activity (+).

E 3.

20.11.35 24-hour Output of Urine = 1,065 ccs.

Urine Oestrone 24-hour Output.

(1) Total Extract 8 ccs. 3 mice. Dose .2 cc.

	M.	E.
22.3.36	.2	.2
23.3.36	.2	.2

25.3.36 Smears read: C $\frac{4}{4}$ (2)

Result: More than 10 m.u. per 24 hours.

(2) Total Extract 8 ccs. 5 mice. Dose .1 cc.

	M.	E.
26.3.36	.1	.1
27.3.36	.1	.1

30.3.36 Smears read: C $\frac{4}{4}$ (3); C $\frac{0}{4}$ (2).

Result: More than 20 m.u. per 24 hours.

(3) Total Extract 8 ccs. 4 mice. Dose .15 cc.

	M.	E.
17.4.36		.15
18.4.36	.15	.15
19.4.36	.15	

22.4.36 Smears read: C $\frac{4}{4}$ (4).

Result: More than 13 m.u. per 24 hours.

URINE OESTRONE: More than 20 m.u. per 24 hours.

E 3.

Urine Gonadotropic Hormone 20.11.35 (16th day).

60 ccs. Urine. 3 mice. Dose .3 cc.

	M.	E.
1.12.35	.3	.3
2.12.35	.3	.3
3.12.35	.3	.3

4.12.35/

4.12.35 Mice killed.
Minute blood spots in ovaries.
Uteri enlarged.
Microscopic Examination of Ovaries showed definite signs of follicular activity (++).

Used mice were inadvertently made use of in this test, which is therefore of no value.

E 4.

26.11.35 24-hour Output of Urine = 1,400 ccs.

Urine Oestrone 24-hour Output.

(1) Total Extract 8 ccs. 5 mice. Dose .1 cc.

	M.	E.
23.3.36	.1	.1
24.3.36	.1	.1

27.3.36 Smears read: C $\frac{4}{4}$ (3); C $\frac{1}{2}$ (2).

Result: More than 20 m.u. per 24 hours.

(2) Total Extract 8 ccs. 3 mice. Dose .2 cc.

	M.	E.
18.3.36		.2
19.3.36	.2	.2
20.3.36	.2	

23.3.36 Smears read: C $\frac{4}{4}$ (2)

Result: More than 10 m.u. per 24 hours.

(3) Total Extract 8 ccs. 5 mice. Dose .05 cc.

	M.	E.
29.3.36	.05	.05
30.3.36	.05	.05

2.4.36 Smears read: C $\frac{4}{4}$ (1); C $\frac{0}{4}$ (4) leucocytes nucleated and non-nucleated epithelial cells.

Result: Less than 40 m.u. per 24 hours.

(4)/

(4) Total Extract 8 ccs. 4 mice. Total Dose .2 cc.

	M.	E.
4.4.36		.05
5.4.36	.1	.05

9.4.36 Smears read: C $\frac{2}{4}$ (2); C $\frac{0}{4}$ (2)

Result: Less than 40 m.u. per 24 hours.

URINE OESTRONE: 20-40 m.u. per 24 hour Output.

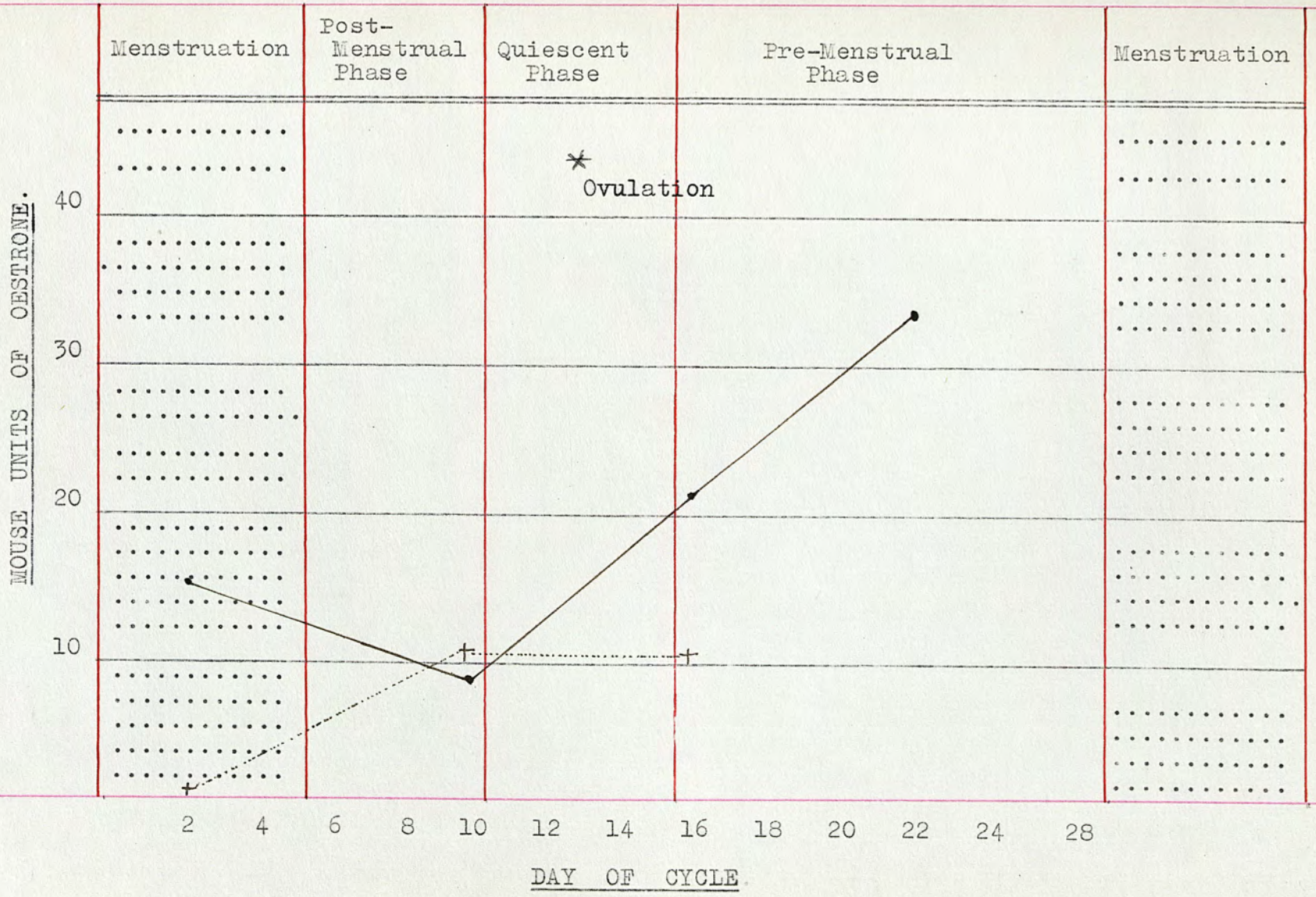
E 4.

Urine Gonadotropic Hormone 26.11.35 (22nd day).

60 ccs. Urine. 3 mice. Dose .3 cc.

	M.	E.
3.12.35		.3
4.12.35	.3	.3
5.12.35	.3	.3
6.12.35	.3	

7.12.35 Mice killed.
Vaginae closed.
Uteri and ovaries unaffected.
Microscopic Examination of Ovaries showed slight signs of follicular activity (+).



Gon. Horm. - - - - - - (0, +, ++, etc.)

Oestrine in Mouse Units - - - - - 10, 20, 30, 40, etc.

Mrs F.

Age - 38 years. Para I.

Menarche - 12 years.

Menstruation - $\frac{3 - 4}{28}$ regular.

L. M. P. - 25:1:35.

Number of Specimen	Date	Day of Cycle	Total 24 Hrs. Urine Output	Oestrine in Mouse Units per 24 Hrs.	Gonadotropic Hormone
F_1	20:1:35	24	680 ccs.	13-20	+
F_2	27:1:35	3	1460 ccs.	13-20	0
F_3	3:2:35	10	1090	< 10	0
F_4	10:2:35	17	1180	13-20	0

F 1.

20.1.35 24-hour Output of Urine = 680 ccs.

Urine Oestrone 24-hour Output.

(1) Total Extract 8 ccs. 3 mice. Dose .15 cc.

	M.	E.
3.3.35	.15	.15
4.3.35	.15	.15

7.3.35 Smears read: C $\frac{4}{4}$ (2); C $\frac{1}{2}$ (1).

Result: More than 13 m.u. per 24 hours.

(2) Total Extract 8 ccs. 5 mice. Dose .1 cc.

	M.	E.
20.3.35	.1	.1
21.3.35	.1	.1

24.3.35 Smears read: C $\frac{4}{4}$ (3); C $\frac{2}{4}$ (2).

Result: More than 20 m.u. per 24 hours.

(3) Total Extract 8 ccs. 3 mice. Dose .15 cc.

	M.	E.
14.11.35		.15
15.11.35	.15	.15
16.11.35	.15	

19.11.35 Smears read: C $\frac{4}{4}$ (2); C $\frac{3}{4}$ (1).

Result: More than 13 m.u. per 24 hours.

URINE OESTRONE: 13 to more than 20 m.u. per 24 hours.

F 1.

Urine Gonadotropic Hormone 20.1.35 (24th day).

60 ccs. Urine. 3 mice. Dose .3 cc.

	M.	E.
24.1.35	.3	.3
25.1.35	.3	.3
26.1.35	.3	.3

30.1.35/

30.1.35 Mice killed.
No effect uteri or ovaries.
Microscopic Examination of Ovaries showed slight evidence of follicular activity (+).

F 2.

27.1.35 24-hour Output of Urine = 1.460 ccs.

Urine Oestrone 24-hour Output.

(1) Total Extract 8 ccs. 5 mice. Dose .1 cc.

	M.	E.
20.3.35	.1	.1
21.3.35	.1	.1

24.3.35 Smears read: leucocytes mainly (5).

Result: Less than 20 m.u. per 24 hours.

(2) Total Extract 8 ccs. 3 mice. Dose .15 cc.

	M.	E.
14.11.35		.15
15.11.35	.15	.15
16.11.35	.15	

19.11.35 Smears read: C $\frac{4}{4}$(2); C $\frac{1}{2}$ (1).

Result: More than 13 m.u. per 24 hours.

(3) Total Extract 8 ccs. 3 mice. Dose .15 cc.

	M.	E.
27.11.35		.15
28.11.35	.15	.15
29.11.35	.15	

3.12.35 Smears read: (1 mouse dead); C $\frac{0}{4}$ (2).

Result: Less than 13 m.u. per 24 hours.

URINE OESTRONE: 13 to 20 m.u. per 24 hour Output.

F 2./

F 2.

Urine Gonadotropic Hormone 27.1.35.

60 ccs. Urine.	3 mice.	Dose .3 cc.
	M.	E.
2.2.35		.3
3.2.35	.3	.3
4.2.35	.3	.3

6.2.35 Mice killed.
Ovaries not affected.
One uterus slightly enlarged.
Microscopic Examination of Ovaries showed no signs of follicular activity (0).

F 3.

3.2.35 24-hour Output of Urine = 1,090 ccs.

Urine Oestrone 24-hour Output.

(1)

Total Extract 8 ccs.	5 mice.	Dose .1 cc.
	M.	E.
20.3.35	.1	.1
21.3.35	.1	.1

24.3.35 Smears read: Leucocytes mainly.

Result: Less than 20 m.u. per 24 hours.

(2)

Total Extract 8 ccs.	3 mice.	Dose .15 cc.
	M.	E.
14.11.35		.15
15.11.35	.15	.15
16.11.35	.15	

19.11.35 Smears read: C $\frac{0}{4}$ (3)

Result: Less than 13 m.u. per 24 hours.

(3)/

(3) Total Extract 8 ccs. 3 mice. Dose .2 cc.

	M.	E.
27.11.35		.2
28.11.35	.2	.2
29.11.35	.2	

3.12.35 Smears read: C $\frac{0}{4}$ (3).

Result: Less than 10 m.u. per 24 hours.

URINE OESTRONE: Less than 10 m.u. per 24 hours.

F 3.

Blood Oestrone 3.2.35 40 ccs. Venous Blood.

Total Extract 1 cc. 1 mouse. Dose .2 cc.

	M.	E.
29.4.35	.2	.2
30.4.35	.2	.2

3.4.35 Smears read: C $\frac{0}{4}$ (1).

Result: Less than 1.25 m.u. per 24 hours.

F 3.

Urine Gonadotropic Hormone 3.2.35.

60 ccs. Urine. 3 mice. Dose .3 cc.

	M.	E.
4.2.35		.3
5.2.35	.3	.3
6.2.35	.3	.3
7.2.35	.3	

8.3.35 Mice killed.
Ovaries negative.
One uterus greatly enlarged: two slightly enlarged.
Microscopic Examination of Ovaries showed no signs of follicular activity (0).

F 4./

F 4.

10.2.35 24-hour Output of Urine 1,180 ccs.

Urine Oestrone 24-hour Output.

(1) Total Extract 8 ccs. 5 mice. Dose .1 cc.

	M.	E.
21.3.35	.1	.1
22.3.35	.1	.1

25.3.35 Smears read: C $\frac{0}{4}$ Leucocytes mainly.

Result: Less than 20 m.u. per 24 hours.

(2) Total Extract 8 ccs. 3 mice. Dose .15 cc.

	M.	E.
14.11.35		.15
15.11.35	.15	.15
16.11.35	.15	

19.11.35 Smears read: C $\frac{4}{4}$ (2); Leucocytes mainly (1).

Result: More than 13 m.u. per 24 hours.

(3) Total Extract 8 ccs. 3 mice. Dose .15 cc.

	M.	E.
27.11.35		.15
28.11.35	.15	.15
29.11.35	.15	

2.12.35 Smears read: C $\frac{4}{4}$ (2); C $\frac{1}{4}$ (1).

Result: More than 13 m.u. per 24 hours.

URINE OESTRONE: 13 to 20 m.u. per 24 hours.

F 4./

F 4.

Blood Oestrone 10.2.35 50 ccs. Venous Blood.

Total Extract 1 cc.	1 mouse. M.	Dose .2 cc. E.
1.3.35	.2	.2
2.3.35	.2	.2

5.3.35 Smears read: C $\frac{0}{4}$ (1).

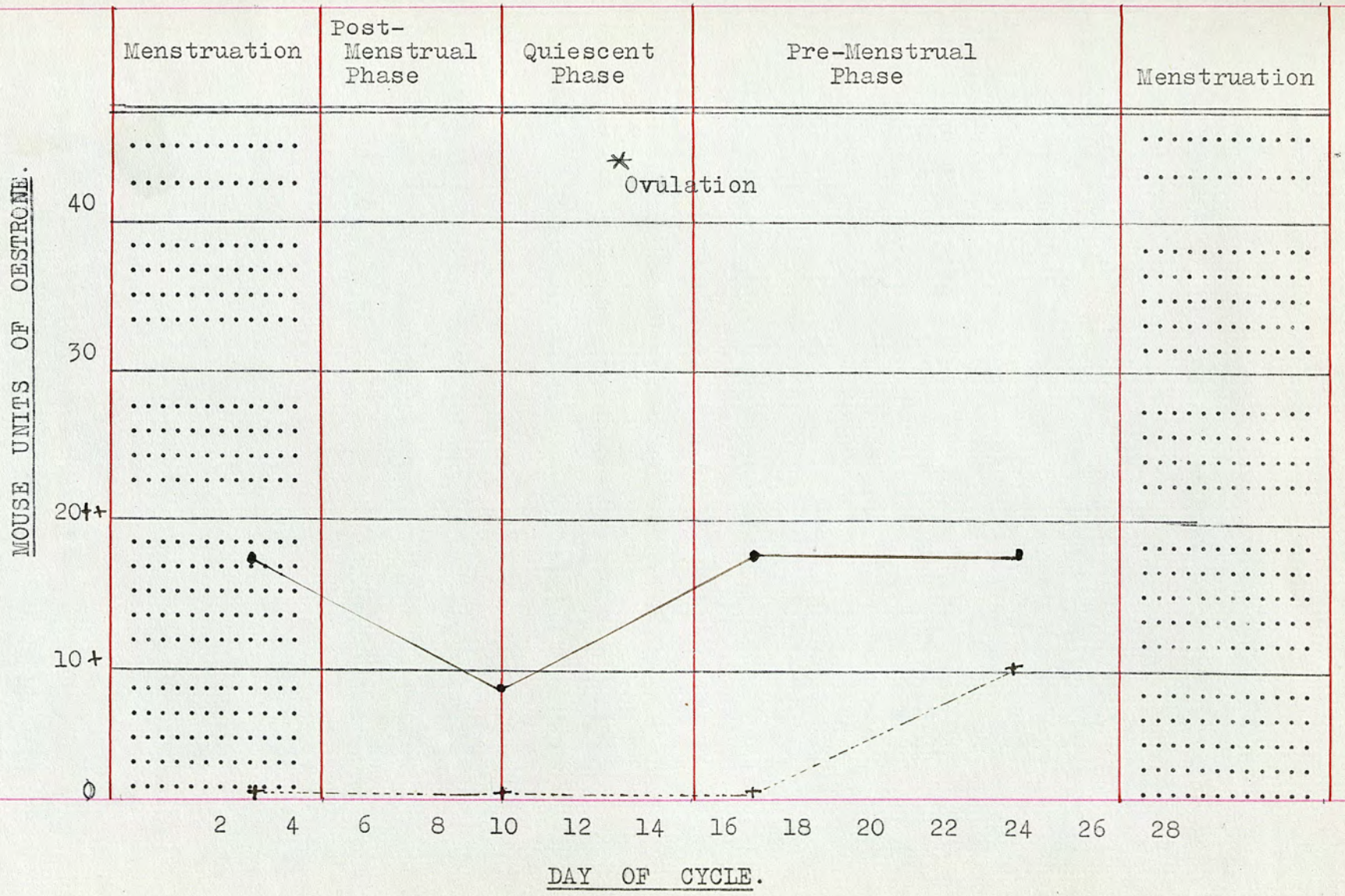
Result: Less than 1.25 m.u. per litre.

F 4.

Urine Gonadotropic Hormone 10.2.35.

60 ccs. Urine.	3 mice. M.	Dose .3 cc. E.
26.2.35		.3
27.2.35	.3	.3
28.2.35	.3	.3
29.2.35	.3	

1.3.35 All died after last injection.
No effect uteri or ovaries.
Microscopic Examination of Ovaries showed no signs of follicular activity (0).



92.

Gon. Horm. - - - - - (0, +, ++).
Oestrine in Mouse Units - - - - - (10, 20, 30, 40).

COMMENTARY ON RESULTS OF HORMONE ANALYSIS.

A. OESTRONE.

A survey of the graphic representations of the urinary oestrone excretion values, at the various stages of the cycle in all the cases investigated, shows curves, which, for the most part, are of a similar nature, except perhaps in one instance, Miss B., where there is very little variation in the oestrone excreted throughout the cycle.

The graphs show relatively high oestrone values in the post-menstrual phase, which decline fairly rapidly as the quiescent phase is reached, and then increase in value during the pre-menstrual phase, up to the time of menstruation.

A study of the table of approximate mean values brings out these findings more clearly. In this table the approximate means of the oestrone excretion values for each seven days of the menstrual cycle have been assessed and graphically represented. This table brings out, even more clearly, the decline in the amount of oestrone excreted in the post-menstrual phase to its minimum, in or about the time of quiescent phase of the endometrium, and then the increase/

OESTRONE EXCRETION VALUES IN NORMAL MENSTRUATION.

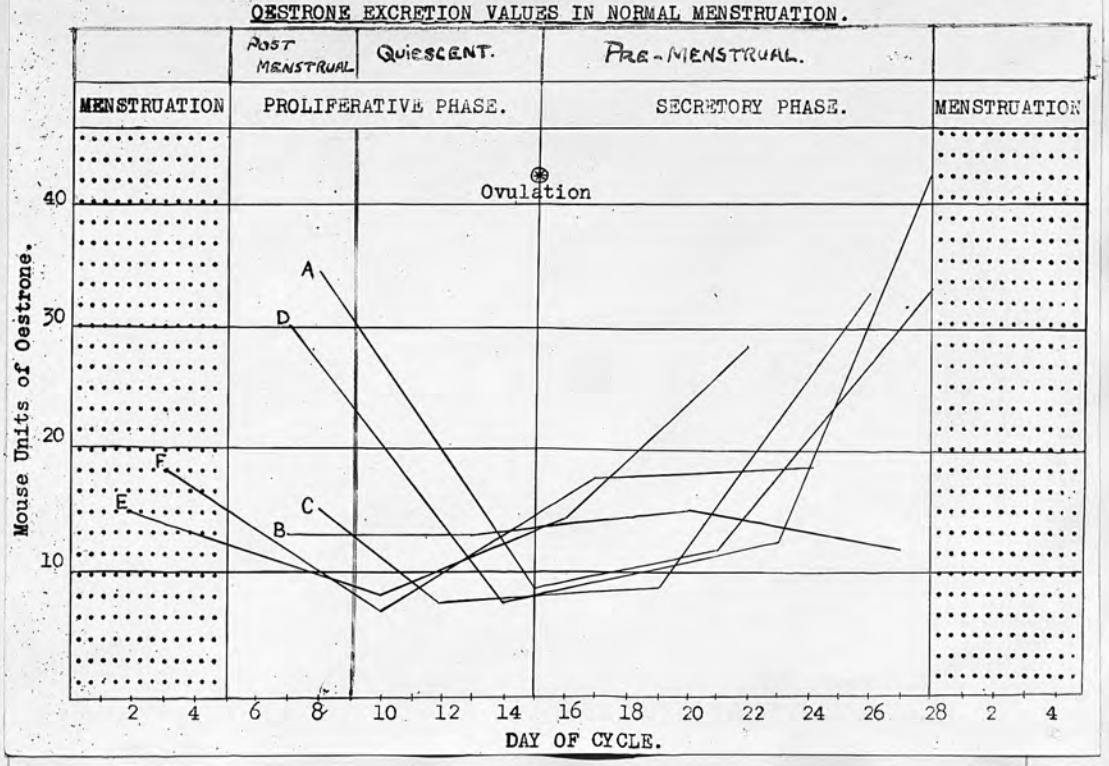
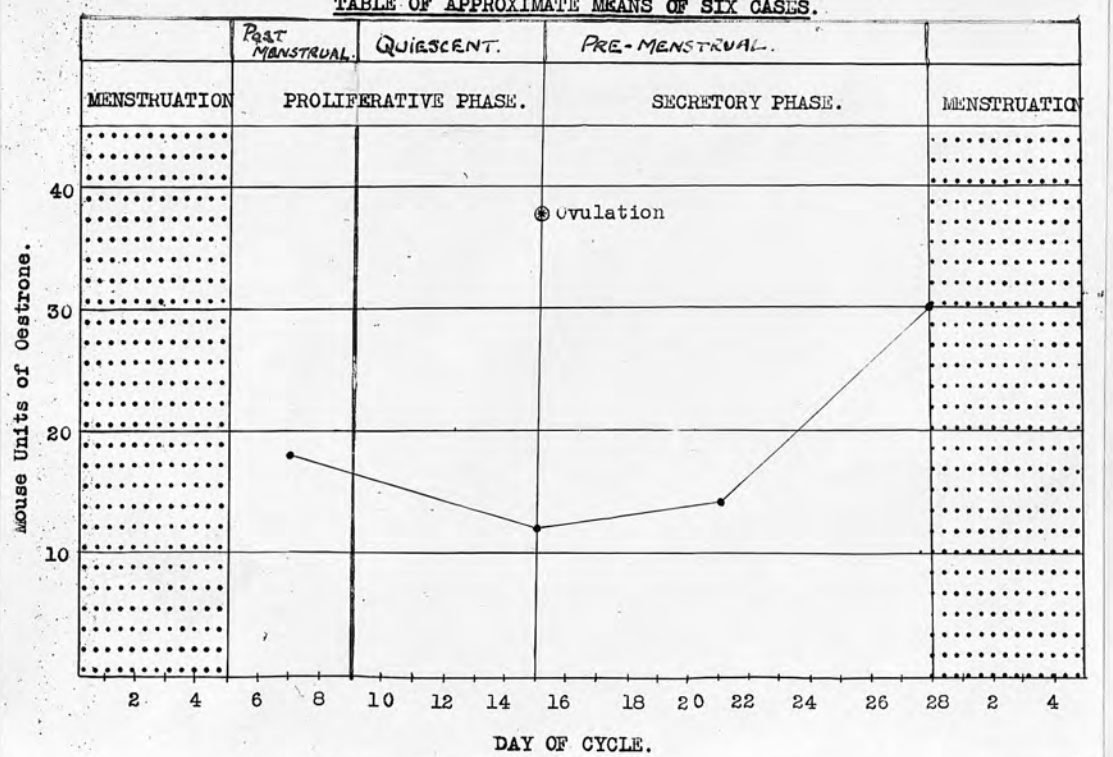


TABLE OF APPROXIMATE MEANS OF SIX CASES.



increase, which occurs in the premenstrual phase, up to the onset of menstruation, when there evidently is a diminution in excretion.

One may therefore, not unreasonably, pretend that the ovary in the post-menstrual phase secretes a sufficient amount of oestrone for the regeneration and repair of the endometrium, after the destructive phase of menstruation has taken place, whereupon the endometrium passes into the resting or quiescent stage and consequently, as much oestrone as was previously produced is not required.

When the post-menstrual period is reached, an increased amount of oestrone is secreted and this is accompanied by the secretion of Progesterone in order to build up the endometrium into a more perfect structure for the possible reception of a fertilized ovum.

If this contingency, however, does not occur, then the hormone production activity of the ovary of oestrone and Progesterone diminishes temporarily.

The approximate mean values of oestrone excretion in (1) the post-menstrual period was found to be 18 m.u. per 24 hours; (2) in the quiescent stage to be 12 m.u. per 24 hours, and (3) in the post-menstrual stage to be 14-30 m.u. per 24 hours.

(b)/

(b) Gonadotropic Hormone.

It was not found practicable to estimate the amounts of gonadotropic hormone excreted owing to these being rather small in the quantities of urine tested.

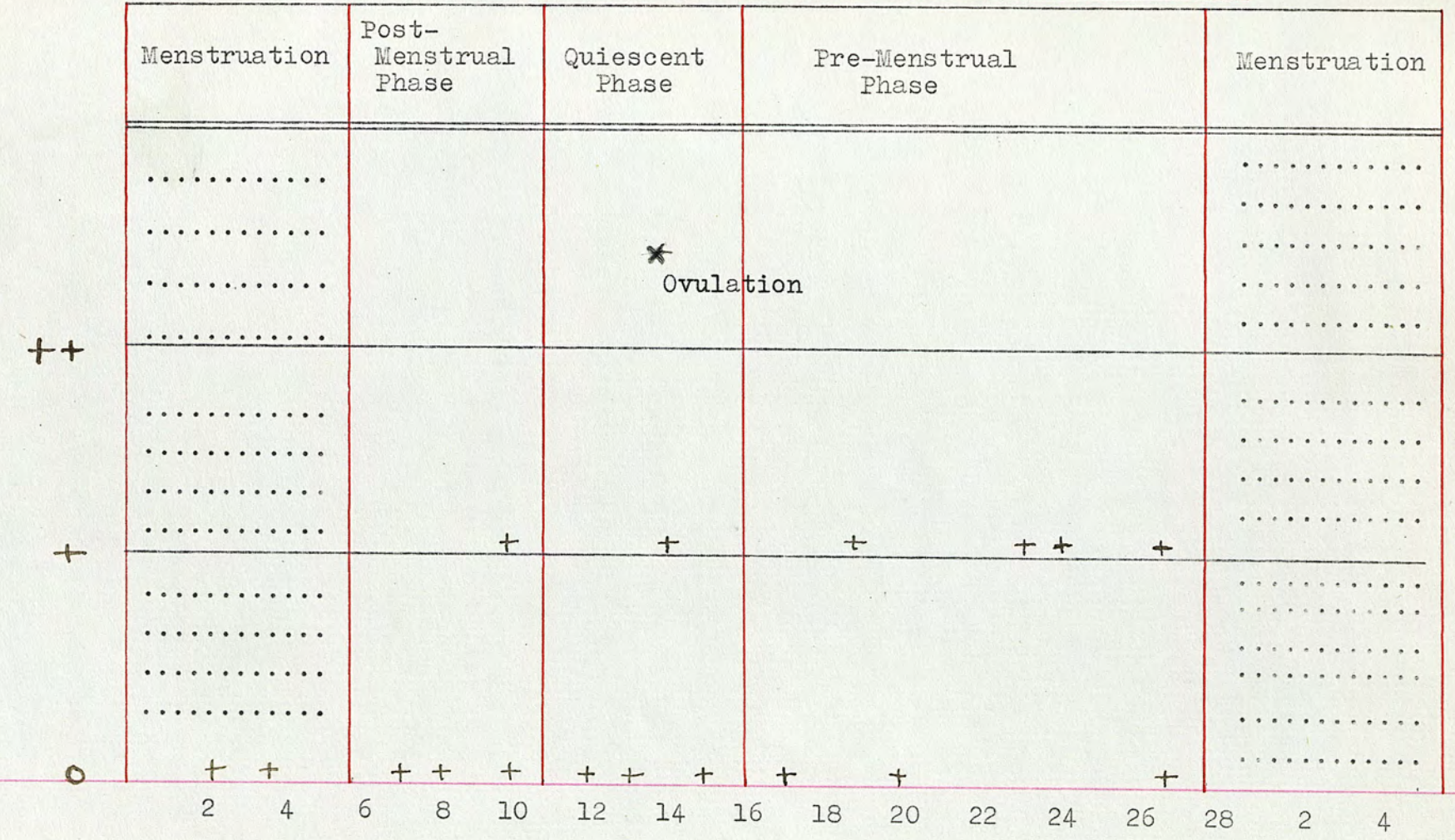
A glance at the Table representing the gonadotropic excretion values shows apparently very low excretion values of the gonadotropic hormone in the post-menstrual phase and in part of the quiescent stage of the menstrual cycle, and also that the values increase in amount in the premenstrual phase.

In all cases investigated no signs of follicular maturation or luteinization or blood spots were observed in the ovaries with the naked eye, and only evidence of follicular maturation was seen under the microscope, which agrees with the view held by Zondek,(56) that only the follicular maturation hormone is excreted in the urine during the menstrual cycle and he gives the following values for the various stages of the cycle:

Post-menstrual Phase - 8 rat units per day.
Quiescent Phase - 25 " " " "
Pre-menstrual Phase - 29 " " " "
Menstrual Phase - 25 " " " "

and/

TABLE REPRESENTING GONADOTROPIC HORMONE EXCRETION VALUES.



0 = no signs of follicular activity.
+ = slight " " " "
++ = definite " " " "

and these values are, according to Zondek, only the effect of the follicular maturation hormone of the pituitary - Prolan A.

Thus, with regard to the cyclic changes in the endometrium, one might state that the pituitary hormone output, in the early stages of the cycle, namely the post-menstrual and early quiescent periods, is relatively low, and that, thereafter, increases in amount until the onset of menstruation, and that throughout, it exerts in a consistent manner, an increasingly stimulative effect on the ovary, causing that organ in turn to secrete the hormones, which provoke the various cyclic changes of the endometrium, until, about the onset of menstruation when its activity temporarily diminishes, which then causes the abeyance of the hormone activity of the ovary and menstruation results.

C O N C L U S I O N.

To recapitulate, the at present accepted theory concerning the mechanism controlling the menstrual cycle is, that it is governed by the anterior lobe, which/

which probably produces two hormones: (1) Prolan A, which stimulates follicular maturation in the ovary, and the secretion of oestrone, which in turn causes the endometrium to develop to the post-menstrual phase, and (2) Prolan B, which causes the formation of the corpus luteum and the secretion of Progesterone, which advances the development of the endometrium to that of the pre-menstrual phase.

The pituitary then might be termed the conductor of the instruments of the menstrual orchestra, directing its harmony, but, from what score must the conductor beat the tempo? What music is to be played?

In plainer terms, is there a higher nervous sexual centre, which controls the hormone function of the pituitary?

Hohlweg and Junkmann[57] have suggested by their experiments on castrated rats, into which implants of hypophyseal tissue have been made, that the pituitary is controlled extrinsically by fine nerve connections to the brain via the pituitary stalk.

Theobald[58] has recently put forward an hypothesis from evidence of a clinical nature, which postulates that there are one or more centres in the brain, probably in the hypothalmic region, which regulate/

regulate the functions of the reproductive tract, through afferent and efferent neuro-hormonic stimuli. He considers the efferent route to be composed by:

(1) The hormones of the anterior lobe, and

(2) Nervous impulses to the generative organs;

and the afferent pathway to be composed of:

(1) The hormones of the ovary.

(2) Impulses transmitted from the external generative organ.

(3) Impulses transmitted from the higher special centres.

This hypothesis is rather novel and attractive, yet it requires the corroboration of more fundamental study.

It, however, causes a swing of the pendulum in the opposite direction, in focussing the attention of investigators of this subject, to a higher centre or centres, which may be the final link in the chain whereby the phenomena of menstruation, ovulation, pregnancy and parturition can be more adequately explained.

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