THE ASCHHEIM-ZONDEK REACTION, AND A CONSIDERATION OF THE ORIGINS AND FUNCTIONS OF THE HORMONES CONCERNED THEREIN

A Thesis

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by

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I N D E X

I N T R O D U C T I O N

The discovery of the Aschheim Zondek test for pregnancy resulted from a large amount of research work which had been performed in the investigation of the hormones concerned in the physiology of the female reproductive system. This work had been carried out by an almost countless number of investigators, in all parts of the world. I shall make no attempt to review the literature on this subject since it has already been done in the monographs by Parkes[130] and Frank[89], and, moreover, it would serve no useful purpose in a thesis of this kind. It is my intention merely to point out the chief events which led up to the foundation of the Aschheim Zondek Reaction as a test for pregnancy.

From time immemorial, it has been recognised that the ovaries are intimately concerned with the onset of puberty, and that their presence is essential for maintaining the integrity, and functional activity of the accessory sex organs. That this ovarian influence is by means of an internal secretion was first proved by Knauer[109], who shewed that the effects of bilateral oophorectomy could be counteracted by ovarian transplants.

All efforts to produce an active ovarian extract either failed, or the results were unconvincing, until 1912 when Adler[2] obtained signs of sexual activity in ovariectomised animals following the injection of watery extracts. From this date onwards, reports of the effects of active extracts were given by workers in all countries. Fellner[73], also in 1912, progressed so far as to be able to describe the preparation of a lipoid which was soluble in ether and alcohol, was thermostabile, and which produced oestrus when injected into ovariectomised animals. This crude product contained the active principle which we now know as oestrin, or folliculin. Herrmann[101] confirmed this work, and also shewed that an indentical hormone could be obtained from the human placenta.

However, all these investigators experienced difficulty in deciding when their test animals were 'on heat' and this decision often meant sacrificing the life of the animal. It was, therefore, an observation of great moment when Stockard and Papanicolaou[166], in 1917, found that the vaginal epithelium of the guinea pig shews cyclical variations corresponding in time to the cyclical changes in the uterus. The importance of this discovery cannot be over estimated, and practically all the work which has since been carried out in connection with ovarian, placental, and pituitary hormones, has been made easier and more exact, by the introduction of the 'vaginal smear technique' as a test

for oestrus.

Until 1923 there had been no means of standardizing these ovarian extracts, but in that year Allen and Doisy[6] devised a method. Subsequently Coward and Burn[49] proved this method to be inaccurate, and proceeded to modify, and improve it.

Before oestrin could be used as a therapeutic agent on human beings, it merely remained for it to be produced in a water-soluble form : this was accomplished by Laquer and his co-workers [114, 115]. Later Butenandt[39], and Doisy[59], working independently, both described processes whereby the hormone could be obtained in a pure crystalline state.

The occurrence of oestrin in the tissues, and body fluids of human beings and animals, has been the subject of numerous articles, but for our purpose we shall only point out that Fels[77], Aschheim and Zondek[14], and others, demonstrated that it could be found in large quantities in the blood and urine of pregnant women. Allen and Doisy[7] in 1927 reviewed the whole subject, expounded the physical and chemical properties of oestrin, and produced good evidence to shew that it was derived from the membrana granulosa cells, and was in fact the active principle of liquor folliculi.

Meanwhile other observers had worked on the hormone

of the corpus luteum. The earliest work was done between 1903 and 1913 by Fraenkel [84,85,86]. He demonstrated that the corpus luteum was essential for the implantation of the ovum, and for the continuance of pregnancy. Leo Loeb[117] was the first to shew that the corpus luteum so sensitized the endometrium that its response to any stimulus was a 'placentoma' formation. Gradually the corpus luteum came to be looked upon as the chief factor in the causation of menstruation : this view originally introduced by Hitschmann and Adler[107], was fully worked out by Corner[47] who experimented on the Macacus rhesus. It was in 1923 that Corner elaborated the theory of menstruation, which, although modified, is generally believed at the present day.

For many years it has been known that the ovary is intimately related to the other endocrine organs. Fröhlich[92] and Cushing[52] both drew attention to the effect of pituitary disease on the female genitalia. That experimental ablation of the anterior lobe of the pituitary leads to hypoplasia of the genitalia was shewn by Crowe, Cushing and Hormans[51] and also by Aschner[19]. Blair Bell[23] confirmed their findings, and Smith[159] demonstrated conclusively the dependence of the genitalia upon the integrity of the anterior hypophysis.

After failing to produce any effects by feeding animals on fresh, or dried, anterior pituitary lobe, Evans and Long [67,68] in 1924, published the results of injecting

immature female rats with alkaline hypophyseal extracts. The following changes occurred :

1. True gigantism
2. The uterus and fallopian tubes remained infantile
3. The ovaries shewed an inhibition of follicular ripening and ovulation, together with a luteinization of unruptured follicles
4. There was a delayed onset of puberty and inhibition of oestrus.

These experiments were repeated by Smith and Engle[160,161], but instead of using extracts, they treated the immature rats with daily transplants of anterior lobe. They found that these transplants, instead of delaying the onset of puberty, hastened it.

The changes occurring in the genitalia were :

1. Ovary (a) Increase in size and weight
   (b) Ripening of follicles and super-ovulation
   (c) Complete absence of atretic follicles
2. Uterus (a) Increase in weight and size
   (b) Increase in the thickness of the musculature
   (c) The lining epithelium became columnar, and the cavity was filled with secretion
3. Vagina (a) Patency was established
   (b) The mucosa became cornified.

These effects, they shewed, were only obtained if the injected animal had ovaries in situ : pituitary transplants into ovariectomised rats produced no changes in the uterus, and oestrus was not precipitated.

At the same time, but independently of these workers, Aschheim and Zondek[12] performed similar experiments. They obtained identical results while using the anterior hypophysis of male or female, human beings or animals : they also shewed that the pituitaries taken from immature, or senile, animals, produced exactly the same effects as those taken from mature animals. The ovarian responses which they obtained differed slightly from those of Smith and Engle, in so far that they described the production of corpora lutea atretica, as well as ripe follicles : again, some of the follicles became haemorrhagic and shewed up to the naked eye as brownish red spots on the ovary.

In view of the marked differences in results obtained by Smith and Engle, and Aschheim and Zondek, as compared with those obtained by Evans and Long, it was postulated that the anterior pituitary contained two hormones which acted on the ovary. Bellerby[26] was the first to suggest this possibility, and although it was discredited at first, it became more generally accepted when Evans and Simpson[69] shewed that the follicular ripening factor, and the luteinizing factor, had different solubilities. The presence of at least two ovary

stimulating hormones in the anterior hypophysis is now believed to be a fact : to these Aschheim and Zondek apply the names Prolan A and Prolan B, the former being the follicular ripening hormone, the latter being the luteinizing factor. Wiesner[174,175] refers to the same substances as rho 1, and rho 2, respectively.

As early as 1898, Comte[45] demonstrated gross enlargement of the anterior lobe of the pituitary during pregnancy. The definite histological changes which occurred were later described by Erdheim and Stumme[65] and Blair Bell[23]. In view of the hypertrophy of the hypophysis during pregnancy, it was reasonable to suppose that its functional activity was also increased at that time. Aschheim and Zondek[13], in 1928, demonstrated that this supposition was correct, and that the pituitary hormones were produced in such large quantities that they were easily demonstrable in the urine of pregnant women. The demonstration of these substances in the urine has thus become the Aschheim Zondek test for pregnancy. In the same year Siddall[155] shewed that these same hormones occurred in large amounts in the blood during pregnancy.

THE ASCHHEIM ZONDEK TEST

Principle

The Aschheim Zondek reaction as a test for pregnancy, depends for its principle on the demonstration of the presence or absence of Prolan A and Prolan B in the urine. This is done by injecting the urine into immature mice, and noting the effects produced on their genitalia.

It must be emphasized at the outset that although this reaction is usually referred to as a test for pregnancy, this is not strictly true, and it is my intention in this thesis to regard it as a test for the presence of living chorionic elements in the body.

Technique

(1) As described by Aschheim and Zondek[13]

In the original technique the animals used were immature female mice aged 21 to 28 days. Five mice, and one control, were used for each test, and for preference, these mice were all taken from the same litter. An early morning specimen of urine was obtained from the patient in question, and was injected subcutaneously into the animals ; each one received six injections within 48 hours. The mice were killed and examined 100 hours after the first injection. The amount of urine injected was varied in each of the five mice according to the following plan :

Animal 1	6 injections of	0.2 cc.	urine
" 2	"	0.25 cc.	"
" 3	"	0.3 cc.	"
" 4	"	0.3 cc.	"
" 5	"	0.4 cc.	"

(2) As employed in this series of experiments

With a view to simplifying the procedure, and making it a more practical test, the following technique was employed :

Animals. Immature female mice were used as test animals. Their ages varied from 21 to 28 days, and they weighed between 6 and 8 grams. The animals were obtained from dealers so that no reliance could be put on their exact age. To save expense, only three mice were used for each test : this did not affect the reliability of the test. The economy in animals had one disadvantage in as much that when a toxic urine was injected, there was less chance of at least one mouse surviving until the completion of the test. This occasionally meant that the test had to be repeated, but the ultimate saving in the number of animals was considerable. At first, a control mouse was used : after some experience of the test however, this procedure was abandoned as being unnecessary.

Dosage. If the dose is varied with each animal as recommended by Aschheim and Zondek, a very complicated

method of animal marking is required. I therefore gave each mouse 0.5 cc. of urine at each injection, and so no distinction between them was necessary. This modification produced no incorrect results.

Injections. It was found inconvenient to inject animals three times in one day, so in the majority of the tests, the mice were injected morning and evening only. Five injections were given, so each mouse received a total of 2.5 cc. of urine within 48 hours. In a few cases however, circumstances forbad rigid adherence even to these times of injection, and on occasions, the animals received only one dose in a day, while at other times two doses were given within three hours. In these tests, the animals always received their full quota of urine within 56 hours. Although these variations in no way interfered with the results obtained, yet it seems advisable that, whenever possible, the doses should be evenly spaced over a period of 48 hours.

All injections were made subcutaneously into the abdominal wall, a procedure which, with practice, can easily be performed without the help of an assistant.

Examination of the Animals.

The mice were killed between 96 and 108 hours after the first injection. In a few cases, when a diagnosis was required quickly, one of the animals was killed before this

time : this was never done until a vaginal smear showed the cells characteristic of oestrus. The vaginal orifice usually became patent about 72 hours after the commencement of the test, but it was rare to find ovarian changes so early. It is stated that if only one of the test animals shews the typical effects, the test should be reported as positive : this has been found to be true although in actual practice, if the reaction is positive, it is usual for all the mice to show the characteristic genital changes.

The uterus and ovaries were examined by the naked eye, and if haemorrhages were present in the ovaries, no further examination was required. In the absence of these haemorrhages, microscopic sections of the ovaries were cut, since it was found to be unwise to rely on a macroscopical diagnosis of the presence, or absence, of corpora lutea. Zondek[183] found microscopical examination necessary in 12 per cent. of cases : in 18 per cent. of our series of tests, it was impossible to arrive at a diagnosis on the naked eye appearances alone. This high figure is due to the fact that we examined many more urines which gave a negative reaction, than those which gave a positive reaction.

Urine

An early morning specimen of urine was obtained whenever possible : this has the advantage of containing

a higher concentration of the hormones. No preservative such as chloroform, or trieresol, was added, but in the intervals between the injections the urine was kept in an ice-chest at -4° C. Before each injection it was slightly warmed. At one period, in an attempt to make the test an even more simple procedure, the urine was left entirely untreated, and kept at room temperature. This method was abandoned since the urine usually became toxic and killed the mice.

Methods used for assessing the presence of the various hormones in the urine

Before stating that any urine contained any special hormone, it has been required to satisfy one or more of the conditions given under the heading of the hormone concerned.

1. Oestrin

   a. The production of the uterine and vaginal changes associated with oestrus, without there being any microscopical evidence of follicular ripening.

   b. The production of oestrus in a spayed mouse or rat.

   c. The fact that previous extraction of the urine with ether deprived it of its oestrus producing power.

2. Prolan A

a. The production of oestrus changes in association with ripening of the Graffian follicles in the mice ovaries. The ovarian changes were only accepted on microscopical evidence.

b. That no effects were obtained on spayed animals.

c. That previous extraction of the urine with ether did not deprive it of its ability to produce oestrus.

3. Prolan B

a. The production of corposa haemorrhagica in the ovaries, recognised macroscopically, or microscopically.

b. The production of corpora lutea atetrica as shewn in microscopic sections.

Quantitative Estimation of the Hormones.

Although many investigations have been carried out in connection with the standardization of these hormones, yet quantitative work is surrounded by difficulties. Most experiments in this direction have been performed with oestrin, and the difficulties encountered have been well illustrated

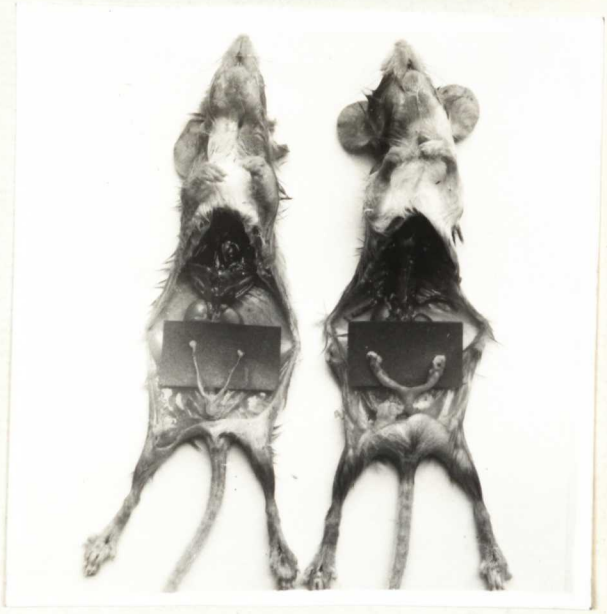
in a series of papers by Coward and Burn[49], Marrian and Parkes[123], and Allan, Dickens and Dodds[4]. The chief obstacle is the variation in individual response of different animals, to the same amount of hormone. Parkes[130] sums up the situation by saying "It would seem, therefore, that unless prohibitive numbers of animals are used..... the assay of oestrin cannot become exact with the present methods."

In view of this I made no effort to estimate the occurrence of hormones quantitatively. In one or two cases I attempted roughly to estimate the amount of oestrin present in the urine : this was done by injecting varying quantities of urine into spayed rats, or mice, and noting the least quantity which would produce cornification of the vagina. This method has been rightly criticized by Coward and Burn, and the results obtained are not put forward as being accurate.

Ovariectomy in Animals

In order to test for the presence of oestrin, it was necessary to use animals from whom both ovaries had been removed. This operation was performed on mice and rats under ether anaesthesia. In the mouse, a horizontal midline dorsal incision was used, while in rats it was found more convenient to use two vertical dorsal incisions, one on either side of the erector spinae muscles.

To make sure that all ovarian tissue had been removed,



Control Test Mouse

Fig.1. A positive Aschheim Zondek reaction shewing enlargement of the ovaries and corpora haemorrhagica, also hypertrophy and dilatation of the uterus.

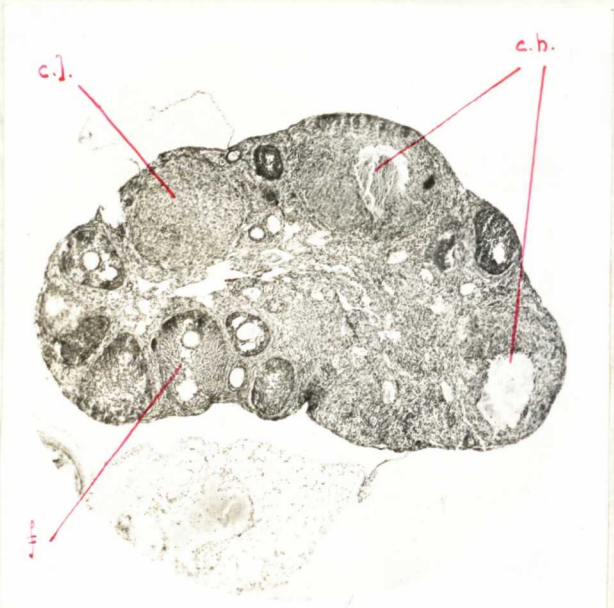
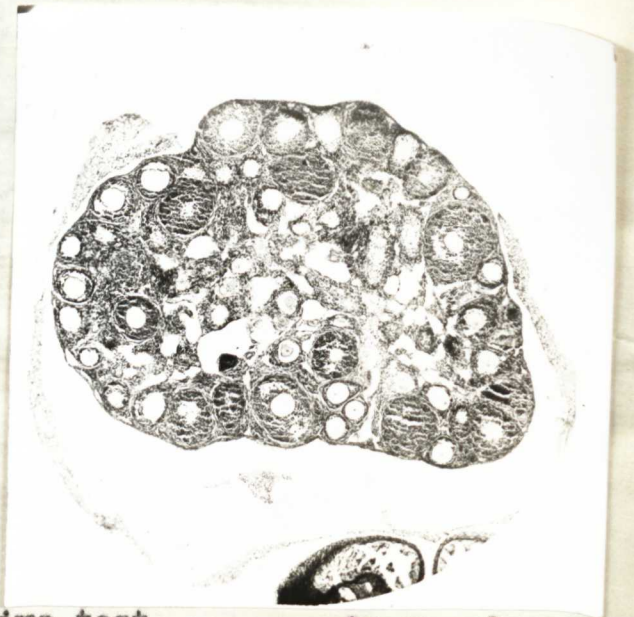


Fig.2 Mouse ovary from a positive test x 45



Ovary of normal immature mouse x 45

c.l. = corpus luteum atreticum
c.h. = corpus haemorrhagicum
f. = follicle in early stage of ripening.

and that none had regenerated, the animals were never used for an experiment unless their vaginal smears had been negative for oestrous on seven previous successive days.

The Changes in the Immature Mouse Genitalia produced by the injection of the urine of pregnant women, and their causation.

A. Ovarian Changes (see Fig.2)

1. Increase in size and weight (see Fig.1). This enlargement is noticeable to the naked eye. Microscopically this sign is valueless, since it is obvious that a section cut through the periffery of a large ovary may be smaller than a section cut through the centre of a small ovary. This enlargement is due to the presence of large ripe follicles, and of corpora lutea atretica.

2. Increase in vascularity. The dilated blood vessels can be seen coursing over the surface of the ovary. This change is also apparent in microscopical sections (Fig.4).

3. Ripening of Follicles. This is known as the Anterior Pituitary Reaction One (A.P.R.I) by Aschheim and Zondek[13]. The mature follicles can be seen, in section, as large follicles distended with liquor folliculi (Fig.3). Although a ripened follicle is easy to distinguish, it is much more difficult to recognise the early stages of ripening. It has been said that the first stage is

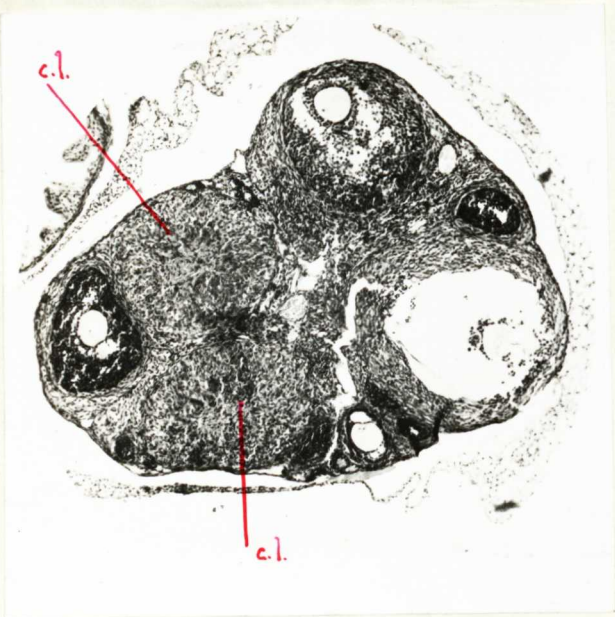


Fig.3. The ovary shews follicles in all stages of ripening, also two corpora lutea atetrica (c.l.) x 60.

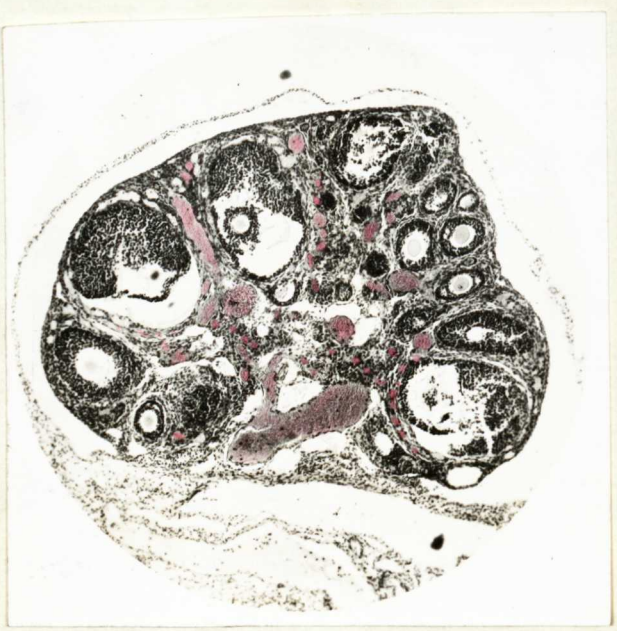


Fig.4. Shewing increased vascularity of the ovary, and follicles in the process of ripening. Blood vessels coloured pink. x 55.

represented by an increase in the number of layers of cells which comprise the membrana granulosa. This change, however, cannot be recognised with certainty, since the apparent number of layers varies according to the depth at which the section is cut through the follicle. For the signs of early ripening it seems best to rely on two features, namely :

(a) The increase in size of the follicle as shewn by distortion of the shape of a neighbouring follicle (Fig.4).

(b) The formation of liquor folliculi producing a space amongst the granulosa cells.

The cause of this follicular ripening is generally assumed to be Prolan A.

4. Luteinization (A.P.R.III).

By the term luteinization one means "the well known changes that sometimes occur in unruptured follicles as evidenced by enlargement of the granulosa and theca interna cells, by an increase in the thickness of the follicular wall and by the ramifications of the capillaries..... Imprisoned between the hyperplastic lutein cells is the degenerated ovum" (Kraul[112]).

These corpora lutea atretica can be seen by the naked eye as yellowish brown bodies, but it is unwise to rely on the macroscopical appearance in diagnosing their presence (see Case 85). Microscopically, these corpora

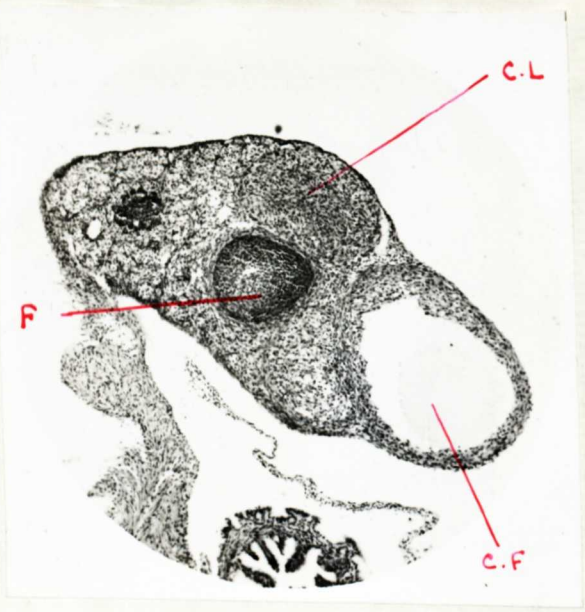


Fig.5 (a) Section shewing histological differences in appearance between granulosa cells and lutein cells. x 55

F = unripe follicle. C.L. = corpus luteum atreticum. C.F. = cystic follicle.

Fig.5 (b) High power view of part of the same section. The swollen, faintly staining, lutein cells are well seen. x 280.

lutea present the same features as those found in the human ovary, their cells staining more faintly than the granulosa cells, and having a swollen appearance (Fig.5). The degenerate ovum may, or may not, be seen, depending on the degree of luteinization, and on the level at which the section is cut. This ovarian change, according to present beliefs, is due to Prolan B, and corresponds to the effects produced by Evans and Long[68] with their alkaline extracts of anterior pituitary.

5. The Formation of Corpora Haemorrhagica

Haemorrhage into a follicle often occurs and gives rise to the appearance of reddish brown dots on the surface of the ovary (Fig.6). These were described by the originators of the test as "Blutpunkte". They can usually be seen by the naked eye, but occasionally, unless care is taken, one may mistake a sharp bend in a superficial blood vessel for one of these haemorrhages. If the haemorrhages are small, or are deeply situated, they are sometimes not seen unless a microscopic examination is made.

The factor which causes these haemorrhages is a matter of dispute. Kraul[112] advances the view that oestrin is responsible : his evidence is that a similar effect can be produced by injecting adult rabbits with oestrin. However, I have often injected urines, proved to contain oestrin, yet no Prolan A or Prolan B, and have

Fig.6. Photographic enlargement of the genitalia of a mouse shewing a positive reaction. The ovaries shew increase in size and corpora haemorrhagica : the uterus is enlarged.

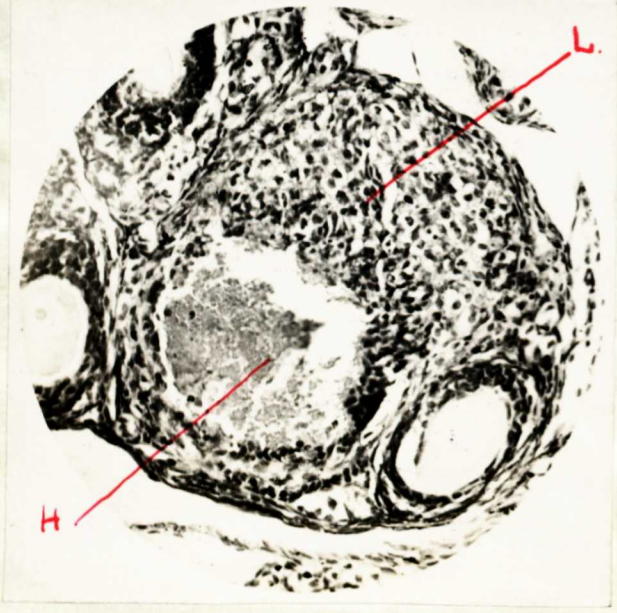


Fig.7. Shewing haemorrhage (H) in association with luteal cells (L) x 200

failed in every case to produce any changes in the ovaries. To prove this conclusively, a urine of a pregnant woman was taken and extracted repeatedly with ether until it failed to produce oestrus in ovariectomised mice. It was then injected into normal immature mice, and it produced haemorrhages in exactly the same way as before the removal of oestrin. This experiment was repeated with three different urines, and similar results were obtained.

I thought at first that Prolan A was probably the causal factor. It will be shewn later that Prolan A, unaccompanied by Prolan B, can be demonstrated in the urine in conditions other than pregnancy : in all of those cases in which it occurred, I failed to find a single ovarian haemorrhage.

There remains, finally, Prolan B, and many things point to this hormone as being the essential factor in the production of corpora haemorrhagica.

(i) No haemorrhages ever occurred except following injection of urines obtained from pregnant women.

(ii) Prolan B was never found in the urine except during pregnancy

(iii) The occurrence of haemorrhages, and the formation of corpora lutea, are the only two specific changes which are diagnostic of pregnancy. Prolan B is responsible for one of these, and therefore is probably intimately concerned in the production of the other.

(iv) Microscopical examination of the haemorrhages shews that luteinization is usually occurring in adjacent areas of the follicular wall (Fig.7).
Engle[63] has also pointed out this feature.

But Evans and Long[68], who obtained luteinization by injecting alkaline extracts of the pituitary, failed to produce these haemorrhages. Aschheim and Zondek[12] however, with their pituitary transplants, produced follicular ripening, luteinization and corpora haemorrhagica. It seems therefore, that the haemorrhages are most probably due to a combined action of Prolan A and Prolan B. Neither hormone can produce them in the absence of the other, and since they are only found together in the urine during pregnancy, it is understandable that the occurrence of corpora haemorrhagica should be diagnostic of that condition.

Fellner[76] has suggested that this phenomenon is due to a non-specific irritant impurity in the urine : this idea can hardly be tenable since it is obvious that they must be produced by some substance which is only present in the urine during pregnancy.

B. Uterine Changes

The thread like uterus of the immature animal becomes converted into the large thickened organ as seen in a mature animal at oestrus (Figs.6 & 8). The detailed

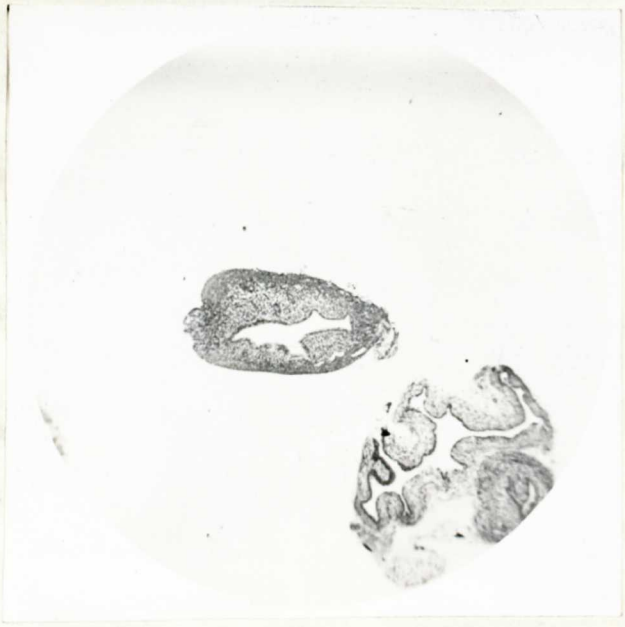


Fig.8 (a). Cross Section of an immature mouse uterus.
x 35.

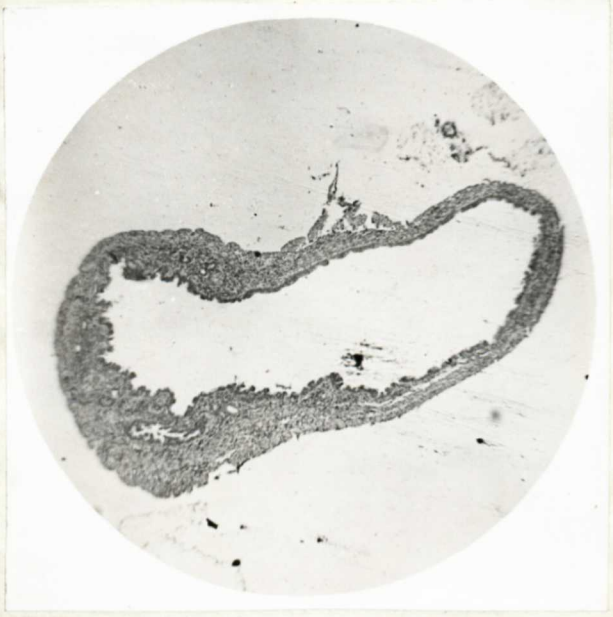


Fig.8 (b) An immature mouse uterus as seen after injections of pregnancy urine. There is hypertrophy of the walls, and distension of the lumen.
x 35.

changes are :-

(i) Increased Vascularity

This is recognized macroscopically by the turgid congested appearance : the dilated blood vessels can be seen standing out in marked contrast to the pale background of the uterine wall.

(ii) Hypertrophy of the Musculature

The muscle coats undergo hyperplasia and are definitely increased in thickness. This effect is probably due to the action of oestrin.

(iii) Endometrium

The endometrium is thickened, and the glands are increased in number and become active. The epithelial cells lining the uterine cavity change from the immature cubical to the high columnar type. This endometrial proliferation, as Allen[8] has pointed out is due in the first place to oestrin, but in its final stages, results from the superimposed action of the corpus luteum hormone. Clauberg[41] is of the opinion that oestrin alone is responsible for this change.

Decidual reaction has never been seen in microscopical sections : this is in accordance with the findings of Gander[93] .

(iv) Formation of Secretion

The secretion, which is colourless, and rather viscous, collects in the uterine cavity, and causes

a dilatation, thus producing more enlargement of the uterus than could be obtained by muscular hypertrophy alone. The secretory stage of the uterus is said by Mazer and Hoffmann[125] to be due to the corpus luteum hormone. However, I have shewn that this secretion is produced if urines containing only oestrin are injected, and in those tests in which the urine contained large amounts of this principle, it was noticed that excessive dilatation of the uterus by secretion was produced.

The oestrin producing these effects is derived from two sources :

(a) It is present in large quantities in the injected urine.

(b) It is produced by the ripening Graffian follicle of the mouse : this is secondary to the ovarian response to Prolan A.

The corpus luteum hormone has never been demonstrated in the urine during pregnancy so it must originate from the lutein tissue produced in the mouse ovaries by the action of Prolan B. That this abnormal lutein tissue is capable of function, can be shewn by the fact that a deciduoma reaction can be obtained in the uterus of a mouse treated with alkaline extracts of pituitary (Teel[168]).

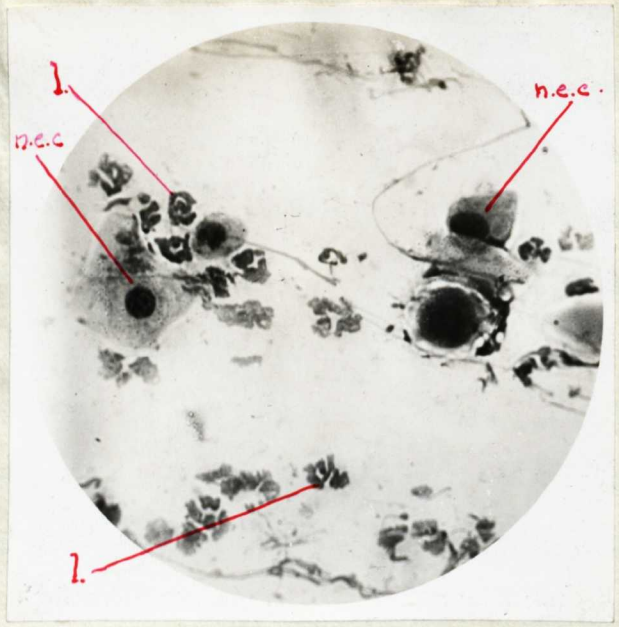


Fig.9. Vaginal smear during dioestrus. n.e.c. nucleated epithelial cell. l. leucocyte. x 400.

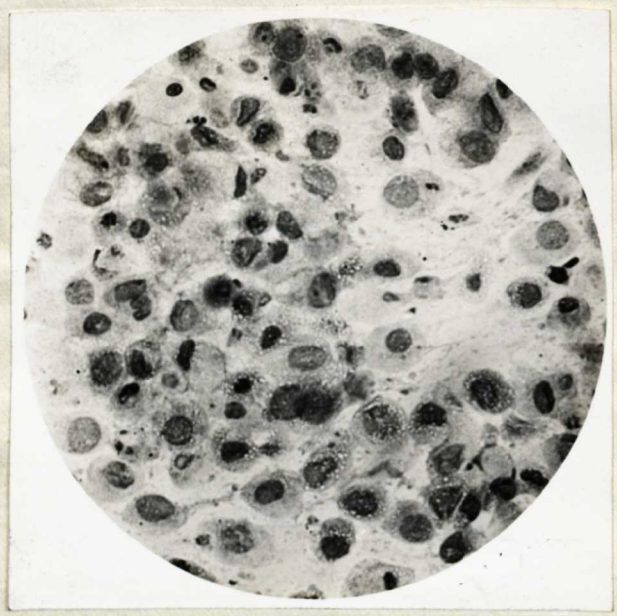


Fig.10. Vaginal smear during pro-oestrus. This consists almost entirely of small nucleated epithelial cells. x 300.

C. Vaginal Changes

The vagina in an immature mouse is represented by a solid cord of cells. This becomes canalized at puberty. After injections of urine the following responses are obtained :

(i) Premature canalization of the Vagina

The vagina is usually seen to be open about 72 hours after the first injection. When full oestrus is reached, the vaginal orifice is not only open, but appears unusually large, congested, and free from secretion.

(ii) Changes in the Vaginal Smear

The changes occurring are those characteristic of the onset of oestrus. The cyclical variation in the vaginal epithelium of a guinea pig was first described by Stockard and Papanicolaou [166]. In brief, the various stages in the cycle of the mouse are represented by the following appearances in the vaginal smear.

Dioestrus (Fig.9)

There is a moderate amount of vaginal secretion. The smear shows some large nucleated epithelial cells, and a considerable number of polymorphonuclear leucocytes. In addition there are usually a few non-nucleated squamous cells.

Pro-oestrus (Fig.10).

The vagina is filled with a mucous secretion.

Fig. 11. Vaginal smear during early oestrus shewing large epithelial cells with nuclei in various stages of degeneration. X 300.

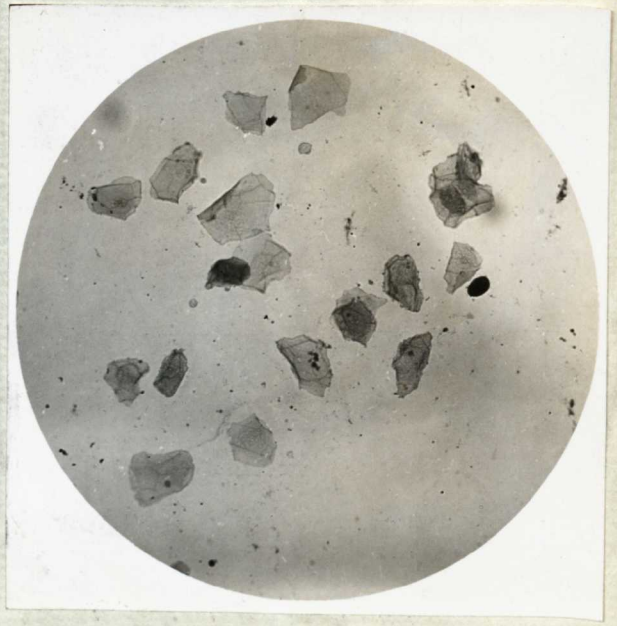


Fig. 12. Smear at oestrus. There are only large non-nucleated epithelial cells present. X 250.

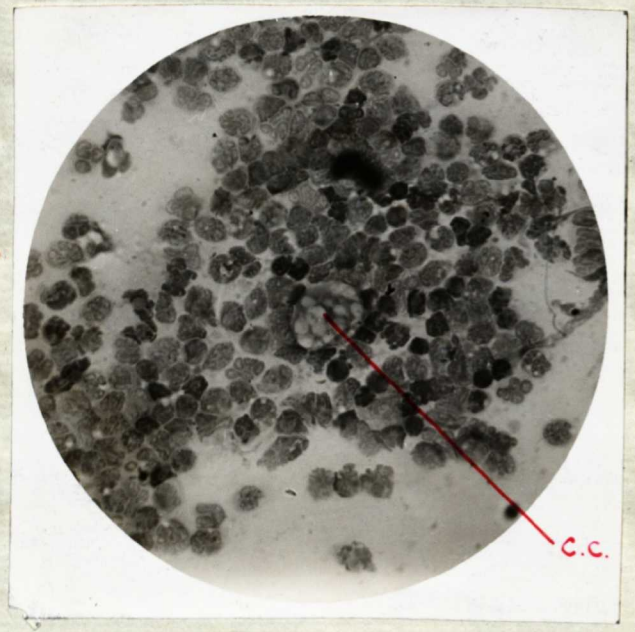


Fig. 13, Vaginal smear during metoestrus. The field shews a mass of leucocytes: in the centre is a degenerated cornified cell. (c.c.) X 350.

The leucocytes disappear from the smear, and nothing is seen except small nucleated epithelial cells. These occasionally shew mitotic figures. As oestrus comes on, the cells become larger and the nuclei gradually disappear (Fig.11).

Oestrus (Fig.12)

The smear shews very large non-nucleated squamous cells only : these stain pink with eosin, and cannot be mistaken. The vagina at this stage is abnormally dry, and the contents have the "cheesy" appearance described by Stockard and Papanicolaou.

Metoestrus (Fig.13)

At this stage, there is a leucocytic invasion, and a smear shews a mass of polymorphonuclear cells, while here and there, a non-nucleated squamous cell remains.

At times, an intermediate stage between oestrus and the leucocytic invasion, has been noticed ; on these occasions the vagina becomes filled with a mucous secretion, and a smear shows streaks of this material, but very few cells of any type. This appearance probably corresponds to that obtained in the third stage of oestrus by Stockard and Papanicolaou. The secretion, they say, is composed of the products of the destruction of the epithelial cells by the action of the leucocytes.

The terms used in denoting the stages of the oestrus cycle are those introduced by Heape[99].

All these vaginal changes are known definitely to be due to oestrin, this hormone, as pointed out above, being derived from an exogenous and endogenous source.

The Criteria of a Positive Diagnosis.

From what has been said previously, it is seen that the changes occurring in the mice genitalia, when pregnancy urine is injected, are due to at least three hormones - Prolan A, Prolan B and oestrin. All the effects described however, are not necessarily produced in every test, and it is essential to know on which specific response one should base a diagnosis of pregnancy.

Oestrin has been shown to be present in the urine of non pregnant women. Allen and Doisy[7] found it in the urine in amounts which varied according to the stage in the menstrual cycle. Frank and Goldberger[88] found that in some cases of amenorrhoea, oestrin was excreted in moderate amounts, and this fact led Mazer and Hoffmann[125] to suggest that the cause of the amenorrhoea in these patients was a lowered renal threshold for this hormone. I have confirmed these findings in two cases of secondary amenorrhoea (Nos.52 and 85), and oestrin was also present in the urine just before menstruation, in a patient who suffered from irregularity

of the menstrual cycle (No.67). In addition, Zondek[179] shewed that the hormone could be found in the urine of patients who had ovarian cysts producing menorrhagia.

In view of these findings, it is highly important to neglect all oestrin effects in arriving at a diagnosis on the question of pregnancy.

It will be shewn later that Prolan A occurs in the urine in conditions such as malignant disease, menopause and after bilateral oophorectomy. Follicular ripening is, therefore, valueless as an indication of a positive reaction.

Aschheim and Zondek insists that A.P.R.II or A.P.R.III must be obtained before a positive diagnosis is given. Allan and Dickens[3] also make this statement. Experience in the test only makes one desire to emphasize this fact more strongly, and as an illustration of this basis principle I quote three cases :

1. Case 85 (see page 39) Here an error in diagnosis was made because a decision was reached before microscopical examination revealed complete absence of luteinization in the ovaries.
2. Case 81 (see page 41) The details of this test were as follows :- Three mice were used ; one died 72 hours after the first injection and shewed no genital changes. The remaining two were killed at the appointed time and examination revealed :-

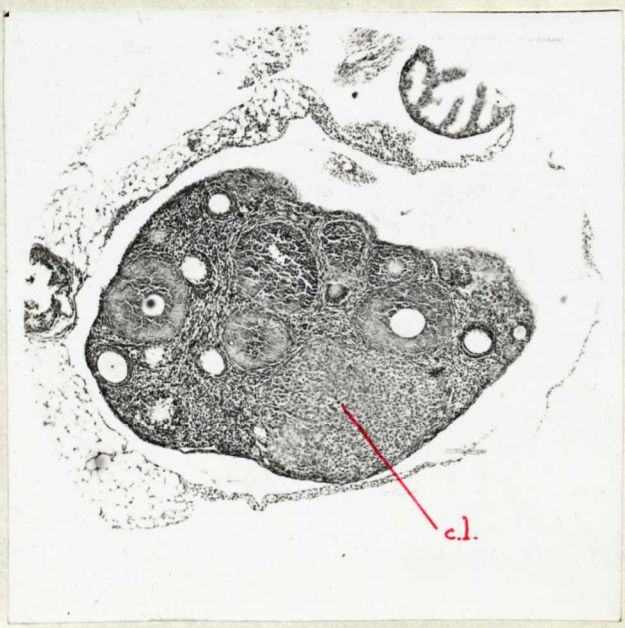


Fig.14. Mouse ovary from case No.81. Here a positive diagnosis was made on the appearance of a single corpus luteum (c.l.) The remainder of the ovary is typically immature. x 60.

Animal 1. Vagina closed. Doubtful uterine enlargement. No ovarian change.

Animal 2. Vagina just open, but smear negative for oestrus. Uterus slightly enlarged. Ovaries doubtfully enlarged, but one corpus luteum in the right one.
Microscopical examination of the ovaries shewed no increased vascularity, and no evidence of follicular ripening. Both ovaries contained one corpus luteum (see Fig.14).

In spite of complete absence of signs of oestrous in the animal, the test was reported positive, and after events shewed it to be correct. The case was one of a tubal mole which, no doubt, had still some living villi present.

3. The macroscopical appearances obtained in this test are shewn in Fig 21. Gross uterine and ovarian enlargements are seen, the vaginal smear contained cornified cells only. There were no corpora haemorrhagica, and the ovaries were sectioned. The microscopical picture (Fig.22) shews follicular ripening, but complete absence of luteinization. This, therefore, was a negative reaction : the urine was from a patient who had had both ovaries removed.

R E S U L T S

In the performance of tests for pregnancy, 105 urines were examined. These urines were, for the most part, received from patients in whom a clinical diagnosis of the presence or absence of pregnancy was uncertain. A few, however, were obtained from patients known to be pregnant: these were tested in the course of an investigation of the effects produced by variations in technique. An endeavour was made, in every possible case, to find out the main symptoms and physical signs before performing the test.

With these 105 specimens of urine, 148 tests were carried out. All the patients have been subsequently traced, and their final diagnoses ascertained.

The results are given in detail below. The notes on the after histories of the patients, or the operative findings, are given under the column headed 'remarks'. In those cases in which the medical attendant of the patient concerned has merely reported that the diagnosis had been correct, without giving the details of the after events, I have inserted 'diagnosis confirmed'.

RESULTS

No.	Clinical History	Result of Test	Remarks
1.	None	Positive.	Ectopic pregnancy at operation.
2.	12 weeks amenorrhoea. Mass in Pouch of Douglas.	Negative.	Patient died from T.B. peritonitis.
3.	Abdominal Tumour.	Negative.	Broad Ligament Fibroid.
4.	Enlarged Uterus ? fibroid ? pregnancy.	Negative.	No difference in physical signs on examination 3 months later.
5.	Period 3 days overdue. ? Ectopic.	Negative.	Recommenced menstruation 3 weeks later.
6.	None.	Positive.	Retroverted Gravid Uterus.
7.	22 weeks uterus ? dead foetus pre-eclampsia.	Positive. Excess oestrin.	Macerated foetus passed 4 days later.
8.	Age 46. Uterine Tumour.	Positive.	Diagnosis confirmed.
9.	Amenorrhoea. 6 weeks.	Positive.	Diagnosis confirmed.
10.	? Incomplete abortion.	Negative.	All symptoms disappeared menstruation became normal.
11.	12 weeks pregnancy. Hyperemesis.	Positive.	
12.	Early pregnancy. Bleeding p.v.	Positive. Excess oestrin.	Abortion occurred one week later.
13.	Age 44. 20 yrs. since last child. Fibroids with amenorrhoea.	Positive.	Confirmed.
14.	20 weeks uterus ? dead foetus.	Negative. Test 1 Negative. Test 2.	Uterus emptied of macerated foetus.

No.	Clinical History	Result of Test	Remarks
15.	Age 43. Amenorrhoea.	Negative. Prolan A present.	Diagnosed as onset of menopause.
16.	Sterility for 12 years. Amenorrhoea for 12 weeks.	Positive.	Diagnosis confirmed.
17.	Amenorrhoea followed by bleeding.	Negative.	No clinical signs of pregnancy supervened.
18.	16 weeks amenorrhoea. Morning vomiting and frequency.	Negative.	Uterus normal in size.
19.	Sterility for 8 years. Uterus 23 weeks but containing fibroids.	Positive.	Diagnosis confirmed.
20.	Pregnancy. ? death of foetus.	Positive. Oestrin in excess.	Normal pregnancy progressing.
21.	32 weeks uterus. ? death of foetus.	Positive. Oestrin in excess.	Macerated twins passed 6 days later
22.	Pregnancy. ? death of foetus.	Positive. Oestrin in excess.	Macerated foetus passed subsequently
23.	12 weeks amenorrhoea.	Negative.	Clinical diagnosis of Menopause.
24.	? Incomplete abortion.	Negative.	No clinical evidence of pregnancy.
25.	Abortion 12 days previously. placenta retained.	Positive. Oestrin in excess.	
26.	Ruptured Ectopic Pregnancy. Urine received 24 hours after Operation.	Positive.	Section shewed active villi.
27.	Amenorrhoea 6 weeks, followed by 3 weeks bleeding.	Positive.	Aborted before completion of test.
28.	Tubal mole. Urine received 24 hours after operation.	Negative.	No active villi seen in sections

No.	Clinical History.	Result of Test.	Remarks.
29.	26 weeks pregnancy. Pre-eclampsia. ? foetal death.	Positive. No excess of oestrin.	Foetal Heart heard subsequent-ly.
30.	Amenorrhoea 10 weeks. Bleeding p.v. Uterus 17-18 weeks.	Strongly positive.	Hydatidiform mole evacuated 2 days later.
30.	Test repeated with same urine.	Strongly positive.	
31.	12 days after evacuation of Hydatidiform mole.	Positive.	Hysterectomy showed portion of mole still present.
32.	Enlarged uterus found at Laparotomy.	Positive.	Diagnosis confirmed.
33.	8 weeks amenorrhoea followed by 3 weeks bleeding.	Negative.	At operation follicular cysts of ovaries found but no evidence of pregnancy.
34.	Amenorrhoea 17 weeks. Uterus 12 weeks. Bleeding vaginally.	Test 1. Negative. Test 2. Negative. Test 3. Negative. Oestrin present.	missed abortion evacuated subsequently.
35.	Tubal Mole. Urine received 48 hours after operation.	Test 1. Positive. Test 2. Positive. Oestrin present in excess.	No sections of mole were examined.
36.	Uterus found enlarged at Laparotomy.	Positive.	Abortion 6 weeks later.
37.	Amenorrhoea 6 weeks followed by 4 weeks bleeding and abdominal pain.	Test 1. Negative. Test 2. Negative. Oestrin present in small amounts.	At operation Tubal Mole was found.
38.	Age 44. Uterus enlarged. Bilateral appendage swellings.	Positive.	Operation. Pregnancy with ovarian fibromata.

No.	Clinical History.	Result of test.	Remarks.
39.	18 weeks pregnancy. Both ovaries removed.	Test 1. Positive.	
40.	25 weeks pregnancy. Both ovaries removed.	Test 1. Positive. Oestrin in excess.	
41.	32 weeks pregnancy. Both ovaries removed at operation.	Positive.	
42.	18 weeks pregnancy. Bleeding. ? hydatidiform mole.	Positive. No excess of oestrin.	Bleeding stopped. F.H.S. heard.
43.	Urine received 24 hours after evacuation of Hydatidiform Mole.	Test 1. Mice died. Test 2. " Test 3. Strongly positive.	
44.	Uterus 12 weeks. ? missed abortion.	Test 1. Mice died. Test 2. Negative. Oestrin present.	missed abortion evacuated later.
45.	Uterus 12 weeks. ? missed abortion.	Test 1. Mice died. Test 2. Negative. Oestrin present.	missed abortion evacuated later.
46.	Uterus 18 weeks with abdominal tumour.	Test 1. Mice died. Test 2. Positive.	At operation: pregnancy with ovarian cystadenoma.
47.	Age 50. Amenorrhoea. Uterus enlarged.	Negative. Prolan A present.	Fibroid Uterus. Menopause.
48.	Age 51. Uterus 28 weeks.	Test 1. Negative. Test 2. Negative.	Fibromyoma Uteri.
49.	Midline abdom: swelling.	Negative.	Dermoid cyst of ovary.
50.	Amenorrhoea 6 months. Uterus 10 weeks in size and retroverted.	Negative.	Carneous mole evacuated.
51.	12 weeks pregnancy.	Test 1. Positive. " 2. Positive. " 3. Positive. " 4. Positive. " 5. Positive.	

No.	Clinical History.	Result of Test.	Remarks.
52.	7 months amenorrhoea. ? missed abortion.	Negative. Small amount of oestrin.	Curettings shewed no evidence of pregnancy.
53.	Age 48. Amenorrhoea followed by bleeding.	Negative.	Diagnosed as menopausal haemorrhage.
54.	Age 42. Bleeding following 8 weeks amenorrhoea.	Negative.	Bilateral cystic ovaries.
55.	12 weeks amenorrhoea separated from husband.	Positive.	F.H.S. heard subsequently.
56.	20 weeks pregnancy. Bleeding.	Test 1. Mice died. " 2. Positive. Oestrin not in excess.	Menstruated regularly up to 32nd. week of pregnancy.
57.	Pelvic abscess. ? following attempts to produce abortion.	Test 1. Mice died. " 2. Negative.	No clinical evidence of pregnancy.
58.	Uterus 12 weeks. ? carneous mole.	Test 1. Negative. " 2. Negative. " 3. Negative. Excess Oestrin and Prolan A present.	Carneous mole evacuated. Section shewed living villi.
59.	4 weeks after removing mole to exclude chorion epithelioma.	Negative.	No clinical evidence of chorion epithelioma.
60.	8 weeks after removing mole.	Negative.	"
61.	7 weeks amenorrhoea.	Negative.	Menstruation recommenced.
62.	Abdominal Tumour.	Negative.	Unilocular Ovarian Cyst.
63.	40 weeks pregnancy. In labour.	Mice died.	
64.	Tubal Mole. Urine received immediately after operation.	Negative.	No active villi found in section.

No.	Clinical History.	Result of Test.	Remarks.
65.	Bleeding p.v.	Negative.	Pelvic Infection found at Operation.
66.	5 weeks amenorrhoea followed by bleeding and abd. pain.	Negative.	Tubal Mole found at subsequent operation.
67.	6 weeks amenorrhoea.	Test 1. Negative. Test 2. Negative.	Menstruation recommenced later.
68.	24 weeks pregnancy. ? foetal death.	Mice died.	F.H.S. heard before the test was repeated.
69.	Large abdominal tumour.	Test 1. Mice died. Test 2. Negative.	Ovarian Cyst at operation.
70.	6 weeks amenorrhoea.	Test 1. Mice died. " 2. Mice died. " 3. Mice died.	
71.	Abdominal Tumour.	Negative.	Fibromyomata Uteri.
72.	One missed period followed by bleeding.	Negative.	Incomplete abortion at operation.
73.	No amenorrhoea. Secretion in breasts. Tubal swelling.	Negative.	Left Tubal Mole.
74.	16 weeks amenorrhoea but bleeding vaginally.	Test 1. Mice died. " 2. Positive. " 3. Mice died.	Reported with normal 36 weeks pregnancy.
75.	40 weeks pregnancy.	Positive.	
76.	40 weeks pregnancy. In 1st. stage of labour.	Positive. Oestrin in excess.	
77.	10 weeks amenorrhoea. Uterus enlarged. Appendage swelling.	Test 1. Positive. " 2. Positive.	Five months later reported with normal pregnancy.

No.	Clinical History.	Result of Test.	Remarks.
78.	36 weeks amenorrhoea. 10 weeks uterus.	Mice died.	Missed abortion evacuated.
79.	4 weeks amenorrhoea. 16 weeks uterus. ? Hydatidiform Mole.	Test 1. Positive. " 2. Positive. " 3. Positive. " 4. Positive. No evidence of Hydatidiform mole.	Reported subsequently as Normal pregnancy.
80.	6 weeks amenorrhoea. Uterus bulky. Appendage swelling.	Mice died. Reported Negative.	Aborted 1 week later.
81.	Appendage swelling, and bleeding p.v.	Positive.	Tubal Mole. No section cut.
82.	Midline abdominal tumour.	Negative.	Carcinoma of ovary.
83.	8 weeks amenorrhoea followed by bleeding. Appendage swelling.	Negative.	Cystic ovary.
84.	Normal 18 weeks pregnancy.	Test 1. Positive. " 2. Positive. " 3. Positive. " 4. Positive. " 5. Positive. " 6. Positive. " 7. Positive. " 8. Positive.	
85.	8 weeks amenorrhoea.	Reported Positive.	Not pregnant. Menstruation recommenced after 5 months.
86.	Normal 20 weeks pregnancy.	Test 1. Positive. " 2. Positive. " 3. Positive. " 4. Positive.	
87.	Age 43. Uterus 26 weeks. No growth in 12 weeks. ? Pregnant. ? Foetal death.	Positive. No excess of oestrin.	Macerated Foetus passed two weeks later.
88.	Amenorrhoea followed by bleeding.	Negative.	Follicular cysts of ovaries.

No.	Clinical History.	Result of Test.	Remarks.
89.	8 weeks amenorrhoea with repeated losses vaginally.	Test 1. Positive. " 2. Positive. No excess of oestrin.	Abortion 2 weeks later but interference was suspected.
90.	Fibroid uterus. ? Pregnant too.	Positive.	Definite signs of pregnancy 4 weeks later.
91.	Two abdominal swellings.	Positive.Test 1. Positive.Test 2.	18 weeks pregnancy with a parovarian cyst.
92.	Urine received 72 hours after passing hydatidiform mole.	Test 1. Mice died. " 2. Positive.	
93.	10 weeks amenorrhoea followed by bleeding.	Mice died.	Patient aborted before the test could be repeated
94.	Bleeding for 6 months after confinemeamt. ? Placental Polyp.	Negative.	No villi found in curettings.
95.	10 weeks amenorrhoea.	Positive.	Diagnosis confirmed.
96.	8 weeks amenorrhoea. Menstruation always irregular.	Positive.	Diagnosis confirmed.
97.	? Ectopic Pregnancy.	Negative.	Tubal Mole at Operation.
98.	6 weeks amenorrhoea.	Positive.	Diagnosis confirmed.
99.	9 weeks amenorrhoea. Retroverted uterus and no physical signs of pregnancy.	Positive.	Confirmed subsequently.
100.	Vomiting. Uterus retroverted and fixed.	Positive.	Confirmed subsequently.

No.	Clinical History.	Result of Test.	Remarks.
101.	7 weeks amenorrhoea.	Positive.	Physical signs of pregnancy at subsequent examination.
102.	Age 32. Irregular menstruation. 12 weeks amenorrhoea.	Negative.	Patient recommenced to menstruate.
103.	22 weeks pregnancy with pyelitis.	Positive.	
104.	Definite 16 weeks pregnancy.	Positive.	
105.	Metrostaxis 9 months after passing a hydatidiform mole. ? chorion epithelioma.	Positive. Negative in dilutions more than 1 in 3.	Hysterectomy revealed the presence of a submucous polyp. Microscopically this shewed hydatidiform mole but no definite evidence of chorion epithelioma.

It will thus be seen that a summary of these results shows :

Total No. of Tests	Positive finding	Negative Finding	No result
148	77	53	18

The fact that in 18 tests no result was obtained because the mice died, needs explanation. This, as well as the high mortality occurring in later experiments, was due partly to the fact that at one time no precautions were taken for the preservation of the urine, and partly because only three mice were used for each test. Although no result was obtained in 18 tests, yet out of the 105 urines examined there were only five in which no diagnosis was returned. Of these, there were four on which only one test was performed, a repeat being made unnecessary by subsequent events. In one case only (No.70), did I fail completely, in spite of all modifications, to obtain a result.

In addition to the above series of tests carried out in the diagnosis of pregnancy, a large number of urines were examined with a view to determining whether any of the hormones concerned were excreted in the presence of other conditions. 75 tests on urines from women patients were performed : 60 gave a negative reaction and in 15 the mice died. 27 tests were carried out on urines from male

patients : 23 gave a negative test for pregnancy, and in 4 the animals died. The tabulated results, according to the clinical diagnoses of the patients, are given below.

In stating that all these urines gave a negative pregnancy test, I do not infer that there was always a complete absence of hormones. Prolan A was found in many, but in none was any evidence of Prolan B, the specific hormone for pregnancy, found.

Disease	MALES		FEMALES	
	Negative Result	No result	Negative Result	No result
Malignant Disease of Genitalia	3	1	17	7
Malignant Disease of other organs	9	2	7	5
After Bilateral oophorectomy			22	1
Addison's disease	1			
Diabetes Insipidus	1			
Exophthalmic Goitre	1		2	
Parenchymatous Goitre	1			
After Partial Thyroidectomy	6	1	7	1
Menopause			3	1
Acromegaly	1			
Fibromyomata Uteri			1	
Follicular Cysts of Ovaries			1	
Total	23	4	60	15

Thus taking the two series together, the results obtained were :-

Number of Tests	Positive Reaction	Negative Reaction	No Result
250	77	136	37

An incorrect diagnosis of pregnancy was given in two instances, namely Cases No.80 and No.85. The negative reactions reported in some cases of carneous mole, dead foetus, and tubal mole, are not regarded as fallacies, since it has already been stated that the test is one for living chorionic villi, and not one for pregnancy. Since in 213 tests a result was obtained, the percentage error was 0.94 per cent. This figure is very low, and is in agreement with the statement of Ettinger and his co-workers[66] who, in April 1931, collected results of 4,000 tests reported by various workers, and found that the percentage errors were less than 2.0 per cent.

The following are the details of the two cases in which an error was made :

Case No.80. E.M.J. Age 31. Vomiting and vaginal bleeding. Cervix firm. Uterus size of 6 weeks pregnancy. R. appendage swelling ? cystic ovary.

Result of Tests. The urine was toxic, and no mouse received more than four injections. The last two mice died 72 hours after the first injection.

Findings. Doubtful uterine enlargement.
Ovaries (microscopically) shewed early follicular ripening but no definite luteinization.

There was a shortage of mice at the time, otherwise the test would have been repeated.

After History. Patient aborted one week later.

Case No.85. B.C. In hospital for treatment of rheumatoid arthritis.
Amenorrhoea for 8 weeks. No symptoms of pregnancy.

Results of Test. 4 mice received 5 injections.
2 died, and 2 were killed at 96 hours after the first injection.
The vaginae were closed, but the uteri were definitely enlarged. The ovaries appeared to be enlarged and naked eye appearances suggested the presence of corpora lutea.
There were no haemorrhages.

Since a result was required urgently, a positive diagnosis was given. Subsequently the ovaries were sectioned, and, although there was follicular ripening, no corpora lutea (the only diagnostic sign) were seen.

After History. Patient commenced to menstruate after 5 months amenorrhoea.

In the first case, no opinion should have been given without a repeat test, while the second case merely illustrates the danger of attempting to be certain of the presence of corpora lutea in the ovaries without a microscopical examination. In both instances, therefore, the fault lay in the technique which was used. This admission merely emphasizes the reliability of the Aschheim Zondek reaction as a test for the presence of chorionic elements. As further evidence on this point, I quote six cases in detail.

Case No.48 M.McS. Aet.51.

Clinical Features. Complaining of an abdominal swelling. Amenorrhoea for 28 weeks, accompanied by menopausal symptoms.

Physical Signs . Smooth, regular, midline swelling size of a 28 weeks pregnancy, arising out of the pelvis.

The patient was diagnosed as an ovarian cyst, and was operated on. At the operation, the uterus was found to be regularly enlarged, smooth, and cystic, with areas of hardness resembling foetal parts. Since the uterine enlargement corresponded exactly with the period of amenorrhoea, the abdomen was closed without interference. As a matter of interest an Aschheim Zondek test was performed and a negative result was obtained. At this, an X.ray photograph was taken, but failed to show foetal parts. The urinary test was repeated, and once more was negative.

The abdomen was then opened for the second time, and a uterus, full of degenerating fibromyomata, was removed.

Cases No. 81 and 82.

The urines from both these cases were handed in at the same time. The first patient was said to have an appendage swelling which was probably due to pelvic infection, the second was said to have a midline abdominal tumour which was probably a pregnancy. I tested both urines and reported that the first gave a positive reaction, while the second was negative.

After Histories. Both patients were operated on, and the first had not a pelvic infection, but an ectopic pregnancy, while the second had a carcinoma of the ovary, but no evidence of pregnancy.

Case No.14. Mrs.G.

Clinical Features. The patient was definitely pregnant, but the clinician suspected foetal death.

Results of Test. Test 1. Negative

Test 2. Negative

A report was given saying that, providing a diagnosis of pregnancy was certain, then not only the foetus, but the placenta also, was dead.

After History. Bougie induction resulted in the passage of a very much macerated foetus.

Cases No.30 and 31. Mrs.H.

Clinical Features. Amenorrhoea for 10 weeks. Bleeding vaginally. Uterus 17-18 weeks in size.

Test (No.30). Strongly positive reaction.

After History. The uterus was evacuated of a Hydatidiform mole. It was feared that all the mole had not been removed, so that, 12 days later, a further specimen of urine was examined.

Test (No.31). Again a positive reaction was obtained.

Further History. Hysterectomy was performed, and examination of the specimen revealed that portions of the mole still remained attached to the uterine wall.

The Occurrence of the Hormones in the Urine at Different Stages of Pregnancy

(i) At the commencement

Observers differ on the question as to how early in pregnancy a positive reaction can be obtained. Allan and Dickens[3] reported a case in which a positive diagnosis of pregnancy was made 9 days after the woman had missed a period. Aschheim[16], and Zondek[183] found that the urine from women in whom a period was 4 to 5 days overdue, contained all three hormones, whereas Magrath and Randall[119] obtained positive tests between 18 and 21 days after coitus. Recently Eberson and Silverberg[60] claimed to have detected microscopical changes in the mice ovaries, when the animals were injected with urine from women who had

had normal menstrual periods 12 to 17 days previously. Presuming that fertilization occurred about the tenth day after menstruation, this would mean that the embryo was only 2 to 7 days old at the time. It seems unlikely that a positive result should be obtained so early, and these workers admit that the only changes produced in the mice were microscopic evidences of early follicular activity. However, the same observers obtained a typical reaction when menstruation was only one day overdue.

Stewart[164] found that the urine collected 5 days before a missed period, produced oestrus in the mouse, but no definite ovarian changes : follicular haemorrhages were produced five days after a missed period. He points out that there is a gradual transition, and the amount of hormones present in the urine, increases gradually.

In the present series of experiments, no urines were obtained from patients who had not missed at least one period. Some of the earliest pregnancies examined were those of ectopic pregnancies in which the condition had progressed for at least three weeks. It is, for obvious reasons, difficult to obtain a specimen of urine from a pregnant woman, prior to her missing a period.

It is, therefore, impossible to give the exact period of pregnancy at which hormones appear in the urine since :

(a) Their appearance is a gradual process

(b) The exact date of conception in never known

(c) There are probably individual variations

The bulk of evidence shows, however, that the reaction is definitely positive, just about, or immediately after, the time that the next period is due. Thus, for all practical purposes, the test is of value as soon as pregnancy is ever suspected clinically.

(ii) <u>During the course of the Pregnancy</u>

From the first month to the termination of pregnancy the urine rarely, if ever, fails to contain Prolan A, Prolan B and Oestrin. However, Wiesner[173] has recently recorded a case in which the hormones did not appear in the urine until the seventh month of pregnancy. Aschheim and Zondek[13] have shewn that during the early stages, while the placenta is most active, there is a relative excess of Prolan A and Prolan B over oestrin, while at term there is more oestrin than pituitary hormones present. It is interesting to note that Fanz and Gault[71] have pointed out that the diminution in the amount of pituitary hormones in the latter half of pregnancy, corresponds in time to the gradual disappearance of Langhan's cells of the villi. Again, they say that the syncytium only becomes well established by the third month, but

continues its development throughout pregnancy : this increasing activity coincides exactly with the oestrin curve.

(iii) <u>During Parturition</u>

I tested the urine from patients in the first stage of labour. Corpora haemorrhagica were rarely seen, but a positive result was always obtained. However, the effects due to oestrin were more noticeable in the mice, than those due to the pituitary hormones.

(iv) <u>In the Puerperium or After Abortion</u>

Every observer agrees that the hormones rapidly disappear from the urine as soon as the pregnancy is terminated. Aschheim and Zondek[13] stated that both oestrin and the pituitary hormones, gradually decrease in amount, and finally disappear about the eighth day of the puerperium. Stewart[164], however, reported that at 34 hours after the termination of pregnancy, only oestrin remains, while no hormones at all are present in the urine 10 hours later. Magrath and Randall[119] found that the urine gave a negative reaction 4 days after parturition. In our series, urine was received from two cases of ectopic pregnancy, 24 hours and 48 hours after salpingectomy had been performed : a positive reaction resulted in both instances. It has also been found that in all cases after labour, oestrin tends to

persist in the urine for a much longer period than Prolan A and Prolan B.

One case was investigated in order to determine the exact end point as nearly as possible. This patient had an abdominal hysterectomy and evacuation of the uterus performed at the 16th week of pregnancy. It was, therefore, certain that all the contents of the uterus had been removed, and the exact hour of the operation was known. Eight-hourly specimens of urine were collected, and all oestrin was removed by repeated extraction with ether. Its absence was confirmed by testing on ovariectomised mice.

Results

Up to 28 hours : A typical positive reaction occurred.

From 28 to 60 hours : Follicular ripening and early luteinization was seen in microscopical sections of the ovaries.

From 60 to 200 hours: There was no evidence of Prolan B, but Prolan A effects were seen.

After 200 hours : No effects were produced in the genitalia.

Prolan A, therefore, persists for a longer time than Prolan B, and oestrin is found in the urine after both of these hormones have disappeared.

It would seem that the length of time for which the hormones remain in the urine depends on their

concentration in the body previous to the termination of the pregnancy. At the end of pregnancy there is a relative excess of oestrin, hence this hormone persists for a longer period than the hypophyseal hormones. Again in one case of hydatidiform mole a strongly positive test was obtained three days after complete evacuation of the uterus. Fanz and Gault report a similar finding. The hormones are known to occur in greater amounts in such conditions of chorionic hyperactivity. Moreover, in case No.7, in which an excess of oestrin was found in the urine prior to the passage of a macerated foetus, oestrin was still present in the urine 11 days after delivery.

From this, I conclude that although Prolan A is present in the urine up to 200 hours after the removal of a 16 weeks pregnancy, yet it may not persist for that period of time after a normal confinement: this statement is made in view of the fact that more pituitary hormones are found at the 16th week of pregnancy than in its later stage. Wiesner[173] produced further evidence in favour of this when he stated that a positive reaction was obtained with a urine received from a patient nine days after an abortion.

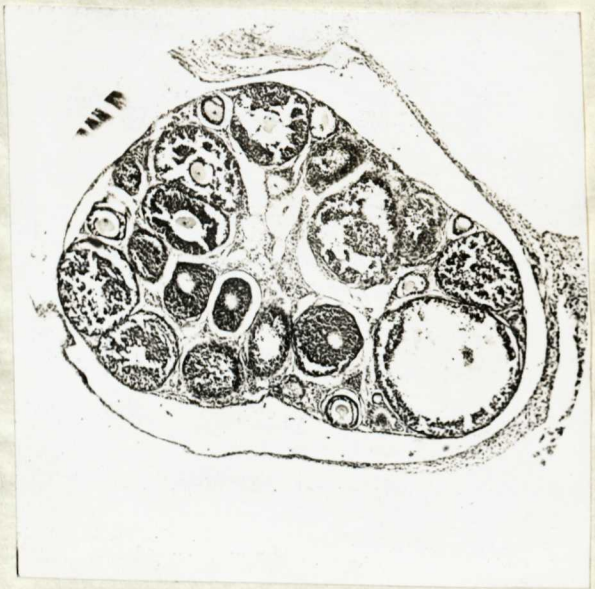


Fig.15. Ovary of mouse from test No.58 : this shews follicular ripening due to the presence of Prolan A in the injected urine. x 50.

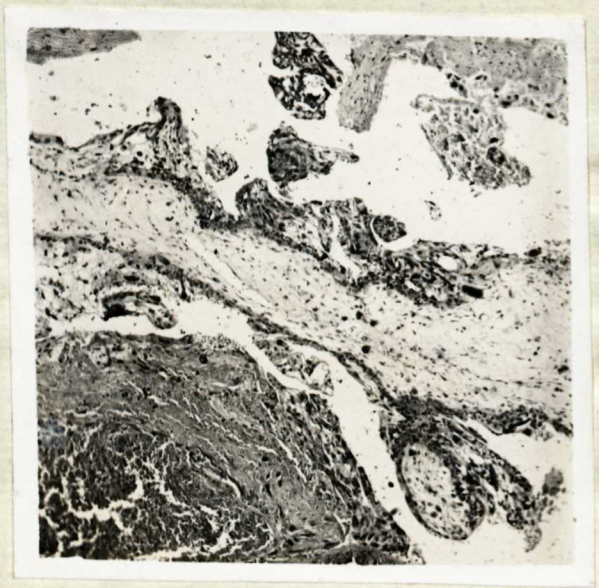


Fig.16. Section of the carneous mole obtained from case No.58. This shews the presence of one large active villus which was no doubt responsible for the presence of Prolan A in the urine. x 55.

The Aschheim-Zondek Reaction in Pathological Conditions associated with Pregnancy

I. Missed Abortion and Carneous Mole

Cases No. 34, 44, 45, 50, 58, 70.

Urines from six cases of missed abortion were examined. Four of these gave a negative reaction although three contained traces of oestrin. The moles from these patients all shewed degenerate villi in microscopical sections. In case No.58, again a negative result was obtained, but the urine contained considerable amounts of Prolan A (see Fig.15). Microscopical examination of two different sections resulted in the finding of only one large villus : this appeared to be active and the cells were proliferating (Fig.16) : it is difficult to understand why this did not produce a Prolan B effect too. It is of interest to note that this patient had had a hydatidiform mole some years previously. In view of this fact, further specimens of urine were examined at monthly intervals, but immediately after evacuation of the mole, the Prolan A disappeared from the urine, and no hormones were found subsequently. In the sixth case, the mice died and no result was obtained.

The occurrence of traces of oestrin in the urine is due, I believe, to the fact that after every abortion or

delivery, this hormone is the last to disappear from the urine. This is born out by the fact that in Case No.34, when the foetus had only been dead about five weeks, the pituitary hormones had already gone from the urine, but oestrin still remained in large quantities - much more than in those cases where a true carneous mole had had time to form.

The fact that a negative reaction is obtained in these cases is not a disadvantage, but rather enhances the value of the test. It is usually possible to decide clinically whether the patient is pregnant, but it is much more difficult at one examination alone, to say that the pregnancy has ceased to progress. In those cases a negative reaction makes the diagnosis certain, whereas a positive result means that the placenta is still alive in part at least, but does not necessarily infer that the pregnancy is progressing.

II. Incomplete Abortion

Cases No.25 and 72.

Only two cases of definite incomplete abortion appear in this series. The first (No.72) gave a negative reaction, and no oestrin remained in the urine : sections of the curettings showed villi which were all degenerate. In the second case (No.25), the whole placenta was retained. Urine received on the 12th day after expulsion of the foetus, still

gave a positive reaction, shewing how intimate is the relation between the presence of placental tissue and a positive result. The placenta was not obtained for section : this was unfortunate since it would have been more convincing to have been able to demonstrate villi which were still healthy.

III. Ectopic Pregnancy

Cases 1, 26, 35, 81, 28, 37, 64, 66, 73, 97.

Urines from ten cases of tubal pregnancy were examined. In four of these a positive reaction was obtained in the remaining six the test was negative. The histories and operative findings of all those cases which gave a negative test, shewed them to be tubal moles which had probably been dead some considerable time. Of these six moles, three were secured for microscopic examination : in one no villi were found, in the others villi were present, but stained faintly, and were obviously dead. Of the four urines which gave a positive test, two were from cases of tubal rupture requiring emergency operations : these urines were received 48 hours and 24 hours after operation respectively. Sections of these pregnancies shewed active villi present. The remaining two were cases of tubal mole with a comparatively short history : neither specimens were obtained for examination, but in view of the positive reactions, one must presume that there must have been some villi still alive.

IV. Pyelitis of Pregnancy

Case 103.

Only one case of this disease was examined. In spite of the urinary infection the urine was no more toxic than the average specimen. There was no difference in the effects produced in this case from those obtained in cases of normal pregnancy.

V. Toxaemias of Pregnancy

(a) Hyperemesis Gravidarum. Cases No. 71 and 100.

Urines from both the patients gave positive reactions, and neither was more toxic than urine from patients with normal pregnancies.

(b) Pre-eclampsia. Cases No. 7. and 29.

Here again the urines showed no increased toxicity, and both produced normal reactions.

Mauriac[124] has recently suggested that the hormones circulating in the blood during pregnancy are important factors in the aetiology of the toxaemias of early pregnancy. If this were true one might possibly expect some difference in the effects produced by injecting urines from such cases. One can only say that the only difference noted was that the reactions were strongly positive in the two cases of hyperemesis. This may have some bearing on the aetiology of pernicious vomiting, but it seems more likely that this effect was due to the fact that the patients were secreting less, but more concentrated, urine.

VI. Hydatidiform Mole

De Snoo[54], Aschheim[15], and Philipp[135], were the first to report that cases of hydatidiform mole gave a positive reaction. Since that time, numerous reports have been given, and it is generally acknowledged that the effects produced are more pronounced than those found in normal pregnancy. Nürnberger[129] shewed that in normal pregnancy the urine never contained more than 50,000 M.U of pituitary hormones per litre. whereas in hydatidiform mole and chorion-epithelioma 200,000 M.U of these hormones per litre had been demonstrated. Vozza[170] also reported the presence of 100,000 M.U per litre in cases of vesicular mole. Considering that the test is one for chorionic activity, these results are to be expected. Three cases of hydatidiform mole are included in our series of tests. The first (Case No.30) gave a very positive reaction, there being at least six haemorrhages in each ovary. In the second case (No.92), the urine was not received until 72 hours after the evacuation of the mole : even at that time, a positive result was obtained with urine diluted to half strength. The urine from case No.43 was very toxic, but a positive test was obtained in a mouse which only received three injections, and which died 54 hours after commencing the test. This would not have occurred if the urine had been obtained from a normal pregnancy, and is strong evidence of the concentration of the hormones. Two urines were sent from patients

suspected of having hydatidiform mole (Nos.42 and 79). In neither case did urine which was diluted more than one in three, give a positive test, and a report was given saying there was no evidence of mole formation. In both instances the foetal heart sounds were heard subsequently.

In order to diagnose a hydatidiform mole it seems that a positive test must be given by urine diluted at least one in five.

Urines obtained from these cases were very much more toxic than normal urine : of 37 mice injected, only 15 survived to the end of 100 hours.

This test has now become valuable as an indication as to whether, after evacuation of the uterus, any of the chorionic elements have been left in utero. After a complete evacuation I have obtained a positive reaction three days later : after an incomplete evacuation the urine contained the hormones 12 days later (Case No.31). Stewart[164] found that the urine gave a negative reaction 5 days after complete removal of the mole, but Vozza[170] and Reeb[141] say that a positive test can be obtained from 14 to 25 days after emptying the uterus. This figure seems to be too high, and such results would lead one to suspect that all of the mole had not been removed.

VII. Chorion Epithelioma

In practically every medical publication one now

finds reference to the possibility of obtaining a positive test in cases of chorion epithelioma. Raisz[140], Vozza[170], Ehrhardt[61], Reeb[141] and Ginglinger[96] are only a few of the workers who have described this phenomenon.

The hormones are usually present in the urine in greater quantity than in pregnancy. Ehrhardt[61] shewed that urine diluted ten times gave a positive reaction, while Reeb[141] found the hormones present in 10 to 300 times the usual amount.

Not only is the test reliable in this disease, but it is now recognised as being a much safer, and more efficient, diagnostic procedure than curettage : cases illustrating this point have been described by Vozza[170]. In addition, all workers have emphasized its value in prognosis : it has been found that if the hormones persist in the urine after operation, then either the growth has been incompletely removed, or there are secondaries present in the body. Moreover, if the hormones disappear from the urine, their reappearance is the first sign of a recurrence of the tumour.

The fact that there is an excess of Prolan B in the body fluids in cases of hydatidiform mole and chorion-epithelioma, led Aschheim[15] to suggest that this hormone is responsible for the production of true lutein cysts in the ovaries. These cysts have long been known to be intimately associated with such conditions, and Aschheim[15]

has now supplied the explanation.

VIII. Foetal Death and Threatened Abortion

These conditions are grouped together since it has been found that both tend to produce a similar type of reaction. When the Aschheim-Zondek test was first introduced, it was hoped that it would be of help in the diagnosis of foetal death. It failed completely, and it is now known that it will diagnose death of the placenta, but not death of the foetus alone. Case No.14 was an exception to the usual findings, and in this case death of the foetus was diagnosed because the placenta was dead too.

Recently, however, Eberson and Silverberg[60] state that they find a different microscopical picture of the mice ovaries in those cases in which the foetus has died in utero. They say "that detection of the death of the foetus is possible notwithstanding the fact that positive signs in rats examined post mortem may be seen as late as seven days after the death of the foetus in utero. The microscopic picture in the corpora lutea is characteristic of retrograde changes, and is different from the typical luteinization in these cells". It appears unlikely that a certain specimen of urine will commence the formation of corpora lutea, and yet that the same specimen will cause retrograde changes to supervene. Moreover, the

Fig.17. The Aschheim Zondek reaction obtained by the injection of the urine of a woman who was about to abort. There is enormous enlargement and distension of the uterus due to a relative excess of oestrin. The ovaries, although enlarged, shew no corpora haemorrhagica.

photographs in this article are unconvincing, and one feels that such claims will only tend to bring a reliable, but limited test, into disrepute.

It is, therefore, with caution that one suggests that the reaction obtained is cases of threatened abortion and foetal death, are often different from those obtained with urines of normal pregnancies. However, in many of these cases, the test has shewn that an abnormally large amount of oestrin is present in the urine. This manifests itself by producing in the mice an enormously enlarged and dilated uterus, filled with secretion (Fig.17). At the same time, although there is some luteinization present in the ovaries, as well as an occasional haemorrhage, yet the Prolan B effect is not so noticeable as in normal pregnancies. Acting on these observations, it has been possible on six occasions to forecast that the pregnancy was about to terminate and that the foetus was probably dead. In all of these cases abortion occurred soon afterwards, or a macerated foetus was evacuated prematurely. In three other cases, urine was sent from patients in whom foetal death was suspected : in all of these there was no excess of oestrin, and this fact was reported. In each instance, the pregnancy was afterwards stated to be progressing normally.

It seems probable that this appearance of such large quantities of oestrin is not, strictly speaking, related in

any way to the death of the foetus, but is rather an indication of an upset in the hormonic balance which is the cause of, or occurs because of, a premature termination of the pregnancy. Since death of the foetus is, in most cases, followed by its expulsion, the sign has sometimes been regarded as rather suggestive of foetal death : this, I feel sure, is an incorrect point of view.

Following on this line of thought, urines from patients in the first stage of labour were tested : in every case exactly the same type of reaction was produced.

It is not intended to put forward this observation as an infallible test, since in many cases abortion may be caused by conditions other than an upset in the endocrine regulation. Again, there is considerable difficulty in being accurate as to the amount of oestrin present : one can only judge by a 'general impression'. In one case of death of the foetus, no excess of oestrin was noticed, while one pregnancy which shewed an excess of the hormone proceeded to term in a normal manner.

I quote one interesting case (No.38, 39, 40 and 41). This was a case of pregnancy in which both ovaries were removed at the 16th week. 6 weeks after the operation a rough estimate of the oestrin content of the urine shewed that it was about 1,000 M.U per litre. Three weeks later, it was over 2,000 M.U of oestrin per litre, and subsequent events proved that the foetus died about that time.

The Effect of Physical Agents on the Hormonic Content of the Urine

(i) Time

In view of the fact that some time may elapse between the collection of a specimen of urine, and its receipt at a pregnancy diagnosis station, it is of importance to know whether such delay results in a diminution of its hormonic content. Bourg[31] found that a urine kept as long as 10 to 15 days still gave a positive reaction. In case No.31, urine received from a patient with a hydatidiform mole, was kept 85 days, in a coloured glass vessel, and at a temperature of -4° C. The coloured glass container was used because ultra violet light rays are said to destroy the hormones. At the end of that time, the effects produced by its injection into mice differed in no way from those obtained when the urine was first tested.

(ii) Filtration

It has been often stated that the hormones are readily adsorbed on to a filter paper. Allen and Dpisy[7] found that oestrin extracts lost their potency if they were filtered three times. In many cases treatment of the urine which may involve filtration, is desirable ; it was therefore determined to see what effects filtration had. In each case a Whatman Filter Paper No.1 was used.

Results :

(a)	Filtered once.	Normal positive reaction with haemorrhages.
(b)	" twice	Positive reaction. No haemorrhages.
(c)	" three times	" " " "
(d)	" four times	Oestrin present, and traces of Prolan A and Prolan B.
(e)	" five times	Oestrin present in small amounts. No evidence of pituitary hormones.

There appears to be no doubt therefore that filtration tends to remove the hormones ; but oestrin is less affected by the process than Prolan A and Prolan B. However, since five separate filtrations are required to remove the greater part of the hormones, it seems permissible to filter a specimen of urine once, if occasion should demand it.

(iii) Heat

Prolan A and Prolan B are known to be readily destroyed by heat : Wiesner[174] and Murata and Adachi[127] found that rho 1 is totally destroyed by boiling for one minute, and that rho 2 can only survive at that temperature for a slightly longer period of time. Oestrin is thermostabile.

The urine from a woman who was 20 weeks pregnant was repeatedly extracted with ether until all the follicular hormone was removed. Different portions were then heated to different temperatures, and maintained at their respective temperatures for a period of five minutes. On testing

these fractions the following results were obtained :

Heating to 30°C	Normal positive reaction	
" " 40°C	" "	"
" " 50°C	Prolan A present.	Prolan B in small quantities.
" " 60°C	Prolan A present.	Prolan B in small quantities.
" " 70°C	No hormones present.	

(iv) <u>Dilution with Distilled Water</u>

In the diagnosis of Hydatidiform mole, and chorion epithelioma, it is often essential to dilute the urine, and to demonstrate that a positive reaction can be obtained in greater dilutions than is possible with the urine from a normal pregnancy. For this reason it was decided to investigate what dilutions of a normal pregnancy urine would contain demonstrable quantities of Prolan A and Prolan B. The following results were obtained with the urine from a woman 20 weeks pregnant :

<u>Dilution</u>		<u>Effects</u>	
(a) 1 : 1	$\frac{1}{2}$ strength	No haemorrhages.	Prolan A and Prolan B present.
(b) 1 : 2	$\frac{1}{3}$ "	" "	Prolan A present ; Prolan B in small amounts.
(c) 1 : 4	$\frac{1}{5}$ "	" "	Prolan A present ; No evidence of Prolan B.
(d) 1 : 6	$\frac{1}{7}$ "	" "	? small amounts of Prolan A ; no evidence of Prolan B.
(e) 1 : 8	$\frac{1}{9}$ "	" "	No evidence of any hormones present.

Oestrus was produced in the animal in all experiments ; in the larger dilutions this was due entirely to oestrin. No macroscopic ovarian responses occurred if the urine was less than one third strength, although Prolan A effects were seen microscopically when the urine was diluted to one fourth strength. It may be assumed, therefore, that if typical ovarian responses are obtained with urine diluted as mush as one fifth, the presence of abnormally over active chorionic villi should be diagnosed.

The difficulties and disadvantages of the Aschheim-Zondek Test

(I) Time

Five days at least must elapse from the time a specimen of urine is received until a diagnosis can be made. This delay reduces considerably the value of the test. However, it may be overcome by one of many modifications of technique :

(a) Sacrifice one of the mice as soon as its vaginal smear is characteristic of oestrus. By this means it is possible, at times, to reach a conclusion on the 4th day : in these instances only a positive reaction is accepted. Ettinger, Smith and McHenry[66] also record the possibility of giving a positive diagnosis for pregnancy on the 4th day.

(b) Concentration of the urine : Zondek[178] was the first to devise a means of obtaining the hormones in greater

concentration. Since this method has been adopted in many of the later experiments, it will be described in detail.

60 cc. of urine is slightly acidified with one drop of glacial acetic acid. It is then shaken up with 240 cc. of 96 per cent. alcohol, and the mixture is allowed to stand for 24 hours. A flocculent yellowish grey precipitate forms, and the supernatant fluid can be decanted off. Occasionally it is necessary to centrifuge in order to obtain the residue. This precipitate is now shaken up with 20 to 30 cc. of ether for 5 to 10 minutes. The ether is poured off, the last traces being removed by centrifuging, or evaporation at 30°C. 12 cc. of distilled water is added to the precipitate, and the whole is well shaken. Separation is again effected, and the watery extract is used for injection into mice. This method serves to concentrate the hormones five times. If one is adopting this procedure in order to reach a speedy diagnosis, instead of leaving for 24 hours after the addition of the alcohol, the precipitate is separated from the supernatant fluid by centrifuging at the end of one half-hour.

In testing such extracts, Zondek recommended that the mice should receive six injections of 0.3 cc. within 48 hours : in this series of experiments five doses of 0.5 cc. were always given.

In the first place Zondek described this procedure for the purpose of demonstrating small amounts of Prolan A

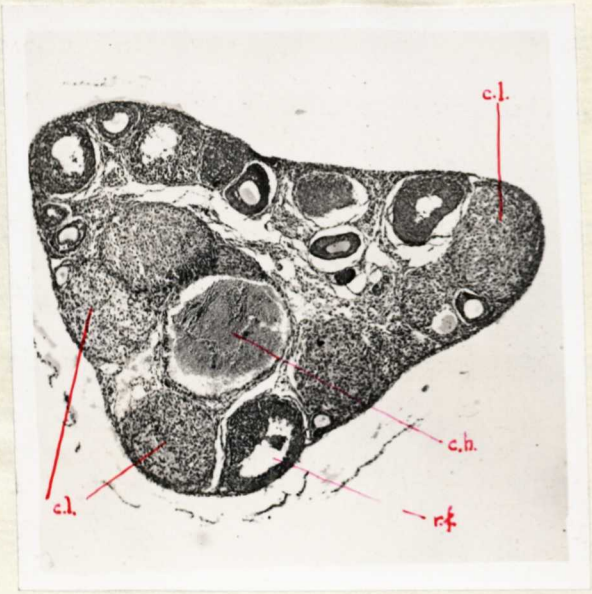


Fig.18. Mouse ovary shewing the typical ovarian responses produced by the injection of pregnancy urine which had been treated by the concentration process.
x 50.

c.h. = corpus haemorrhagica.

c.l. = corpora lutea atetrica.

r.f. = ripening follicle.

in the urine of non-pregnant patients. I have used it for a similar purpose, but have also investigated the effect of such treatment on urine received from cases of pregnancy. It has been found that :

(i) The final watery extract contains both hypophyseal hormones and produces the typical changes in the mice ovaries.(Fig.18). These effects are more pronounced than those obtained by injecting unconcentrated urine. The changes due to oestrin are very slight since most of this hormone is removed by the treatment with ether. This loss is an advantage rather than the reverse.

(ii) The alcohol fraction, I have shewn to contain a substance identical with Prolan A, and on injection into animals produces follicular ripening and the onset of oestrus. This does not necessarily infer the presence of yet another hormone, but is probably due to the fact that, since Prolan A is soluble in alcohol all of this hormone is not thrown out of solution. The exact nature of the precipitate is unknown, but whatever it is, it seems likely that the Prolan A is not an essential component, but is merely adsorbed on to it. This would explain why some of the Prolan A still remains in the supernatant fluid. Collip[42] is of this opinion.

(iii) If the ether fraction is evaporated down at a temperature of 30-40°C, under reduced pressure, and the residue dissolved in water, it is easy to demonstrate that oestrin is present in large amounts.

It is evident, therefore, that such treatment serves to concentrate both Prolan A and Prolan B. Zondek[177] used it in his pregnancy diagnosis tests, and thereby obtained results in 51-57 hours after the first injection. However, a negative reaction under such conditions is of no significance.

In the tests described previously, speed was never necessary, so that the accuracy of the test was never imperilled by an attempt to make an early diagnosis. However, positive reactions were noticed on the third and fourth days after the commencement of injections. Eberson and Silverberg[60] using a similar method of concentration, injected rats instead of mice, and claim to have obtained positive reactions from 24 to 36 hours after the first injection.

(c) A third method of obtaining a diagnosis quickly has been described by Friedman[90]. This is really a modification of the Aschheim-Zondek reaction, but is now known as 'Friedman's test'. It consists in the use of adult segregated female rabbits as test animals, instead of mice or rats. The injections of urine are given intravenously in single doses of 5-15 cc. A positive reaction is determined by the finding of haemorrhagic follicles, or recent corpora lutea, in the ovaries

I have had no experience of this test, but Friedman claims to have obtained positive results in 18 to 24 hours. Reinhart and Scott[144] and Magrath and Randall[119] have repeated his experiments and have confirmed his statements with regard to the speed, and accuracy, of the method. Working on similar lines Schneider[150] states that he was able to make a positive diagnosis of pregnancy within 12 hours of the commencement of injections. However, Wiesner[173] has recently found that this test is not as reliable as reports seem to indicate, and using the same specimen of urine he obtained a positive reaction in mice although he failed to do so in the rabbit.

II. Death of the Animals

Of all the adverse criticism which has been levelled against this test, the most justifiable seems to be that occasionally the animals die and no result is obtained. It has been found that certain urines are toxic while others are non toxic to the mice. As to what substance in the urine is the cause of its toxicity opinions are varied and nothing definite is known. Vozza[169] observed that 11 per. cent. of all animals injected died before the end of the appointed time. In this series of experiments a high animal mortality was experienced, and approximately 30 per cent. of test animals died before the conclusion of the tests. This figure is high because

(i) The methods of keeping the urine were varied purposely

in order to note effects of different procedures.

(ii) A large number of experiments were performed on urines from cases of malignant disease. Such urines are exceptionally toxic.

Although all attempts to find the cause of the death of the mice failed, certain interesting observations were made.

(a) Once a urine is toxic it tends to remain toxic

In case No.38, 39, 40 and 41, specimens of urine were received from the same patients at various times over a period of four months. On every occasion the urine was toxic and no animal survived unless the urine was first treated by a method to be described later.

(b) Relation of the toxicity of urine to its constituents.

(i) Albumin

It was suspected that the injections of proteins might have resulted in an animal's death. Examination of the urines failed to show any relation between the presence of albumin and the death of the mice. Moreover, in other experiments, injections of blood sera and placental extracts, all containing large quantities of protein, failed to effect the test animals adversely.

(ii) Bacteria

Stewart[164] states that infected urines are more toxic. No definite evidence of this was found, in fact

on many occasions, urines which contained large numbers of pus cells and organisms, had no ill-effects, while perfectly clear urines were very deadly. In case No.103 the urine was from a patient suffering from pyelitis : on this occasion no test animals died. Again, in one instance while experimenting with rabbits, one of the animals developed so severe a cystitis that its urine was almost 'solid' with pus and organisms, and as a result of the infection the animal developed a paraplegia. This urine was practically harmless whereas normal rabbit urine is usually very toxic.

In spite of these facts, it would appear that organisms do at times play a part in causing the mice to succumb: Böhne[29] blames the bacillus coli in particular.

(c) Relation of the Toxicity of Urine to its Reaction

The reactions of 22 toxic urines were taken, and the reports shewed :

Acid	4
Slightly Acid	8
Neutral	4
Slightly Alkaline	4
Alkaline	2

These figures seem to indicate that there is no association between the death of the animals and the reaction of the urine. The greatest number of specimens

were 'slightly acid' : this is the finding if any groups of urines are tested.

(d) Relation of the Toxicity of Urine to the Disease of the Patient.

In those cases of pregnancy from which urines were examined, no pathological conditions such as pre-eclampsia, pyelitis, missed abortion, had any noticeable relationship to the animal mortality. However, in testing the urines of patients, male and female, suffering from malignant disease, it was found that such urines were most fatal to the animals. Altogether 61 mice were injected with non-treated urine received from such patients : of these, no less than 49 died before the completion of the test, and usually after only 2 or 3 injections. The mortality rate was thus 80 per cent. No cause for this could be found and one can only conjecture that such urines possibly contain products of tissue breakdown, or of altered cell metabolism.

It is of interest to note that many observers have found that the urines from cases of hydatidiform mole and chorion epithelioma are more toxic than those of normal pregnancy. I found this to be true with respect to the former but had no opportunity of examining urine from a case of chorion epithelioma.

Post mortem examination of the animals which died revealed no evidence to help in the solution of this problem. The skin over the sites of injection was usually thickened, hard, and indurated, but apart from this there was no obvious pathology.

Although so far it has been assumed that there is only one factor concerned in this question, yet it is possible that there is more than one condition, or constituent of the urine, which is able to have this untoward effect on the test animals.

Methods for obtaining a result when a urine is toxic

One of many alternative procedures may be adopted.

(i) Change of Test Animal

Bourg[31] while using rats had only 2 deaths among 120 experimental animals. Eberson and Silverberg[60] claim to have had no mortality while using the same species of animals. Rabbits, too, are more resistant to this toxic action of urine, and the adherents of 'Friedman's Test' point this out as one of the advantages of that technique.

(ii) The Use of Blood Serum

On occasion it may be possible to substitute blood serum for urine. This procedure robs the test of some of its simplicity, but may be of great help. Siddall[155] first shewed that the pituitary hormones

could be demonstrated in the blood during pregnancy. In one case only have I used serum, and in this a typical positive reaction resulted : the mice ovaries shewed corpora haemorrhagica and corpora lutea.

(iii) Detoxication of the Urine

The methods for detoxicating the urine are mostly empirical since no one knows what toxic substance is to be removed or destroyed.

(a) Treatment with Ether

In many of the experiments, the urine was rendered innocuous by shaking for 5-10 minutes with an equal volume of ether. This was then separated off, and the last traces removed by heating in vacuo at a temperature of 30°C. This method has also been described by Stewart[164] , and by Zondek[177] . In one case 11 mice were injected with different dilutions of pregnancy urine : every one died. The same specimen was treated as described, and 8 more mice injected : none succumbed. Zondek has reported that, by the use of a similar measure, the usual mortality of 6-7 per cent. is reduced to 0 per cent. The results obtained by this procedure do not depend on the number of times that the ether treatment is repeated. A urine exposed to the action of ether once, is rendered just as innocuous as the same urine treated five times in the same way.

Stewart[164] suggests that the efficacy of this procedure depends of the fact that the ether destroys the organisms. In support of this, the following interesting feature was noticed. If, after it has been allowed to mix with a toxic urine, the ether is evaporated down and the residue dissolved in water and injected into mice, it produces no ill-effects. It would appear, therefore, that the ether acts by destroying, or by altering, the composition of some urinary constituent, and that it does not merely remove the toxic agents.

(b) Filtration of the Urine

Zondek[177] reports that filtration of the urine through a Berkefeld filter diminishes the animal mortality. Stewart[164] believes that a Zeitz filter renders the urine less toxic by removal of the organisms.

(c) Centrifuging

Centrifuging the urine is said to diminish its ill effects. I have had no experience of this procedure.

(d) Concentration of the Urine

The method of concentrating the urine as described on page 62 is also efficacious in reducing the animal mortality. This method was used chiefly in cases of malignant disease, since it thus served two purposes : (i) concentration of the hormones

(ii) prevention of animal death.

In every test except one, the injection of this watery extract produced a diminished mortality amongst the mice. It is possible that this procedure depends for its detoxicating action merely on the shaking of the precipitate with ether.

(e) Recently, Wiesner[173] has described yet another method. In this he precipitates proteins with sulphosalicylic acid, and filters off the precipitate through a filter paper. The urine is then neutralized with sodium bicarbonate. He evidently infers that the toxicity is due to proteins. I tried this method in three cases only : in one of these it failed whereas a simple ether extraction was successful in spite of the fact that the urine had been kept for a longer period. As a result of this I abandoned the procedure.

Of all these methods I relied on two only (i) the treatment with ether and (ii) the concentration of the hormones. By using one or other of these modifications I only failed to obtain a result in one case of the pregnancy diagnosis series.

The Aschheim-Zondek Test on the Urine of Pregnant Anima

In view of the fact that pituitary transplants taken from all the common laboratory animals, either male or female, produce the typical ovarian responses, one would expect that the urine of pregnant animals would give a positive pregnancy test. Zondek[181], however, found that of all animals, only the monkey excreted hypophyseal hormones during pregnancy. This finding was confirmed by Ehrhardt[62], but Allen and his colleagues[10] failed to demonstrate the presence of pituitary hormones even in the urine of the monkey.

In order to test the verity of these statements, the following experiments were carried out. It was found that the urine of animals was much more toxic than that of human beings. From their results Allan and Dickens[3] appear to have experienced this too.

Results

Animal	Result of Test
Rabbit 16 days pregnant	No hormones found
" 20 " "	" " "
" 7 " "	" " "
Cat. Near Term	" " "

Believing that in such small animals the hormones were perhaps present, but in much smaller amounts, I performed a second series of experiments using concentrated urine.

Animal	Result of Test
Rabbit 14 days pregnant	No hormones present
" 16 " "	" " "
" 18 " "	" " "
" 22 " "	" " "
Cat. 14 " "	" " "

There was still a possibility, however, that although the hormones were being secreted, they were not being excreted; blood serum was therefore collected and tested with the following results :

Animal	Result of Test
Rabbit 14 days pregnant	No hormones found
" 15 " "	" " "
" 20 " "	" " "
" Near Term	" " "
Cat " "	" " "

In every case I failed to demonstrate the presence of any hormones in the blood stream or urine of the pregnant cat or rabbit. Moreover, no observer has yet demonstrated the presence of the hypophyseal hormones in the larger animals such as the cow and horse, although Cole and Hart[50]

have shewn that oestrin occurs in considerable quantities in the blood serum of the mare during the second half of pregnancy. In connection with this, Kraul[112] makes an interesting comment "In rodents the yellow body (corpus luteum) persists throughout the course of pregnancy, although their placentae do not contain such great amounts of hormone as the human being and their blood serum shows no increase of anterior pituitary hormone during pregnancy. An exception apparently exists in the case of the mare whose blood serum contains high amounts. In this animal, however, the yellow body degenerates before the termination of pregnancy."

Placental Hormones

Halban[97], in 1905, was the first to shew that the human placenta contained a hormone which was capable of producing oestrus in animals. That this effect was due to a hormone which we now know as oestrin, was demonstrated by Herrmann[101]. Since that time all observers have agreed that oestrin occurs in large quantities in the human placenta, and for some years now commercial preparations of the ovarian hormone have been obtained from this source.

In 1920 Hirose[105] gave intraperitoneal injections of placenta into rabbits and was able to shew that this procedure produced an abnormally large number of corpora lutea in the ovaries. Later, Murata and Adachi[127] confirmed this, and in addition to producing luteinization, found that oestrus

occurred in the animal. However, oestrus was not produced in ovariectomised animals and so the hormone concerned in this case was not oestrin.

As a result of the work of Smith and Engle, and Aschheim and Zondek, it became apparent that the effects of placental implants were rather similar to those of anterior pituitary transplants.

Finally Wiesner[174], by means of sulphosalicylic acid extracts of placenta, was able to produce changes in the ovaries of mice and rats, identical with those obtained by the use of hypophyseal extracts. In other words, he shewed that the placenta contained either Prolan A and Prolan B (rho 1 and rho 2), or substances which had exactly the same properties and physiological actions.

In connection with placental hormones Collip[42] has done a large amount of work, and has described in detail the preparation in pure crystalline form of a hormone corresponding to Prolan A : this hormone he calls 'emmenine'.

Hisaw[106] has suggested that the corpus luteum hormone may also be found in the placenta. Kraul[112] has recently injected placental extracts into rabbits ovariectomised 18 hours after copulation : he failed to find any changes in the uterine mucosae, and concludes that the human placenta at term contains none of the active principle of the corpus luteum.

I have made extracts of normal human placenta and also of carneous, and hydatidiform, moles. The methods of extraction were based on those described by Collip[42]. Both alcohol and acetone extracts were made, but it was found that acetone extracts were easier to prepare, and in most cases this medium was used.

Method

The tissue was macerated in an ordinary meat mincer. One and one-half volumes of acetone, which had been previously slightly acidulated with glacial acetic acid, was added. The whole was then pounded together and allowed to remain for 24 hours : it was stirred frequently. At the end of that time the residue was filtered off, and the filtrate heated to 30° to 40° C. at reduced pressure, until all the acetone was evaporated. An aqueous solution, containing the hormones, remained. This was, or was not, repeatedly extracted with ether, depending on whether one wished to have oestrin in the extract. No further purification of the extract was required for my purpose which was merely to demonstrate the presence of the hormones qualitatively.

The extracts were injected by the same technique as was employed in the ordinary Aschheim-Zondek tests.

Results

Cases	Result of A-Z test performed before removal of specimen.	Description of Tissue extracted.	Hormones in Extract.			
			Oestrin	Prolan A	Prolan B	Haemorrhgs. in mice ovaries.
P.23	Positive	6 weeks 'placenta'	+	++	++	++
P.24	Positive	12 weeks placenta	+	++	++	++
P.22	Not done	15 weeks placenta	+	+	+	+
P.21	Not done	40 weeks placenta	+	+	+	0
P.18	Not done	" " "	+	+	+	0
P.17	Not done	" " "	+	+	?	0
P.16	Not done	" " "	+	+	+	0
P.2	Not done	" " "	+	+	+	+
P.1	Positive.Excess oestrin	22 " " (i) Macerated foetus (ii)	++ ++	+ +	+ +	+ +
P.10	Negative.Oestrin present	Missed abortion	++	0	0	0
P.20	Not done	Missed abortion	Mice died			
P.9	Negative.Oestrin present	Carneous mole	+	0	0	0
P.6	Negative. " "	Missed abortion. Dead 5 weeks	++	0	0	0
P.3	Positive	Living tubal pregnancy	+	0	0	0
P.4	Negative. Trace of oestrin	Tubal mole	Trace	0	0	0
P.8	Strongly positive	Hydatidiform mole	+	+	+	+

From these results it is seen that by a comparatively simple method, an active extract can be prepared from the human

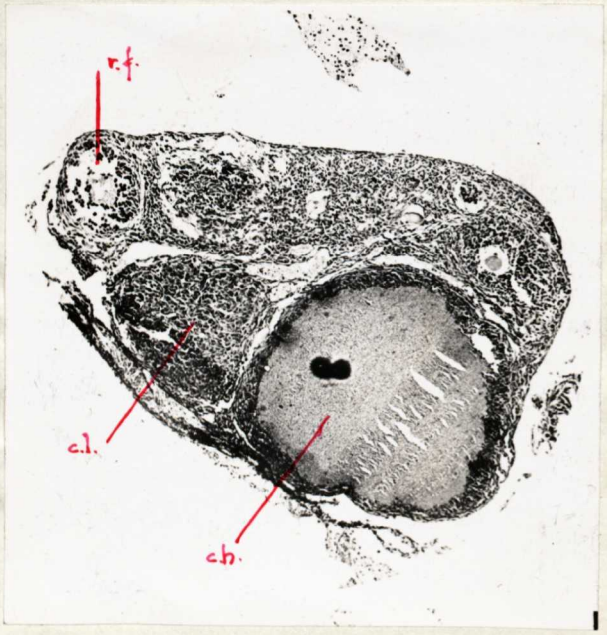


Fig.19. Ovarian responses produced by injection of extracts of human placenta. These are identical to those obtained by injections of pregnancy urine. x 65.

c.h. = corpus haemorrhagica.
c.l. = corpus luteum atetrica.
r.f. = ripening follicle.

placenta at all stages of pregnancy. Such extracts produce effects in the mouse genitalia which are identical to the effects obtained by the injection of pregnancy urine (Fig.19). Extracts of hydatidiform mole also contain the hormones.

I should like to point out that, with but one exception, the responses to injection of a certain tissue extract were identical to the effects produced by the injection of the urine of the patients, received before removal of the tissue. Thus in the early stages of pregnancy both urine and placental extracts produced numerous corpora haemorrhagica and a preponderance of Prolan A and Prolan B effects. In the later stages, the oestrin effects were dominant, and it was rare to find haemorrhages in the mice ovaries. Similarly in one case of foetal death at 22 weeks (P.1), both the urine and the placenta contained a relative excess of oestrin. In those cases of carneous mole when no positive reaction was obtained with the urine, the same result was obtained with the extract of the mole. The exception to this rule was in case P.3 in which, although the urine of the patient had given a positive reaction, the extract of the tube and its products of conception did not contain either Prolan A and Prolan B. : this can be attributed to the small quantity of material which was available for extraction. There seems to be no doubt that, even in this case, a more elaborate method of extraction would have resulted in the demonstration of the presence of the hormones.

A second series of extracts were made from the tissues of animals and the following results were obtained :

Case	Results of injection of serum or urine of animal	Description of Tissues from which extracts were made	Hormones in Extract		
			Oestrin	Prolan A	Prolan B
P.11	Negative for all hormones.	Placentae of rabbit 20 days pregnant	0	0	0
P.12	Negative for all hormones	Uterus of rabbit 20 days pregnant	0	0	0
P.13	Negative for all hormones	Foetūs of rabbit 20 days pregnant	0	0	0
P.14	Negative for all hormones	Placentae of cat near term (i)	Mice died		
		(ii)	0	0	0
P.19	Negative for all hormones	Placentae of cat 14 days pregnant (i)	Mice died		
		(ii)	0	0	0

In every case I failed to demonstrate the presence of any hormones in extracts of the placentae or uterine walls of the cat or rabbit. Parkes and Bellerby[131] also found complete absence of the hormones in the pig, cat, dog, rat and mouse placentae, but demonstrated the presence of oestrin in the placentae of sheep. Allen[9] says that the cow placenta contains oestrin, while Lane-Claypon and Starling[113] claim to have found small amounts of that hormone in the placentae of rabbits.

Although Kraul[112] has recently stated that implants of placentae of rabbits, guinea pigs and cats produce slight follicular ripening and luteinization in the ovaries of test animals, it is the general opinion that the hypophyseal hormones are not to be found in the placentae of animals.

The presence of the Hormones in the Human Foetus

The fact that oestrin and the hypophyseal hormones occur in large quantities in the placenta, infers that either they are produced there, or that they are produced elsewhere and merely collect there from the general circulation. In either case, it is of interest to know if they can pass through the placenta and enter the foetal circulation.

Philipp[136] reports the occasional occurrence of these hormones in the blood and urine of new-born infants, and Brühl[35] says that oestrin can be found in the urine up to the fourth day after birth.

In the investigation of this subject, urine was obtained from still-born foetūs : this in some cases was concentrated, in others it was injected without any modification. The letter C is placed against those cases in which it was concentrated.

	No.	Particulars of Case	Hormones present			Corp.Haem. in ovaries
			Oestrin	Prolan A	Prolan B	
C	1	Male foetus 37 weeks	0	Trace	0	0
	2	" " 40 "	+	+	0	0
C	3	" " 40 "	Mice died			
	4	" " 40 "	+	Trace	0	0
	5	" " 34-35 "	Mice died			
	6	" " 38 "	+	+	0	0
	7	" " 40 "	+	+	0	0
	8	" " 40 "	+	+	0	0
	9	" " 40 "	Mice died			

It is seen from these results that oestrin and Prolan A occur fairly constantly in the foetal urine. Prolan B has never been demonstrated. However, it has been pointed out that towards the end of pregnancy, Prolan B does not occur in very large amounts even in the maternal circulation : this may account for its absence in the foetus at term.

Another series of experiments were performed using blood serum collected from the umbilical cord while the placenta was still in utero. In all, the sera were obtained from cases of normal full-time pregnancies.

Results :

Case	Oestrin	Prolan A	Prolan B	Corp.Haem.
1	++	+	None	None
2	+	+	Trace	"
3	+	Trace	None	"
4	+	+	"	"

These results are identical with those obtained by injections of foetal urine. In one case, however, the mice ovaries shewed a suggestion of luteinization which I regarded as indicating the presence of small amounts of Prolan B.

Brühl[36] states that both oestrin and Prolan A are present in the blood from the umbilical cord, but that the latter hormone is present in relatively small amounts. He failed to find the luteinizing factor in the blood of the mature foetus, but demonstrated its presence in the blood of an eight months baby, and also in the liquor amnii of a four months pregnancy. These findings are in agreement with the view already suggested, that Prolan B probably passes through the placenta in the early stages of pregnancy, but that it is not found in the blood and urine, of the mature foetus, because at the end of pregnancy its concentration in the maternal fluids is comparatively low.

The Origin of the Hormones found in the Urine during Pregnancy.

Oestrin.

In the non-pregnant woman it is generally agreed that the ovary is the organ which secretes oestrin. "Oestrin-like" substances have also been found in the male testes by Maino and Frattini[122] and Dodds, Greenwood and Gallimore[58]. Moreover, Walker and Janny[172] have demonstrated that some plants contain a principle which has the power of producing oestrus in animals.

As to which part of the ovary is responsible for its formation, authorities differ. Allen and Doisy[7], and Laquer and his associates[114,115], have shewn that the hormone is present in large amounts in the liquor folliculi, and they maintain that the Graffian follicle is the sole site of its formation. In confirmation of this, it is quite easy to prove that the contents of follicular retention cysts can produce the onset of oestrus in ovariectomised mice and rats.

Parkes and Bellerby[131], however, found that residual ovarian tissue after removal of the follicles, still contained appreciable quantities of the hormone. Other workers have claimed that the corpus luteum is responsible for its formation : Fellner[74], and Herrmann[102], obtained active extracts from this source, while of late Aschheim and Zondek have obtained positive results. On the other hand, many observers have failed to confirm these findings. For the explanation of this discrepancy in results, we are indebted to Parkes and Bellerby[131] who shewed that it was only in those cases in which recent corpora lutea were used, that potent extracts were obtained. These corpora lutea still contained liquor folliculi, and they were able to prove that whereas the liquid portion contained the hormone, the solid lutein tissue contained no principle capable of producing oestrus.

It seems therefore that oestrin is derived either from the Graffian follicle, or from the interstitial cells of

the ovary. Blair Bell[24] long ago reported that ovarian grafts which contained no follicles were as potent as those which had, in maintaining oestrus in ovariectomised rabbits. This would infer that the interstitial cells can perform this function : against this it has been argued that the human ovary contains little, if any, interstitial gland. In attempting to decide this much debated question, Parkes[130] employed the use of X-rays. By this means he destroyed the follicular apparatus of the ovaries of mice. Brambell, Fielding and Parkes[33] described the effects which this procedure produced. They found that all the follicles were destroyed, and consequently, the corpora lutea too. If an immature animal was used, the ovary became filled with a paranchymatous tissue derived from the germinal epithelium : if the animal was mature a similar tissue appeared, but it was developed from the granulosa cells. Parkes[130] regards this tissue as being comparable to the normal interstitial cells of the ovary. He investigated the effects on the oestrus cycles resulting from exposure of an animal's ovaries to such treatment, and found there was no interference in the onset of puberty in immature mice, and no cessation of the oestrus cycles in the mature mice. Although the cycles were not suppressed, some irregularity in the duration of oestrus and dioestrus occurred at times. In view of these results, Parkes wrote :

"The coincidence of follicular maturation with the occurrence of oestrus, together with the fact that the initial extractions were made from follicular fluid, led to the supposition that the oestrus-producing hormone was essentially elaborated by the mature follicle. More recently, however, the oestrus hormone has been found in situations where it cannot possibly have been elaborated, so that its occurrence in any particular site is not evidence of its origin there. At the time of the first oestrus period, no corpora lutea are present in the ovary, and these structures cannot therefore be considered as the essential site of origin. In the same way, the placenta, also an abundant source of the hormone, is clearly not the essential site of origin. Further, it has been shewn that elimination of the Graffian follicles by exposure to X-rays does not prohibit the formation of the oestrus producing hormone, and it would seem therefore, that, under certain conditions, if not normally, the stromal tissue of the ovary can elaborate the hormone. "

His results were so remarkable that I attempted to confirm them.

Technique.

The animals used were white rats : some of these were just past puberty, others were only approaching maturity. They received varying doses of filtered and

unfiltered X-rays.

A single dose of filtered X-rays was made up as follows :

Kilovolts 185 Milliamps 2.0

Filtration 0.5 mm Zinc + 2.0 mm Aluminium

Distance 10 inches. Time 15 minutes

Dose is equivalent to four-fifths unit skin dose

A single dose of unfiltered X-rays was made up as follows :

Kilovolts 110 Milliamps 1.5

Filtration. Nil

Distance 7 inches Time 14 minutes

Dose is equivalent to four-fifths pastille dose.

All the animals were irradiated from the dorsal aspect. Vaginal smears were examined daily. In this connection it was found that repeated swabbing produced an abnormal condition of the vaginal mucosa. This was evidenced by the presence of a vaginal discharge, and the cyclical changes in the smears disappeared. Damage to the epithelium, and a superimposed infection, was no doubt the underlying pathology : the condition always cleared up if no smears were taken for a few days.

The ovaries were obtained for microscopical examination either on the death of the animal, or at operation.

Fig.20.(a) Shewing the ovary obtained from a rat which had been exposed to x-rays. (Expt.8). There are a few degenerated follicles, but the major part of the organ is filled with parenchymatous 'interstitial' tissue. x 40.

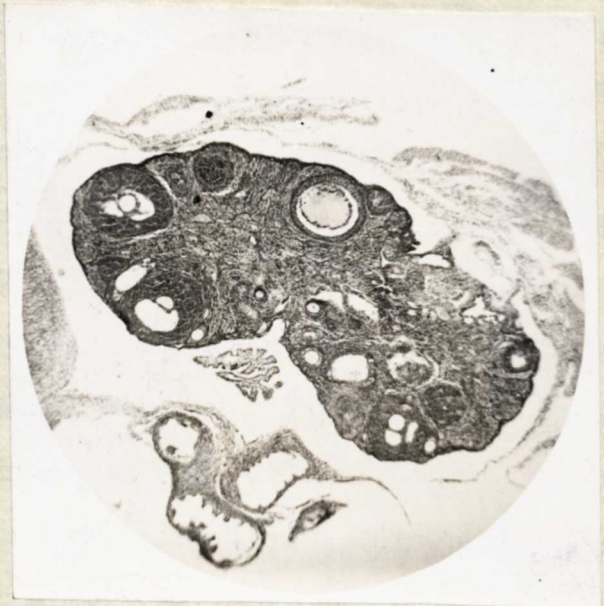


Fig.20.(b) Section of an ovary removed from a normal rat used as a control in Expt. 8. x 30.

Results.

It was found that surprisingly large doses of X-rays were required to cause destruction of the follicular apparatus.

Experiment 1. Animal : Adult rat. Weight 97 grams.

Dosage. Two doses of filtered rays with an interval of 7 days.

Oestrus Cycles. Regular cycles occurred every 5-6 days. Cornification of the vagina persisted 1-2 days except on one occasion when it lasted 3 days.

Ovarian Effects. One ovary was removed 34 days after the second exposure. It showed a few follicles in the process of ripening, and many old corpora lutea. There was a small amount of newly formed 'interstitial' tissue.

Dosage. A third dose of filtered rays was given 5 days after the operation.

Oestrus Cycles. Cycles persisted, but were irregular. Cornification of the vagina sometimes lasted as long as 9 days with a dioestrus interval of only two days.

Ovarian Effects. The second ovary was removed 80 days after the third exposure. There were a few degenerated follicles, but the major portion of the ovary consisted of 'interstitial' tissue.

Experiment 2. Animal : Rat approaching puberty. Weight 87 grams.

Dosage. Two doses of filtered rays with an interval of 7 days.

Oestrus Cycles. The vaginal orifice had not opened 34 days after the second exposure.

Ovarian Effects. One ovary was removed 34 days after second exposure. This appeared to contain a large amount of lutein tissue: Parkes has described this abnormal response which sometimes occurs. Only degenerated follicles were seen.

Dosage. A third exposure to filtered rays was given 4 days after the operation.

Oestrus Cycles. The vagina became patent the same day. Subsequent cycles were irregular, and shewed prolonged periods of dioestrus. This stage sometimes lasted as long as 14 days.

Ovarian Effects. The second ovary was removed 32 days after the third exposure. Although most follicles were destroyed, one was definitely in the process of ripening. Lutein tissue was still present, but in addition, there was a moderate amount of 'interstitial' tissue.

Experiment 3. Animal : Adult rat. Weight 82 grams.

Dosage. One dose of unfiltered rays.

Oestrus Cycles. These were the same as in Experiment 3 except that occasionally true cornification of the vagina did not occur, and the smear only showed cells characteristic of pro-oestrus.

Ovarian Effects. The rat died 31 days after exposure. The ovarian picture was the same as in Experiment 3.

Experiment 5. Animal : Adult rat. Weight 99 grams.

Dosage. Two doses of unfiltered rays with a nine days interval.

Oestrus Cycles. Irregular cycles persisted for 15 days following the second dose.

Ovarian Effects. The rat died 15 days after the second exposure. The ovaries showed old and recent corpora lutea : Some follicles were degenerated, others were in the process of early development.

Experiment 6. Animal : Adult rat. Weight 99 grams.

Dosage. Three doses of unfiltered rays with intervals of 9 days, and 25 days, between them.

Oestrus Cycles. The cycles continued but were very irregular. Oestrus occasionally occurred three times in 7 days : at other times dioestrus persisted for 10 days.

Ovarian Effects. One ovary was removed 44 days after the third exposure. One degenerated follicle was seen : the remainder of the ovary was filled with 'insterstitial' tissue.

Experiment 7. Animal : Immature rat. Weight 64 grams.

Dosage. One dose of filtered rays followed by one dose of unfiltered rays, after an interval of 60 days.

Oestrus Cycles. The vaginal orifice became patent 16 days after

the first exposure. The subsequent cycles were irregular, oestrus persisting for as long as 5 days, and dioestrus for as long as 12 days. After the second irradiation cornification of the vagina did not occur within 20 days, and pro-oestrus smears were only obtained on 3 occasions.

Ovarian Effects. The animal died and the ovaries were lost.

Experiment 8. Animal : Immature rat. Weight 57 grams.

Dosage. One dose of filtered rays followed by one dose of unfiltered rays after an interval of 60 days.

Oestrus Cycles. Irregular cycles occurred. The smears rarely showed cornified cells, but merely the cells characteristic of pro-oestrus.

Ovarian Effects. The animal died 20 days after the second exposure. A few degenerated follicles were present in the ovary, but the major part of that organ was filled with 'interstitial' tissue. (see Fig.20).

Although the number of experiments was small, and they in themselves unconvincing, yet they served to confirm the work of Parkes, inasmuch as they demonstrated that oestrus cycles, although irregular, could occur in the absence of the Graffian follicles.

Schugt[151] repeated Parkes' experiments on mice and obtained similar results. He found that unless the largest X-ray doses were used, oestrus continued although the cycles were often irregular and atypical. He, too, believes that the interstitial cells of the ovary are the source of oestrin. Ford and Drips[83] investigated the action of X-rays on rat ovaries and obtained results identical with the ones described above.

In spite of all this work, I should like to contend that the results are by no means any proof that the Graffian follicle, by reason of its granulosa cells, is not the source of oestrin.

(1) Parkes and others have assumed that the 'interstitial' tissue resulting from exposure to X-rays, is comparable with the normal ovarian interstitial cells. There appears to be no grounds for doing so.

(2) The cells of this tissue are derived from either granulosa cells, or the germinal epithelium which many authorities regard as the ancestor of the membrana granulosa.

(3) If, after treatment with X-rays, mice are injected with alkaline extracts of hypophysis, Parkes[130] found that this newly formed tissue becomes luteinized in exactly the same way as the granulosa cells of the normal ovary. Again Walter[176] made anterior

pituitary transplants into immature mice which had been previously irradiated, and produced precocious puberty in 85-100 hours. If, therefore, this interstitial tissue is derived from granulosa cells, and resembles them so closely that it not only responds to the action of Prolan A, but is also capable of undergoing luteinization, it seems reasonable to suppose that its action does not represent the normal action of stromal tissue, but rather is indicative of the action of the membrana granulosa from which it arose, and whose function it carries on. This view would account for the fact that although the oestrus cycles persist, they are often irregular in the time of onset and duration.

(4) Genther[95] has recently worked with guinea pigs, and reports that by varying the dosage and filtration of the rays, different ovarian effects are produced. She distinguishes four groups :

(a) Ovarian changes similar to those of Parkes resulted in the continuance of irregular oestrus cycles.

(b) The ovaries shewed the presence of only under-developed follicles, together with a small amount of newly formed 'interstitial' tissue. All oestrus cycles ceased.

(c) The ovaries shewed only under-developed follicles. There were no corpora lutea, and the stroma was fibrous and compact. This resulted in complete ablation of the oestrus cycles.

(d) The ovarian changes were the same as in (c), but were temporary, and follicles began to develop later. This produced temporary cessation of oestrus only.

She shewed, therefore, that oestrus never occurred unless there were present in the ovary, either ripening graffian follicles, or a moderate amount of newly formed 'interstitial tissue'. If the granulosa cells were actually destroyed, instead of being converted into a new tissue, all oestrus cycles ceased.

It is not my intention to set out all the evidence in favour of the follicular origin of oestrin : this is well known. It will suffice to mention that Schuschnania[152] has recently brought to light another fact in its favour. He has found that patients suffering from folliculomata of the ovaries have large amounts of the hormone in the blood and urine. It is significant that increased production of oestrin should coincide with a hyperactivity of the granulosa cells.

It would appear therefore, that no evidence has

been produced to disprove the generally accepted view that in the normal animal, oestrin is the secretion of the granulosa cells.

If then, oestrin is produced by the ripening Graffian follicle, one has to explain how this hormone is produced in such large quantities during pregnancy when no follicular ripening occurs. It might appear that this fact alone tends to shew that the interstitial cells of the ovary are perhaps, after all, the site of origin of this hormone. This is not necessarily true, since it is highly probable that the placenta replaces the ovary in as far as the production of oestrin is concerned.

It has been known for many years that the placenta contains large quantities of oestrin, and Smith[163] says that 1 gram of placenta contains almost twice as much of this hormone as 1 cc of blood. One must therefore conclude that either the hormone is produced in the placenta, or that it is produced elsewhere, and is concentrated at that site. Parkes[170] discredits the view that the placenta produces oestrin, and Parkes and Bellerby[133] suggest that the placenta absorbs oestrin from the maternal circulation, in order to protect the male foetus from its action. This theory is not true, since it has already been pointed out in this thesis that the urines of five still born male babies all contained the ovarian hormone, as did also the

blood collected from the umbilical cord in four other cases. This point is interesting, since Herrmann and Stein[103] first, and later, Allen and Doisy[7], and Fellner[75], have all shewn that oestrin inhibits sexual development in males. One might conjecture that this initial handicap is an explanation of the fact that the onset of puberty in a male always occurs later than in the female.

I have been fortunate enough to be able to study the hormonic content of the urine of a patient whose ovaries were removed during pregnancy. The details of the case were as follows :

Patient. Mrs.A.R. Age 44.

Symptoms. Amenorrhoea for 14 weeks. Vomiting.

Physical Signs. Uterus enlarged to the size of a 12 weeks pregnancy. Bilateral, hard, and nodular, appendage swellings.

Aschheim Zondek Test. (27.11.30) Positive.

After History.

15.12.31. (16 weeks pregnancy). The patient was operated on : the diagnosis of pregnancy was confirmed, and bilateral ovarian fibromata was removed. In removing these tumours, all ovarian tissue, including the corpus luteum of pregnancy, was excised.

Further Tests on the Urine.

30. 12. 31. (18 weeks pregnancy). A positive Aschheim Zondek reaction was again obtained and shewed no difference

from that obtained before operation. At the same time, the urine was tested on three ovariectomised mice, and oestrus was produced, shewing that in spite of the absence of the ovaries oestrin was still being excreted in large amounts.

29. 1. 31. (22 weeks pregnancy). The amount of oestrin was estimated by the method stated previously. It was found that the urine contained 1,250 M.U. per litre. Aschheim and Zondek[14] give the normal as being 1,000 M.U. per litre, although in a later publication[13], they state that between 4,000 and 10,000 M.U. per litre can be found.

17. 2. 31. (25 weeks pregnancy). The urine was shewn to contain over 2,000 M.U. of the ovarian hormone per litre. The Aschheim-Zondek reaction was again positive, but shewed less Prolan B effects, and an excessive oestrin response. These findings were significant in view of the after events.

8. 4. 31. (32 weeks pregnancy). Oestrin was present, the Aschheim-Zondek reaction was positive, but not very pronounced.

28. 4. 31. (35 weeks pregnancy). The oestrus producing hormone was still present in large quantities and although no attempt was made to estimate the exact amount, there was at least 800 M.U. per litre.

Further History

At this juncture, the patient's medical attendant reported that the uterus was only 24 weeks in size, and that no foetal movements had been felt since the third week in February. The patient, however, left her native town, and all trace of her was lost with the exception that a report was received from her next medical attendant saying that, on June 6th 1931, a very much macerated foetus was delivered : the sex and age of this were impossible to determine.

It would have been of interest to have examined the urine again after the expulsion of the foetus : however, all attempts to obtain specimens, or to learn more about the case, failed.

Two similar cases have been reported recently by Allan and Dodds[5] , and Szarka[167] : both these observers found that oestrin was excreted in the urine during pregnancy although the patients had no ovarian tissue. Amati[11] has also found the follicular hormone in the blood serum of a pregnant patient whose ovaries had been removed.

Such evidence is undeniable proof that the ovaries, during pregnancy, are not the prime source of oestrin, and it seems permissible to assume that it is the placenta itself which produces it. As to which special part of the placenta is responsible, Fanz and Gault[71] have suggested that there is

an association between the development of the syncytium and the production of oestrin.

Prolan A and Prolan B

These hormones were first demonstrated in the anterior lobe of the pituitary and have become generally known as the pituitary hormones. There is no question that they are produced, and can be found, in the pituitaries of all mammals, male and female, pregnant and non-pregnant, from the time of their birth to their death.

Evans and Simpson[69] in 1928 first put forward the view that the eosinophil cells of the hypophysis are responsible for the production of Prolan B, while the basophil cells are associated with Prolan A formation. Smith[162] also expounded this theory, and Bailey and Davidoff[21] supported it when they pointed out that there is an excess of eosinophils in acromegaly, and that Prolan B sometimes occurs in the urine in cases of that disease. In addition, Wagenen[171] says that the appearance of 'castration cells', derived from the basophils, is an explanation of the occurrence of Prolan A in the urine after bilateral oophorectomy. On the other hand, Blair Bell[23] stated long ago that these castration cells arise from the eosinophils : Philipp[138] is also of this opinion. If this is true it would imply that the production of Prolan A is associated with the acid-staining cells, and not with the basophils.

During pregnancy the pituitary is hypertrophied, and Blair Bell[23] describes an increase both in the eosinophil, and the chromophobe, cells. So which ever of these tissues is responsible for the origin of the different hormones, one would expect an increased production of both Prolan A and Prolan B after conception has occurred. Aschheim and Zondek[17] demonstrated that implants of human placenta into non-pregnant animals caused a similar hypophyseal hypertrophy, while Lehmann[116] described the production of typical pregnancy changes in the pituitary following the injection of placental extracts. These facts serve to explain the intimate association between a positive pregnancy test and the presence of living chorionic villi in the body.

However, the anterior lobe of the pituitary of animals is also hypertrophied during pregnancy, and yet neither Prolan A nor Prolan B have ever been found in their urines. This could be explained by the facts that :

(i) The hormones, although present, are not excreted by the kidneys. This is not true since the hormones have never been found in the blood stream.

(ii) The hormones are present, but in small quantities. Evidence against this is that large animals such as the cow, and horse, do not give a positive reaction, nor does the concentrated urine of smaller animals produce any ovarian response in mice.

(iii) The hormonic regulation of pregnancy may be different in animals from that in the human being.

(iv) It is not the anterior pituitary which is the site of origin of the hormones which are found in the urine of a pregnant woman.

This last suggestion has recently received strong support from Collip[42] , Philipp[137,139] , and Bourg[30] : they are of the opinion that the placenta is directly responsible for the production of the hormones, and not indirectly via the hypophysis. Since it has already been shown that, during pregnancy, oestrin is produced in the absence of the ovaries, it seems feasible that Prolan A and Prolan B should also be produced in the absence of the pituitary. The obvious experiment of performing hypophysectomy on pregnant animals is valueless since animals do not give a positive test.

The evidence in favour of a placental origin for the hormones is :

(i) The human placenta contains considerable quantities of Prolan A and Prolan B, and placental extracts produce exactly the same ovarian responses as pregnancy urine. It has been suggested that this is due to the fact that the placenta merely absorbs the hormones from the general circulation in order to protect the foetus from their action. This has already been disproved by the demonstration of Prolan A in the blood from the umbilical cord, and Siegert and Schmidt-Neumann[156] actually state that the concentration of the hormones in the blood of

the foetus is exactly the same as in that of the mother.

(ii) The placentae of all laboratory animals contain no hypophyseal hormones : this coincides with the negative reactions given by the urines of these animals. It may be that their placentae have no power to absorb these factors, yet this has been discounted by Hill and Parkes[104] who injected hypophysectomised pregnant rabbits with the urine of a pregnant woman, and recovered the hormones from the placentae.

(iii) The relationship between the presence of the hormones in the urine, and placental elements, is so intimate that it seems to imply that the two conditions have a direct connection rather than an indirect one through the pituitary. On removal of the placenta the hormones disappear from the urine more rapidly than one would expect if their disappearance depended on a gradual retrogression of a hypertrophied hypophysis.

(iv) The hormonic content of the body fluids is directly proportional to the activity of the chorion. For example, in hydatidiform mole so much Prolan B is produced that corpus luteum cysts are found in the patients' ovaries. However, Wagner (quoted by Kraul[112]) has described the occurrence of similar cysts in a case of pituitary tumour. Moreover, Novak

and Koff[128] have shewn that excess pregnancy changes are to be found in the hypophysis in cases of hydatidiform mole and chorion epithelioma. After these conditions, they say, this gland takes longer to retrogress and so the hormones persist for a longer time in the urine.

(v) Extracts of chorion epithelioma, or hydatidiform mole, produce ovarian responses in the mice. There seems to be no reason why such neoplastic tissue should absorb hormones. If they merely contain an excess of these principles in view of their vascularity, then why do not other vascular organs, such as the spleen and liver, contain them ?

(vi) Differences in the action of pituitary, placental and urinary hormones have been described :

(a) Evans and Simpson[69] point out that alkaline extracts of the anterior pituitary contain a growth factor as well as Prolan B, whereas acid, or alkaline, placental extracts contain no such principle. Pregnancy urine likewise, produces no growth effects in the test animal.

(b) In experimenting with pituitary transplants, Engle[63] found that they produced not only ovulation but 'superovulation', whereas in the performance of Aschheim-Zondek tests ovulation never occurred in the mice ovaries. Fee and Parkes[72], Jares[108], and recent writers, do not agree with this latter statement.

(c) Collip[42] reports that emmenine, the placental equivalent of Prolan A, never produces abortion in animals, where as Aschheim and Zondek[18], and Engle and Mermod[64], found that ovulation and abortion occurred if hypophyseal transplants were made during pregnancy.

(d) The increase in size of the ovary resulting from pituitary injections is directly proportionel to the amount of gland used. There is no relationship between the amount of urine injected and the ovarian responses obtained (Evans and Simpson[70]).

(e) Pituitary hormones are destroyed by enzymes, and are not effective by the oral route (Smith[161]). Collip[44], however, has shewn that emmenine differs in this respect, and is active when administered by the mouth.

(f) Corner[48] says that lactation can be produced in non-pregnant animals by hypophyseal extracts, whereas injection of pregnancy urine produces no breast changes.

Against the view that the placenta is the site of formation of these hormones there is the following additional evidence :

(a) The fact that Prolan A can be demonstrated in the urine in conditions other than pregnancy.

(b) Zondek[182] says that the hormonic content of the human placenta is only 2-3 per cent of that of the blood and urine.

(c) Zondek[179], Bacon[20], and Philipp[139] have all shown that the human anterior pituitary contains less hormones during pregnancy, than in the non-pregnant state. However, these glands in animals do not have their hormonic content lowered after conception occurs. This would imply that, in the first case, Prolan A and Prolan B are being passed out into the general circulation, while in the second, they are being retainedwithin the hypophysis. This would account for the negative test given by the urine of pregnant animals.

(d) Kraul[112] has found that if an animal is injected with placental extracts prior to removing its pituitary for transplantation, the effects of such transplants are greater than the normal.

It would appear, therefore that there is no definite evidence on which one can base an opinion as to

(i) Whether the hormones occurring in the placenta, pituitary, and urine of pregnancy, are identical substances, or

(ii) Whether the site of origin of the hormones found in the urine during pregnancy, is the placenta or the hypophysis.

However, the points outlined above make one feel that " a theory according to which the placenta is considered the ductless gland of pregnancy ..... would fit the observed facts much better" (Collip[42]). Zondek[183] suggests a dual origin of the hormones : he contends that the pituitary is responsible in the early stages, but in the later stages of pregnancy, its function is taken over by the placenta.

Recently the problem has been complicated by the work of Hill and Parkes[104] who suggest that in the Aschheim-Zondek reaction, the urine acts via the anterior pituitary of the test animal. They claim that ovulation does not occur in rabbits which have neither the pituitary, nor the placenta, in situ. The presence of either of these organs ensures ovulation being obtained. In estimating the value of these experiments, it must be remembered that they were performed on decerebrate animals. The opposite results were obtained by Van Dyke (quoted by Collip[42]), who shewed that emmenine is effective in hypophysectomised animals.

Whether the placenta actually produces the hormones, or whether it merely stimulates the pituitary to form them, one has to explain why negative Aschheim-Zondek reactions are given by the urines of pregnant animals. This, as Allen and Doisy[7] originally stated, is probably accounted for by the differences in structure, and power of invasion, of the chorionic elements in the different species.

The Occurrence of the Pituitary Hormones in the Urine of non-pregnant patients

Prolan B

Throughout this thesis it has been insisted that evidence of the presence of Prolan B in the urine is diagnostic of pregnancy. However, Zondek[184] claims to have obtained A.P.R.II and A.P.R.III in mice injected with urine from two cases of carcinoma cervicis. The same author, in a different publication[178], says that Prolan B may be found in the urine in occasional cases after the patient has had both ovaries removed. Fluhmann[82], using the blood serum of women at the menopause or after castration, sometimes obtained evidence of the presence of this hormone, while Allan and Dickens[3] reported an error in pregnancy diagnosis which arose because the urine from a patient at the menopause produced corpora haemorrhagica in the test animals. Hannan[98] obtained a positive Aschheim-Zondek reaction in two cases of genital carcinoma, and Stewart[164] reported a similar finding in a case of hyperthyroidism.

In the face of such evidence, most of it confirmed by other workers, one cannot but believe that Prolan B can occasionally occur in the urine of non-pregnant women. However, I can only state that in all the tests I have made, there has never been any sign of the presence of the luteinizing factor in the urine, except when the patient has had active chorionic elements in the body.

Prolan A

All observers agree that the follicular ripening factor can be found quite frequently in the blood-stream, or urine, of non-pregnant patients. It is for this reason that evidence of Prolan A in the urine should never be accepted as a positive Aschheim-Zondek reaction.

In investigating the occurrence of this hormone, urine was injected into immature mice, and the technique employed was the same as that used in the Aschheim-Zondek tests. Some of the urines were concentrated, others were not. The results will be classified according to the clinical conditions of the patients whose urines were examined.

I. At the Menopause

At this period, owing to the cessation of ovarian function, there is a necessary readjustment of the endocrine balance in the body. It is not surprising, therefore, that most workers have occasionally found Prolan A in the urine at this time. Hannan[98] actually states that out of 12 cases examined he obtained 4 positive pregnancy reactions. Zondek[179] reports the occurrence of the follicle-stimulating hormone in the urine of menopausal patients : he is of the opinion that it appears late in this physiological process. Fluhmann[82] , who examined blood sera, found that at the onset of menopausal symptoms, 33 per cent. of patients had demonstrable quantities of Prolan A in the circulation. At

a later stage in, or just past, the menopause, he shewed that five patients out of seven had this hormone in the blood-stream.

I examined the urines of 6 women who were at, or about, the menopause, and obtained the following results :

No.	Age	Symptoms	Prolan A	Urine
1	50	Irregular menstruation. "Hot flushes" 2 years	Nil	C
2	43	3 months' Amenorrhoea	+	N.C.
3	42	Irregular menstruation. "Floodings"	Mice died	C
4	50	8 months Amenorrhoea	+	N.C.
*5	48	Menopausal bleeding	Nil	N.C.
°6	47	Irregular menstruation	+	N.C.

C = concentrated urine. N.C. = non-concentrated urine.
* = Case No.52 of pregnancy diagnosis series.
° = Case No.23 " " " "

Of these 6 urines, one killed the mice, three contained the hormone, and two did not. It is to be noted that those patients who excreted the hormone had only had menopausal symptoms for a short time.

Zondek[179] in discussing the reason for the excretion of Prolan A at the menopause says that it must be because :

(1) The ovaries cannot utilize it, or

(2) The functioning ovary normally exerts an inhibitory action on the pituitary.

He points out that since hypophyseal transplants will stimulate the senile ovary of a mouse or rat, the first view cannot be accepted and the second must be the true explanation.

II. After Castration

Fichera[78] in 1905 first described the hypertrophy of the pituitary which follows removal of both ovaries. Blair Bell[23] confirmed this finding and described the microscopical changes which occur. He found that there is a marked increase in the number of eosinophils, and a less noticeable increase in the number of basophils. This variation in cell type has been much discussed, and some observers consider that the changes occurring are degenerative ones. However, Evans and Simpson[70] have found that bilateral gonadectomy, performed prior to removal of an animal's pituitary for transplantation experiments, increases the potency of such transplants. In addition, Zondek[184], and Fluhmann[80,82] have demonstrated the presence of Prolan A in the urine and blood serum of patients following bilateral oophorectomy. These two facts seem to prove conclusively that the pituitary changes are not degenerative, but hyperplastic.

Zondek[184] says that, after castration, the follicular ripening hormone can be found in the urine in quantities as large as 150 R.U. per litre.

An attempt was made to verify these findings and the following results were obtained :-

Results

Case	Age of patient	Operation Notes	Time after operation at which test was made.	Prolan A	Urine
1	42	Panhysterectomy for menorrhagia	14 days	Nil	C
2	31	Bilateral Salpingo-oophorectomy for Pelvic Infection	14 " 6 weeks	Nil +	C C
3	42	Panhysterectomy for cystic ovaries and endometrioma	11 days 22 " 56 "	++ N.R. Nil	N.C. N.C. N.C.
4	41	Panhysterectomy for Pelvic Infection.	3 " 5 " 14 " 24 " 28 "	Nil Nil Nil Nil Nil	N.C. N.C. C N.C. N.C.
5	51 P.M.	Panhysterectomy for Fibro-myomata Uteri	5 " 14 "	Nil Nil	N.C. N.C.
6	55 P.M.	Panhysterectomy for Ovarian Cystadenoma	11 " 18 "	Nil Nil	N.C. N.C.
7	69 P.M.	Panhysterectomy for Ovarian Cystadenoma	20 "	Nil	N.C.
8	38	Panhysterectomy for Fibro-myomata uteri. Ovarian Graft	18 " 28 " 56 " 70 "	Nil Nil Nil Nil	N.C. N.C. C C
9	29	Bilateral Salpingo-oophorectomy for Pelvic Infection. Ovarian Graft	14 "	+	C
10	40	Panhysterectomy for Fibromyomata uteri and ovarian endometriomata. Ovarian Graft.	14 " 42 "	++ +	C C

P.M. - Post-menopause
N.C. - non-concentrated urine C = concentrated urine
N.R. - No result because mice died.

From this table it is seen that 4 patients, under the menopausal age, had bilateral oophorectomy without an ovarian graft. Of these, 2 shewed Prolan A in the urine subsequent to operation. In one, the hormone appeared as early as the 11th day after castration, but was not present later : in the other, it was not found until six weeks after the removal of the ovaries. This small number of cases does not enable one to say at what time the hormone usually appears, and disappears, but Fluhmann[82] obtained a positive result as early as the 9th day, and as late as 14 years after the operation. He also found that some urines which had been negative at first became positive later : this is well shewn by Case 2 of the above series, and is perhaps a reason why negative results were obtained in some of the other cases. In Case 3, Prolan A although present at first, disappeared later : this would seem to infer that its occurrence is merely a temporary effect of the endocrine disturbance.

This variation in the time of the occurrence of the hormone is in accordance with the findings of Rössle[148] who shewed that the changes in the pituitary occurred sometimes as early as five days after castration, while in others a considerable time elapsed before they were manifest. Moreover, Blair Bell[25] in 1913 said "removal of the ovaries appears to exert a certain influence over the secretory function of the pituitary body..... But in my experience the change is moderate and not quite constant.....

..... . I believe the effect is more or less temporary".

This over-activity of the hypophysis, since it occurs both at the menopause and after castration, is obviously due to the removal of ovarian stimulation. As to what ovarian factor is concerned there appears to be some dispute. Kraul[112] quotes other workers who have shewn that injections of oestrin after castration will prevent the changes occurring in the anterior pituitary. However, Fluhmann and Kulchar[81] claim to have shewn that the appearance of 'castration' cells in the hypophysis is not related to a diminished amount of oestrin. Fluhmann[82] found that constant administration of the follicular hormone to castrated rats over a period of three months, failed to prevent the typical pituitary changes. But Mahnert and Siegmund[121] demonstrated that injection of oestrin into a normal animal delayed ovulation, and since the latter event is now known to be dependent on the pituitary, it is probable that an excess of oestrin in the body diminishes the Prolan A output, and vice versa.

On this assumption, it is possible to explain the periodic ripening of the normal Graffian follicle. When no follicle is ripe, and no oestrin is formed, the hypophysis secretes the follicle-ripening hormone. This, having fulfilled its function, and thereby brought about the production of oestrin, the latter hormone reacts on the pituitary preventing further secretion of Prolan A.

Fig.21. Test mouse and control mouse shewing ovarian and uterine enlargements due to Prolan A in the urine of a woman 14 days after castration. These animals were from case 10.

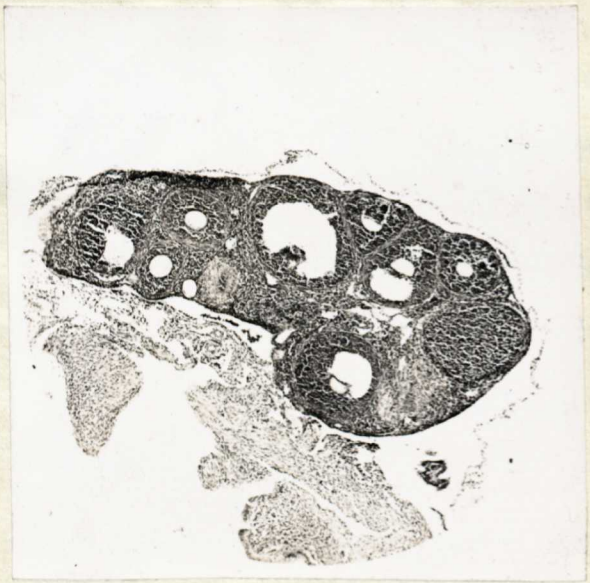


Fig.22 A microscopical section of an ovary of the mouse seen in Fig.21. This shews follicular ripening due to the pituitary hormone. x 45.

Returning to a consideration of the other results that I obtained, it is seen that in three cases the patients were past the menopause before their ovaries were removed. None of these women shewed any Prolan A in the urine : this finding fits in with the above hypothesis.

In the three remaining cases, the patients had an ovarian graft made into the rectus abdominis muscle. One would expect that in these instances no pituitary hormone would have been found in the urine, yet it occurred in considerable quantities in two of the cases (see Figs.21 & 22). However, enquiries into the operative details of these two cases, reveal that in both, the ovary was so diseased that only a very small portion was available for grafting. Although this, in one patient was sufficient to promote the continuance of menstruation, yet one can imagine that there would be a considerable reduction in the amount of functional ovarian tissue.

It is interesting that Fluhmann[82] found that whereas, after operative or radiation castration, at the menopause, and in some patients with prolonged periods of amenorrhoea, the follicle-stimulating hormone appeared in the urine, yet in women with scanty and infrequent irregular menstruation, no hormone was found. This he attributes to the fact that in the latter conditions, the hypo-oophorism is merely secondary to a hypo-activity of the hypophysis. Again he

found Prolan A in some cases of menorrhagia, and severe dysmenorrhoea : this he explains by assuming that the underlying cause of such conditions, is hyperactivity of the anterior lobe of the pituitary.

It seems, therefore, that providing there is no primary pituitary dysfunction, Riddle[146] has expounded the most acceptable view when he says "the gonad hormones, and the gonad-stimulating hormone of the pituitary closely control each other. Injection of gonadal hormones of either type, male or female, depresses the release of the gonad-stimulating hormone by the pituitary..... On the contrary, when the amount of gonad, or its hormone output, is small, the pituitary then furthers its growth and hormone secretion by supplying an effectively large amount of the gonad-stimulating hormone."

In view of these findings, and in view of the fact that animals give a negative pregnancy test, it is of interest to know if pituitary hormones are excreted in the urine of castrated animals. Kraul[112] found that the hypophyses of rabbits after removal of the ovaries, produced greater Prolan A and Prolan B effects when transplanted into immature rats. I removed the ovaries from six rabbits and collected subsequent specimens of urine in a metabolism cage. On testing these specimens on immature mice, the following results were obtained :

Expt.	Age of Animal	Operation	Time after operation	Prolan A in the Urine	Urine
1	Adult	Bilateral oophorectomy	13 days 4 weeks 6 weeks 10 weeks	++ ++ M.D. Nil	C C C C
2	Adult	Panhysterectomy	14 days 4 weeks 7 weeks	M.D. Nil Nil	C C C
3	Adult	Bilateral oophorectomy	16 days	Nil	C
4	12 weeks	Bilateral oophorectomy	9 days	+	C
5	Adult	Bilateral oophorectomy	10 days 10 " 20 " 5 weeks 5 " 6 " 8 "	M.D. Nil M.D. M.D. ?+ Nil M.D.	N.C. C C N.C. C C C
6	Adult	Bilateral oophorectomy. Rabbit died 6 days later.	3 days	M.D.	N.C.

C = concentrated urine.
N.C. = non-concentrated urine.
M.D. = mice died.

One of the rabbits died six days after operation, and so cannot be considered in assessing the results. Of the remaining five, two definitely, and a third doubtfully, excreted Prolan A in the urine subsequent to castration.

The hormone was present as early as nine days after operation, but judging from experiments 1 and 5 it would appear

that its occurrence is only temporary. One of the rabbits (Expt.4) had not reached puberty at the time of the operation : it is, therefore, interesting to notice that the pituitary hormone was found in the urine in this case.

The finding of the hypophyseal hormone in the urine of an animal is remarkable : it at least proves that the pituitary of a rabbit is capable of producing it in quantities sufficient to be demonstrable in the urine. It, therefore, offers a still further link in the chain of evidence pointing to a placental origin of the hormones found in the urine of a pregnant woman.

III. After Thyroidectomy

Schilder[149] in 1911 first shewed that histological changes occur in the pituitary in conditions of thyro-aplasia. Blair Bell[25] in 1913 described in detail the hypophyseal changes following thyroidectomy in animals. He said that there is an increase in active eosinophil cells at the expense of the basophils : there is also an increase in chromophobes such as occurs normally during pregnancy. In view of this, he concludes that "thyroidectomy causes an increase in the secretory activity of all parts of the pituitary body". The same author says that there is more hypophyseal activity following thyroidectomy than following oophorectomy. However, Bryant[37] although finding an increase in chromophobe cells in the pituitary of a rabbit after thyroidectomy, does not believe that the activity of this gland is increased.

Smith[159] shewed that ablation of the anterior pituitary leads to atrophy of the thyroid, and that this effect can be prevented by pituitary transplants. Later, Evans and Simpson[69] went so far as to suggest that the anterior lobe of the hypophysis secretes a separate principle whose sole function is to stimulate thyroid activity : they quote the work of Smith as pointing to an association between the eosinophil cells and this active principle. Crewe and Wiesner[175] have lately produced more evidence in favour of the presence of a separate thyreotropic factor in the pituitary : this they call the 'theta factor'. Within the last few months, Gant[94] , while working with the amblystoma Jeffersonianum, has found that heteroplastic anterior pituitary transplants result in an increased activity of the thyroid gland, and that secretion and discharge of the follicular colloid is stimulated. Silverberg[157] has also shewn that injections of hypophyseal extracts stimulate the thyroid in guinea pigs.

The bulk of evidence, therefore, points to a thyroid-pituitary relationship which is somewhat similar to that of the ovary and the pituitary. In view of this I decided to make tests to see if there was any evidence of the excretion of pituitary hormones following removal of the thyroid influence from the body. Total thyroidectomy cannot be perfofmed in the human being so I chose patients who had had approximately five-sixths of the gland removed for exophthalmic goitre. This choice was made since it was felt that such

patients, more than any others, would be subject to an immediate marked decrease of the thyroid secretion in the body.

Results

Case	Sex of Patient	Time of Test in relation to Operation.	Prolan A in the Urine	Urine
1	Male	Before operation 5 days after operation " " " "	Nil Nil M.D.	N.C. N.C. C
2	Male	10 days after operation 16 " " " 22 " " "	++ + Nil	N.C. C C
3	Male	3 days after operation	Nil	N.C.
4	Male	12 days after operation	?+	C
5	Female	Before operation 9 days after operation	Nil ?+	N.C. C
6	Female	11 days after operation	Nil	C
7	Female	Before operation 9 days after operation 11 days " " 18 " " "	Nil + + +	N.C. C C C
8	Female	7 days after operation	++	N.C.
9	Female	12 days after operation 12 " " "	M.D. Nil	N.C. C

N.C. = non-concentrated. C = concentrated.
M.D. = Mice died.

Out of the 9 cases examined, in three definitely, and in two others doubtfully, a hormone, whose action was similar to that of Prolan A (See Fig.23), appeared in the urine following removal of the greater part of an over-active thyroid. It was present as early as the seventh day, and in Case 2 its occurrence was only temporary. Possibly if the whole gland was removed, all patients, male and female, would excrete the hormone subsequently. It would be interesting to perform total thyroidectomy on animals and to examine their urine subsequently : up to the present, I have had no opportunity for doing this.

Blair Bell[25] shewed increased activity of the thyroid following oophorectomy : in the light of present knowledge, it may be conjectured that this effect is only secondary to the pituitary hyperplasia which follows castration. In the same way it is possible to explain the enlargement of the thyroid which sometimes occurs at puberty in patients with hypo-oophorism. The increased thyroid activity during pregnancy may be the result of the large amounts of Prolan A, or an associated 'theta factor', in the circulation, or may be secondary to a diminished ovarian activity at that time. This theory is borne out by the work of Riddle[145] who shewed that there is a regression in the thyroid of doves during periods of ovarian activity.

Out of this mass of evidence one arrives at a possible explanation as to why thyroid therapy has been found to be

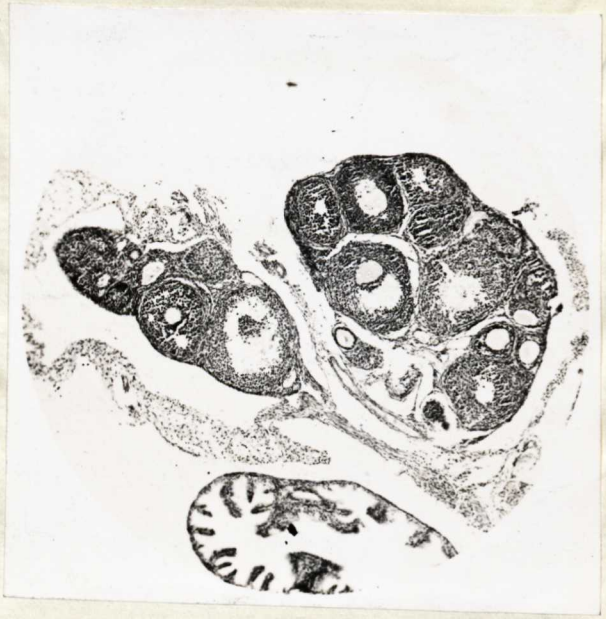


Fig.23.(a) The ovarian responses resulting from injection of the urine obtained from a woman 7 days after partial thyroidectomy (case 8). The follicular ripening infers the presence of either Prolan A, or of a hormone with a similar action. X 45.

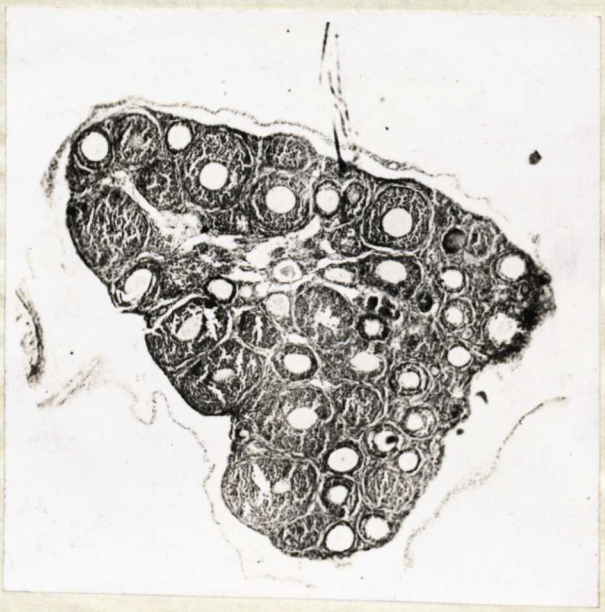


Fig.23.(b) The ovary of a normal immature mouse used as a control in the above test. x 60.

effective in ensuring the continuance of pregnancy to term in those women who have had a series of abortions and premature labours for no obvious reason. The etiology of these conditions may be related to an increased Prolan A, or oestrin, output : in either case administration of thyroid will tend to inhibit the hypophyseal activity, and to diminish its secretion of the follicle-ripening hormone.

IV. In Malignant Disease

Zondek[184] was the first to stress that a malignant neoplastic condition in a patient often resulted in the appearance of Prolan A in the urine. He found that 81.8 per cent. of female patients with genital carcinoma excreted the hormone in the urine. It occurred in amounts as great as 200 R.U. per litre. In cases of extragenital carcinoma, the percentage of positive results was only 36. This writer also maintains that the presence of the hormone had no relation to the age of the patient, and was not associated with the menopause. Moreover, it did not depend on the type of tumour.

Dingemanse and his co-workers[55] found an excess of oestrin in patients suffering from malignant conditions.

I have investigated the hormonic content of the urines of male and female patients suffering from malignant neoplasms in various parts of the body. It has already been pointed out that such urines are very toxic, and in most cases the concentration process was adopted. All the clinical diagnoses were confirmed by operation. or post-mortem examination.

Results

Male Patients

(A) Genital Carcinoma

Case	Disease	Prolan A	Urine
1	Teratoma of Testis Secondaries in Lungs	? +	N.C.
2	Sarcoma of Testis	Nil	C
3	Epithelioma of penis	+	C
4	Sarcoma of Testis	M.D.	N.C.

Out of the three cases in which a result was obtained, one definitely had Prolan A in the urine, and another probably had small amounts. It would be of interest to investigate a case of chorion epithelioma of the testis : no such case has come within my notice since I began this investigation.

(B) Extragenital Carcinoma

Case	Disease	Prolan A	Urine
1	Carcinoma Ventriculi	Nil	C
2	Carcinoma Ventriculi	Nil	C
3	Carcinoma Ventriculi	Nil	C
4	Carcinoma Ventriculi	M.D.	N.C.
5	Carcinoma Ventriculi Secondaries in Liver	Nil	N.C.

Extragenital Carcinoma (contd.).

Case	Disease	Prolan A	Urine
6	Carcinoma Coli	Nil	N.C.
7	Carcinoma Coli	Nil	N.C.
8	Carcinoma of Kidney. Metastases in Lungs	M.D.	C
9	Sarcoma of Mediastinum	Nil	C
10	Retro-peritoneal sarcoma	Nil	C
11	Sarcoma of Femur	Nil	C

A result was obtained in 9 instances : in none was there any evidence of the pituitary hormone.

Zondek[184] found that 13 per cent. of male patients suffering from malignant disease, gave a positive test for Prolan A. Taking both of the above series together, 2 out of 12 patients excreted the hormone in the urine, that is 16.6 per cent.

Female Patients

(A) Genital Carcinoma

Zondek[184] demonstrated Prolan A in the urine in almost all cases of genital carcinoma. He said that

82.5 per cent. of cases of Carcinoma Cervicis were positive.

75.0 per cent. " " " Carcinoma Corpus Uteri were positive.

75.0 per cent. of cases of Ovarian Carcinoma were positive.

100.0 per cent. " " " Epithelioma Vulvae were positive.

I performed 24 tests on the urine of 17 patients. In 7 of these tests, no result was obtained.

Neglecting those tests in which the mice died, my results, summarized, shew that in :-

Carcinoma	Cervicis	16.6 per cent. of patients have Prolan A in the urine.
"	Corpus Uteri	66.6 per cent. of patients have Prolan A in the urine.
"	Vaginae	75.0 per cent. of patients have Prolan A in the urine.
"	Vulvae	50.0 per cent. of patients have Prolan A in the urine.

The one case of carcinoma of the ovaries had no Prolan A in the urine.

The detailed results were as follows :-

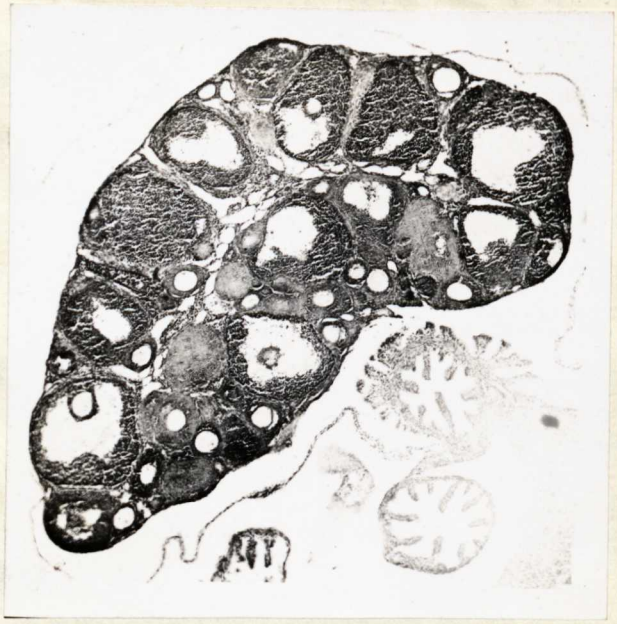


Fig.24.(a) Shewing follicular ripening produced by the injection of urine obtained from a woman suffering from carcinoma cervicis (case 6) x 45.

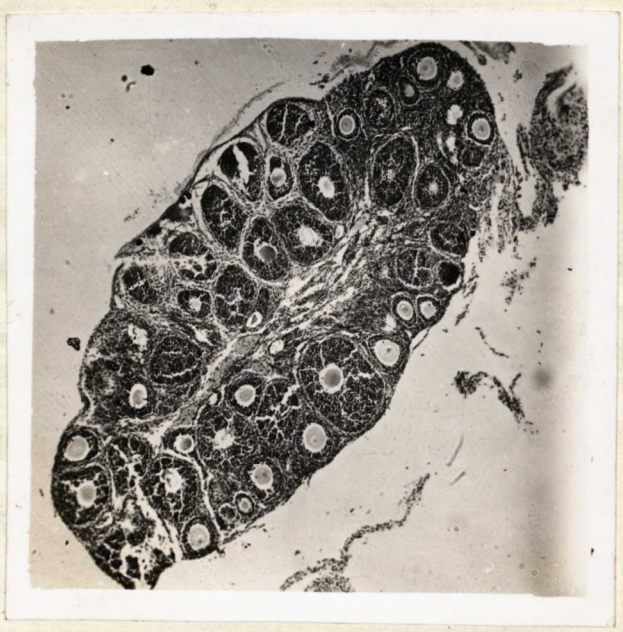


Fig.24.(b) Ovary of a normal immature mouse used as control in the above test. x 45.

Case	Disease	Prolan A	Urine
1	Carcinoma Cervicis	(i) Mice died (ii) Mice died	N.C. C
2	" "	(i) Mice died (ii) Nil	N.C. C
3	" "	(i) Nil (ii) Nil	N.C. C
4	" "	Nil	C
5	" "	Nil	C
6	" "	(i) Mice died (ii) + +	C C
7	" "	Nil	C
8	Carcinoma Corpus Uteri	(i) Mice died (ii) +	N.C. C
9	" " "	(i) Mice died (ii) Mice died (iii) +	N.C. N.C. C
10	" " "	Nil	N.C.
11	Carcinoma Vaginae (Primary)	+	C
12	" " "	Nil	C
13	Carcinoma Vaginae secondary to hysterectomy for Carc. Cervicis	++	C
14	Carcinoma Vaginae secondary to hysterectomy for Carc. Cervicis	+	C
15	Epithelioma Vulvae	Nil	N.C.
16	" "	++	C
17	Bilateral Carcinoma of the Ovaries	Nil	C

See Fig.24

B. Extragenital Carcinoma

Case	Disease	Prolan A	Urine
1	Carcinomatosis Peritonæi ? Primary site	(i) Nil (ii) M.D. (iii) M.D.	N.C. N.C. C
2	Carcinoma Coli	(i) M.D. (ii) +	N.C. C
3	Sarcoma of Spine	Nil	N.C.
4	Sarcoma of Clavicle	Nil	C
5	Sarcoma of Femur	Nil	C
6	Carcinoma Ventriculi	Nil	C
7	Carcinoma Mammae (encephaloid)	(i) Nil (ii) M.D. (iii) M.D.	N.C. C C

Of the female patients suffering from extragenital carcinoma and sarcoma, only one out of seven (i.e.14 per cent.) had Prolan A in the urine.

A final series of tests were made on rabbits innoculated with the 'Brown-Pearce' tumour. The growth was extensive in each animal whose urine was examined. Altogether the urine from 8 rabbits, the serum from another, and ascitic fluid from a tenth, were tested. In every case I failed to demonstrate the presence of Prolan A or any other hormone.

It is, therefore, rare to find Prolan A in the urine of male and female patients suffering from extragenital carcinoma : only once was it demonstrable in such conditions. On the other hand, its occurrence is common when the malignant disease has its site in the genitalia. There is no doubt that the appearance of this hormone is associated with the presence of the growth, since in the two cases of carcinoma corpus uteri which gave positive results, Prolan A disappeared from the urines within ten days of hysterectomy. Moreover, Zondek[184] found that not only did the pituitary hormone vanish from the urine after removal of the growth, but that it reappeared in the presence of a recurrence.

It is difficult to explain why malignant tissue should be associated with the excretion of Prolan A. It may be :-

(i) That the growth actually manufactures the hormone.

Since Blair Bell has expounded the view that the behaviour of malignant tissue resembles that of the chorion, this may be possible. However, I have extracted tumours, both human and animal, and have failed to demonstrate the presence of any hormone in such preparations. Moreover, if this explanation was correct, one would expect that any growth whether it occurred in the genitalia or elsewhere, would produce Prolan A.

(ii) That the growth may stimulate the pituitary to increased activity.

In favour of this view, Zondek[184] states that many investigators have found 'pregnancy changes' in the hypophyses of patients suffering from non-benign neoplasms. Again, Berblinger[27] shewed that injection of any protein would produce pituitary changes, and he considers that in the case of tumours, the products of tissue breakdown can have this effect. One feels, however, that this theory is untenable, since it does not account for the fact that only genital carcinoma produces Prolan A excretion.

(iii) That the presence of the growth in the genitalia acts as an irritant which gives rise to impulses which pass from the genitalia to the pituitary.

It is believed that, in the rabbit, ovulation at the time of coitus is brought about by the anterior pituitary which is connected to the vulva, vagina, or cervix by a mechanism which is probably nervous. It is likely that such a genito-pituitary mechanism exists in all species, including the human being, though not necessarily for the purpose of precipitating ovulation. If this is true it is feasible that the presence of a growth in the vulva, vagina, or uterus will serve to stimulate the transmission of impulses to the hypophysis. This theory will explain why Prolan A for the most part, is only excreted when the genital tract is the site of the tumour.

V. In Benign Neoplasms

Zondek[184] claims to have demonstrated Prolan A in the urine of patients who had innocent growths. He found that 30 per cent. of patients suffering from fibromyomata uteri excreted the hormone, but in no cases of innocent ovarian tumours did he obtain a positive result.

I only investigated such conditions in the course of perfofming pregnancy diagnosis tests : in every case of ovarian cyst or fibromyomata uteri I failed to find any hormone in the urine.

VI. In Diseases of the Endocrine Glands

It is possible that pathological conditions of the thyroid, suprarenal, and pituitary might, on occasion, result in the appearance of the hypophyseal hormone in the urine. From what has been said, one might expect that in hypothyroidism Prolan A would be formed in large amounts. I examined one case and no hormone was found in the urine. All the patients with exophthalmic goitre gave negative results prior to operation. A case of diabetes insipidus, associated with a posterior pituitary tumour, likewise had no demonstrable amount of the anterior lobe hormone in the urine. However, this specimen of urine had such a low specific gravity that the result was of very little significance. No Prolan A was found in a case of Addison's disease. Lutein cysts of the ovaries have been reported in association with anterior pituitary tumours (Kraul[112]) : these were undoubtedly due to

an excess of Prolan B. Other observers have found Prolan A in acromegaly : I tested the urine and blood serum of one male acromegalic and obtained negative results.

The Significance of the Hormones during Pregnancy.

Oestrin and the Corpus Luteum Hormone.

It is well known that, in many respects, the follicular and the corpus luteum hormones are antagonistic. Ovulation does not occur in the presence of an active corpus luteum. Injection of oestrin during pseudo-pregnancy over-rides the action of the corpus luteum, and prevents its sensitizing action on the uterine mucosa. It is difficult therefore, to explain the abundance of oestrin during pregnancy since this is essentially a luteal phase.

A. Growth of the Uterus

In any normal uterus oestrin produces increased vascularity, hypertrophy of the musculature, and some endometrial hyperplasia (Allen and Doisy[7]). Cordua[46] produced intense enlargement of the uterus by ovarian hormone, but found that 1 cc. of pregnancy serum had more growth-promoting power than 40 units of folliculin. It is probable, therefore, that oestrin is the factor responsible for the enormous development of the uterus during pregnancy. This assumption is borne out by Gamder[93] who shewed that this hormone actually produces an increase in length of the muscle fibres.

On the other hand, the corpus luteum hormone, although it may assist in muscular development, acts primarily on the endometrium. This action is to cause a further proliferation, and a development of the glands up to the formation of secretion. In addition, the corpus luteum sensitizes the endometrium so that it reacts to any stimulus by a deciduoma formation. Allen[8] has shewn that this hormone has no action on an endometrium which has not previously been subjected to the influence of oestrin. Clauberg[41] has outlined the modern conception of the actions of these two hormones, and states that the corpus luteum is not essentially a growth-promoting and proliferative hormone like oestrin, but rather a specific hormone of the uterine mucosa in the pre-gravid phase, and evidently only comes into effect if there is proliferation of the mucosa already present.

B. Uterine Contractions

It has been stated that the onset of parturition depends merely on uterine growth. Allan and Dodds[5] have pointed out that this cannot be true since patients with an abdominal pregnancy have a false labour, and moreover, expulsive contractions of the uterus occur in every case of abortion. Uterine contractions are, therefore, probably associated with some factor in the blood stream.

Many workers have shewn that the uterus of a rabbit fails to respond to pituitrin if it has been previously treated with corpus luteum. It is also a well-known fact

that the yellow body is essential to the continuation of pregnancy during its early stages. Knaus[110] has demonstrated that pituitrin has no effect on the rabbit uterus during the early stages of pregnancy and during pseudo-pregnancy, but that during the latter half of pregnancy, and associated with a diminished corpus luteum activity, the sensitivity of the uterus gradually increases, and reaches a maximum at term.

Blair[28], and Frank and his colleagues[87] have found that the contractions of an isolated uterus vary according to the time in the oestrus cycle at which the organ is removed. Another interesting fact is that in such animals as the mouse, guinea-pig, and rabbit, oestrus always occurs immediately after parturition. This infers that, at that time, there are large amounts of oestrin in the circulation. Smith[163], and Parkes and Bellerby[132] have produced abortion at all stages of pregnancy by injections of oestrin into rats and mice. Brouha and Simonnet[34] have shewn that this is due to an increased sensitivity of the uterus to the action of pituitrin.

Oestrin and the corpus luteum hormone are therefore antagonistic in their effects on uterine muscle : the first increases its sensitivity, the second diminishes it. This fact has lead to the development of a theory explaining the onset of labour : this says that pregnancy continues while Prolan B and the corpus luteum are in the ascendancy, but

that if oestrin becomes dominant the pregnancy is terminated. Allan and Dodds[5] expounded this view, and pointed out that the artificial production of an abnormal quantity of luteal tissue at the end of pregnancy interferes with the normal mechanism of parturition, and so leads to a prolonged gestation period. Miklos[126] also obtained this effect by the injection of corpus luteum extracts into animals near term. Burn and Bourne[32] elaborated the theory and reported a series of experiments on the isolated guinea pig uterus. In these they shewed that oestrin and pituitrin had a synergic effect on uterine contractions. They demonstrated that the addition of oestrin alone caused a slight increase in tone and produced better contractions. The combined action of the follicular hormone and pituitrin produced a muscular contraction almost twice as great as one obtained with pituitrin alone.

Since I had found excess of oestrin in the urine of patients in the first stage of labour, it was decided to perform a similar type of experiment. I intended to demonstrate that a synergic effect could be obtained by the use of pituitrin together with the urine of a patient in labour. It was expected that different results would be produced by such specimens of urine as compared with the results obtained if the urine was taken from non-pregnant patients, or patients in the early months of pregnancy.

The isolated uteri of the guinea pig and the cat,

Fig.25. Urine B. was obtained from a patient in the first stage of labour. The tracing shews that such urine produces a contraction on its own (b) : when it is added together with pituitrin, a contraction is obtained (c) which is almost twice as great as that obtained by pituitrin alone (a & d). Identical tracings were obtained with urines from non-pregnant women, and male patients.

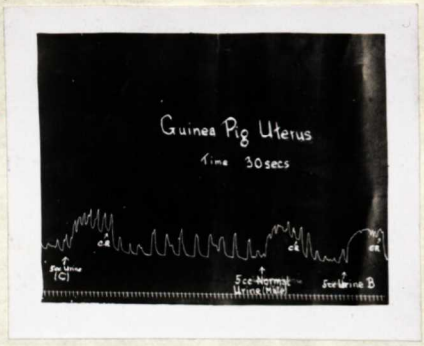


Fig.26. Uterine contractions produced by the addition of urine alone. The urines of patients in early pregnancy (C), of males, and of women in the first stage of labour (B), all gave the same results.

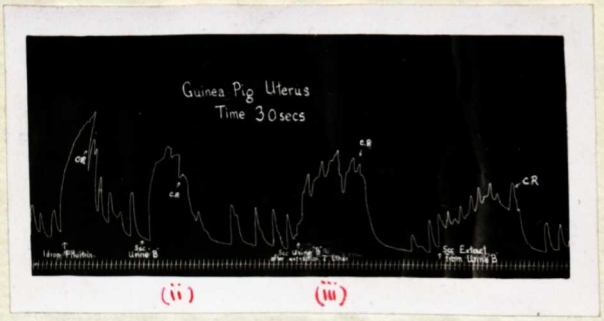


Fig.27. Shewing that if the urine of a patient in labour is deprived of oestrin by ether extraction, it still produces a contraction (iii), and that this contraction is practically the same as the one obtained by the addition of the urine prior to its extraction (ii).

pregnant and non-pregnant, were used. The muscle preparations were suspended in 100 cc. of Ringer's solution, and quantities of urine, varying from one drop to 5.0 cc., were added. All the experiments were repeated several times and the same results were obtained on every occasion.

Experiment 1.

Urine from a patient in the first stage of labour was used. When this was added to the Ringer's solution, a rise in muscle tone resulted. Addition of one spot of pituitrin immediately afterwards produced a contraction which was almost twice as great as the contraction obtained by pituitrin alone (Fig.25). These tracings were almost identical to the ones published by Bourne and Burn. However, this result could not be accepted without a control experiment.

Experiment 2.

Here, urines from males, and non-pregnant women, were used. It was found that not only did such specimens produce contractions when administered alone (Fig.26), but a synergic effect was noticed when they were used in combination with pituitrin. The tracings were exactly the same as those obtained in experiment 1.

Experiment 3.

Ether extracts of the urines of parturient, and non-pregnant women, were prepared by a standard technique. It was found that such extracts all had the same effect in increasing the motility of the uterus no matter whether they contained oestrin or whether they did not (Figs. 27,28 and 29).

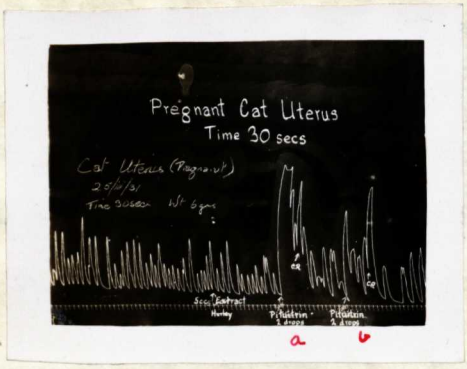


Fig.28. There is a synergic effect obtained if pituitrin is added to a pregnant cat uterus which has been previously exposed to the action of an ether extract of parturient urine (a). The contraction is greater and more prolonged than the one obtained with pituitrin alone (b).

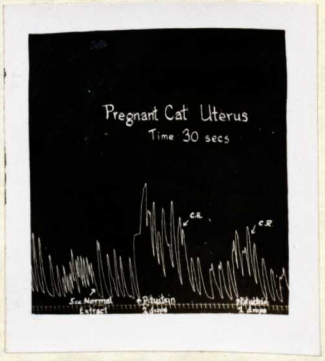


Fig.29. An identical synergic effect is produced if an extract of a non-pregnant patient's urine is added prior to the addition of pituitrin.

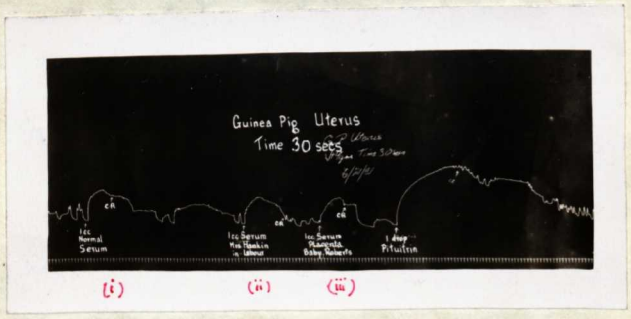


Fig.30. Shewing the effect of sera on the motility of a guinea pig uterus. The contractions are similar even if the sera are obtained from (i) a non-pregnant patient (ii) a patient in labour (iii) the umbilical cord.

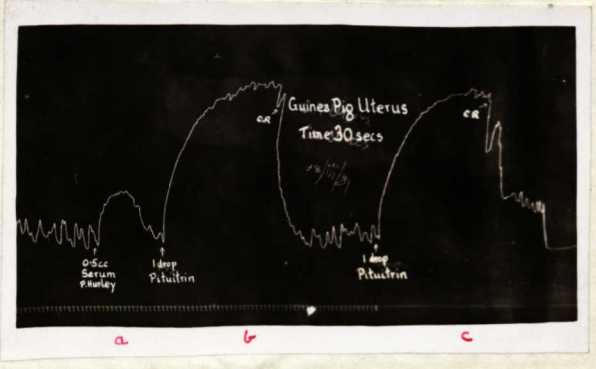


Fig.31. Addition of the serum of a patient in labour produces a small contraction (a). If pituitrin is added immediately afterwards, the contraction resulting (b) is no greater than that obtained with pituitrin alone (c).

However, Perez[134] in 1930 shewed that injections of blood taken from a patient in labour, would induce the onset of parturition in 50-60 per cent. of women in the last weeks of pregnancy. In view of this, experiments with urine were abandoned, and those with blood serum substituted.

Experiment 4.

Blood sera obtained from non-pregnant women, patients in labour, and from the umbilical cord, were used. In all cases, and irrespective of their oestrin content, addition of sera alone produced small uterine contractions (Fig.30). The contractions obtained by a combination of serum and pituitrin were no greater than those obtained with pituitrin alone (Figs.31 and 32). Bourne and Burn[32] pointed out that the synergic effect was best seen on a fatigued muscle. Figure 33 shews that no syneric effect was obtained in these tests even when such a muscle preparation was used.

Having failed completely to obtain the expected results, I repeated the original experiments of Bourne and Burn. The oestrin preparation used by these workers was menformon, which, they admit contains small quantities of protein. In the following tests theelin, an aqueous solution of the pure crystalline hormone, was used : this contains 50 R.U. per cc.

Experiment 5.

Quantities of theelin, varying from one spot to 0.3 cc were added to the Ringer's solution. Addition of this

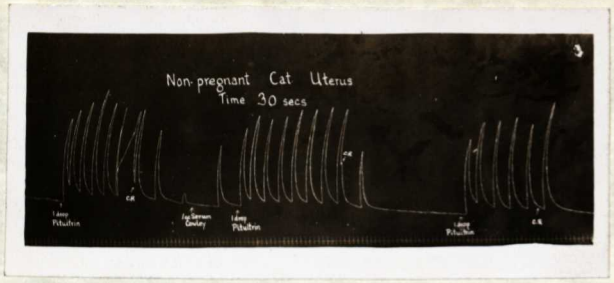


Fig.32. Shewing absence of any synergic effect between pituitrin and the serum of a patient in labour : the test object was the non-pregnant cat uterus.

Fig.33. Shewing absence of any synergic effect between pituitrin and the serum of a patient in labour even when they were tested on a fatigued guinea pig uterus.

Fig.34. This tracing shews that no synergic effect is demonstrable when pituitrin and 0.3 cc. of theelin are used together : the contraction produced (c) is identical to the ones produced by pituitrin alone (a & d).

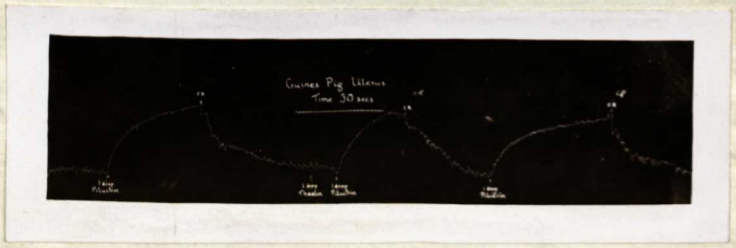


Fig.35 In this experiment one drop of theelin was used. Again no synergic effect is seen.

principle alone, produced no obvious change in the uterine motility, except on one occasion when 0.3 cc. caused a slight contraction (Fig.34). The addition of pituitrin subsequent to that of theelin produced contractions which differed in no way from the contractions obtained by pituitrin alone. No synergic effect was seen (Fig.35).

It would appear, therefore, that the effects obtained by Bourne and Burn were due to impurities which were present in the ovarian preparation used. Since performing these experiments I have found that Heller and Holtz[100] who used theelin, also failed to confirm the results of the above workers.

From this, it might be assumed that oestrin is perhaps not so important a factor in the onset of labour, However, in face of all the other circumstantial evidence, it does not appear to be permissible to contend that such experiments, performed in vitro, prove that the follicular hormone does not sensitize the uterus and so bring about the onset of labour. One feels that work in this connection should be performed on a uterus in situ : in support of this, I quote the work of Reynolds and Friedman[142], who shewed that intravenous injections of the serum from a woman in the early months of pregnancy inhibited uterine contractions. Moreover, Reynolds[143] has recently studied the contractions of uterine fistulae in rabbits, and has shewn that :-

(1) The uterus in situ always contracts more strongly during oestrus.

(ii) The contractions cease completely if the ovaries are removed.

(iii) The contractions return if an ovariectomised animal is injected with theelin.

In view of these experiments, together with the facts cited previously, there appears to be no doubt that oestrin is intimately concerned in uterine contractions. Moreover, the occurrence of oestrin in large amounts in the urine during early pregnancy, we still believe to be significant of the fact that the pregnancy is likely to terminate in the near future. As to the exact role which oestrin plays in this process, there is still some doubt, since my experiments have shown that the work of Bourne and Burn[32] was fallacious.

It is interesting to note that Kosaka[111] has lately found that the uterine contractions in a rabbit ceased after removal of the pituitary. These contractions returned if such animals received injections of placental extracts. He concludes that the placental hormones affect primarily the posterior pituitary, and through its secretion, the uterine motility leading possibly, in pregnancy, to labour contractions. However these results can be explained quite easily in terms of oestrin. Hypophysectomy would stop ovarian function, and the oestrin of the placenta would

be sufficient to cause a return of uterine contractions.

C. Lactation

Castration results in breast atrophy. Moreover all the earliest workers found that the ovarian hormone produced hypertrophy of the mammary glands in a non-pregnant animal. Parkes[130] reviewed the literature on the subject and pointed out that the hypertrophy obtained in these experiments was only equivalent to that found at oestrus : lactation never ensued.

Knaus[110] shewed that proliferative changes occurred in the breasts of a rabbit up to the sixteenth day after coitus. The mammary condition then remained stationary until after delivery. This proliferation period coincided with that of corpus luteum activity. It is now generally believed that although oestrin commences the breast changes, it is the corpus luteum hormone which is responsible for the true development which occurs during pregnancy.

Cutler[53] in discussing 'painful breasts' due to a persistent corpus luteum said that the "corpus luteum..... overstimulates the breast epithelium and the connective tissue, and causes excessive hyperplasia". Rosenburg[147] also stated that the corpus luteum of menstruation, as well as that of pregnancy, causes mammary enlargement.

However, in the human being, cases have been reported in which the corpus luteum of pregnancy has been removed, and yet mammary development continued. In the instance I have cited (page 96), from the time that both ovaries were removed, until the time of the death of the foetus, the breasts continued to enlarge and secretion was formed. Therefore, although the corpus luteum may cause initial mammary development, one cannot believe that it is essential for the breast changes of the later months of pregnancy, nor for the onset of lactation.

Recently, Corner[48] found that, although the mammae of non-pregnant spayed rabbits could not be made to proliferate beyond the normal pubertal degree of development by the administration of corpus luteum extracts, yet transplants of a sheep's hypophysis did cause proliferation and lactation resembling that of a full time gestation. No previous sensitization by the corpus luteum was necessary. However, Stieker and Grueter[165] caused lactation during pseudo-pregnancy by anterior pituitary extracts, and inferred that a primary corpus luteum action was essential. It is also interesting to note that Carnot and Bouttier[40] reported a case of acromegaly in which the patient had galactorrhoea for three years in spite of periods of amenorrhoea.

It would appear therefore, that there is much evidence to suggest that the final hyperplasia of the breast, and the onset of lactation, is associated with pituitary activity. Bugbee, Simond and Grimes[38] have even postulated the existence of a separate anterior pituitary hormone whose function is to stimulate milk secretion.

Parkes[130] produced lactation in animals by injection of hypophyseal extracts : however he concluded that this effect was secondary to the production of a large amount of luteal tissue in the ovaries. This may be true, but, on the other hand, his pituitary extracts may have acted directly on the mammary glands.

If then a'pituitary'hormone is directly concerned in this physiological process, it may have its origin either in the placenta, or in the hypophysis. F.de Freitas Simoes[158] failed to produce breast changes by feeding castrated guinea pigs with placenta. This evidence is of no value since it is now known that Prolan A and Prolan B are ineffective if administered orally. Presuming that the placenta is the site of the hormone concerned in the mammary proliferation, lactation must be the result of the removal of this influence. If, however, the pituitary is responsible, one may suppose that this gland, suddenly released from its duties towards

the genitalia, directs all its attentions towards the mammary glands and precipitates lactation.

D. The action of oestrin and the corpus luteum hormone on the pituitary.

Various workers have suggested that the follicular and the corpus luteum hormones have a direct action on the pituitary gland. Baniecki[22] found that injections of ovarian extracts produced histological changes in the pituitary of a non-pregnant animal : these were similar to those found during pregnancy. However, Berblinger[27] said that such changes could be effected by injections of peptone or any protein body. Moreover, good evidence has already been given to show that it is absence of oestrin which results in hyperplasia of the anterior lobe of the pituitary, and that excess of the hormone probably inhibits the activity of this gland.

Kraul[112] demonstrated that the anterior pituitary of an animal which had been treated with corpus luteum extracts produced excessive Prolan B effects when transplanted into a rat. This again, is difficult to explain since it infers that the corpus luteum stimulates Prolan B production : if this were true, one would expect that a corpus luteum would always persist in the ovary even in the absence of pregnancy. A theory which assumes that the corpus luteum

inhibits Prolan B production, would better explain the cyclical formation of luteal tissue in the ovary.

With reference to the posterior lobe of the pituitary, Dixon and Marshall[56] said that oestrin stimulates the secretion of pituitrin and so brings about the onset of labour. In view of the findings given previously (page 134), it is still possible that the excessive oestrin the body is related to the onset of labour by its action on the posterior lobe of the pituitary.

Prolan B

The function of Prolan B is, undoubtedly, to stimulate the formation of the corpus luteum : it does so in every menstrual cycle. In pregnancy, the placenta either produces, or stimulates the hypophysis to produce, an excess of Prolan B : so the corpus luteum persists and the pregnancy progresses.

Prolan B, therefore, is the true hormone of pregnancy.

Prolan A

It is difficult to explain why Prolan A should occur in such large quantities during pregnancy. It has been shown that hypophyseal transplants produce ovulation and abortion in pregnant animals. Also, Loesser[118] found that injections of acetone extracts of anterior pituitary produced ovulation

in pregnant rabbits. However, there is some doubt whether the follicle-ripening hormone of pregnancy urine has similar effects. Friedman[91] stated that ovulation follows the injection of the urine into pregnant rabbits. Jares[108] confirmed this but failed to obtain the same results while working with guinea pigs. Moreover, Collip[42] claims that emmenine, the placental equivalent to Prolan A, does not terminate pregnancy.

Since Prolan A is known to stimulate ripening of the follicles, it must be assumed that it is present only in quantities insufficient to enable it to over-ride the Prolan B effects.

If one believes that oestrin is formed in the interstitial cells, it seems feasible that the function of Prolan A is to stimulate the production of this hormone, and so indirectly, influence uterine growth. Gander[93] shewed that Prolan A, acting via the ovaries, produced greater uterine enlargement than oestrin. However, in the human being, the ovary is not essential to the continuation of pregnancy after the first few months.

Assuming that Prolan A has its origin in the anterior pituitary, it is likely that its production is the result of the diminished ovarian activity which occurs during pregnancy.

However, even if the ovary is less active, there are large amounts of oestrin in the circulation. This leads one to infer that when it is said that removal of ovarian 'influence' stimulates Prolan A formation, one does not necessarily mean that the 'influence' is oestrin itself.

But it is more probable that these explanations are not needed, and that Prolan A arises directly from the placenta. If this is true, Collip[42] has offered the best solution of the problem as to the function of this hormone during pregnancy. He infers that it is of potential, rather than immediate, value, and says "our conception of the physiological significance of this active principle is that it is produced in the placenta throughout pregnancy for the specific purpose of assuring the continued functioning of the ovary". In other words, it represents a precaution, taken on the part of the body, to prevent a temporary ovarian disuse leading to a persistent atrophy.

Conclusion

In conclusion, it is my intention to attempt to trace the hormonic changes which occur in the body during pregnancy. In doing so, I shall endeavour to give those views which seem best substantiated by the evidence at our disposal. The various events will be stated dogmatically since it would seem that so disorderly is our present state of knowledge, that no further advances can be made without a working hypothesis.

Beginning with an unripe follicle in the ovary : this secretes no oestrin. The absence of oestrin, or the presence of the corpus luteum from the previous ovulation, stimulates the pituitary to cease Prolan B production, and to commence the formation of Prolan A. This principle causes the follicle to ripen and to secrete oestrin. Meanwhile the corpus luteum has degenerated. The follicular hormone acts on the uterus causing increased vascularity, hypertrophy of the musculature, and endometrial hyperplasia. As the amount of oestrin in the circulation increases, so the pituitary stops producing Prolan A and commences to secrete Prolan B.

Rupture of the follicle occurs : this is undoubtedly effected by the influence of the hypophysis, but it is impossible to say whether it is the end-result of Prolan A action, whether it is due to a combined effect of Prolan A and Prolan B, or whether a separate pituitary factor exists for this purpose.

After ovulation, Prolan B, which is now in the ascendancy, stimulates the formation of a corpus luteum. The active principle of this yellow body acts on the endometrium which has previously been subjected to the influence of oestrin. As a result of this, the endometrium undergoes further hyperplasia and becomes capable of a decidual reaction.

The ovum is meanwhile fertilized, and passes to the uterine cavity. It impinges on the wall, a decidual reaction occurs and implantation takes place. The chorionic villi now either produce Prolan A and Prolan B, or stimulates the pituitary to do so. Prolan B is the dominant factor and this acts on the ovary causing the corpus luteum to persist. This structure continues secreting its active principle and thereby :-

(i) Prevents ovulation

(ii) Inhibits menstruation

(iii) Inhibits uterine contractions

Under these conditions the pregnancy progresses normally.

At the same time, oestrin is formed by the developing placenta and also enters the circulation. There are now Prolan A, Prolan B, oestrin, and the corpus luteum hormone all present, but the quantity of each is so regulated that Prolan B and the corpus luteum hormone are dominant.

As the pregnancy progresses, the follicular hormone, together with that of the corpus luteum, produce the initial breast changes. However, either the anterior lobe of the

pituitary, or the placenta, soon takes over this function. In the later months, oestrin begins to assume the ascendancy over the other hormones. The amount of Prolan B in the body fluids diminishes and the corpus luteum retrogresses. As a result of this relative increase in the follicular hormone, the uterus becomes more sensitive and contracts to any stimulus. A certain stage is reached when the uterus becomes sufficiently sensitive to respond, either to the normal amount of 'pressor substances' in the blood, or to an increased amount of pituitrin which is produced by the posterior lobe of the pituitary in response to the stimulation by oestrin. Whichever of these two mechanisms is responsible, parturition commences and the foetus is expelled.

The breasts by this time are fully developed. Lactation is precipitated either by the removal of the placenta, or by the action of the pituitary, whose attentions, previously divided now become directed solely towards the mammary glands.

References

1. Abraham, G. Med.Klin., 1930, vi, 164.
2. Adler, L., Arch.f.Gynäkol., 1912, xcv, 349
3. Allan,H. and F.Dickens, Lancet 1930, i, 39.
4. Allan, H., F.Dickens and E.C.Dodds, Journ.Physiol. 1930,lxviii, 348.
5. Allan, H. and E.C.Dodds, Journ.Obst. & Gyn.Brit.Emp.,1930, xxxvii, 447.
6. Allen, E. and E.A.Doisy, Journ.Amer.Med.Assoc., 1923,lxxxi,819.
7. " " Physiol.Rev., 1927, vii, 600.
8. Allen, W.M., Amer.Journ.Physiol., 1930, xcii, 612.
9. Allen, E., Proc.Soc.Exp.Biol. & Med., 1927, xxiv, 608.
10. Allen, E., W.P.Maddux and J.W.Kennedy, Proc.Soc.Exp.Biol.and Med., 1931, xxviii, 403.
11. Amati, G., Zentralb. f. Gynäk., 1928, xlii, 2639.
12. Aschheim, S. and B.Zondek, Zeitschr. f. Geb. u. Gynäk.,1926, xc, 372

    Deutsch. Med. Woch.,1926,viii,343.

    Arch. f. Gynäkol.,1927,cxxx, 1.
13. Aschheim, S. and B.Zondek, Klin.Woch.,1928,vii,8,1401,1453.
14. " " Klin.Woch.,1927,vi,248,1321,1322.
15. Aschheim, S., Zentralb. f. Gynäk., 1928, lii, 602.
16. Aschheim, S., Amer.Journ.Obst. & Gyn., 1930,xix, 352.
17. Aschheim, S. and B.Zondek, Brit.Med.Journ.,1930, i, 551(review)
18. Aschheim, S. and B.Zondek, Endokrinologie, 1928, i, 10.
19. Aschner, B., Arch. f. Gynäkol., 1912, xcvii, 200.

20. Bacon, A.R., Amer.Journ.Obst. & Gyn., 1930, xix, 335.

21. Bailey, P. and L.M.Davidoff, Amer.Journ.Path., 1925, i, 185.

22. Baniecki, H., Arch. f. Gynäkol., 1928, cxxxiv, 693.

23. Bell, W.Blair, "The Pituitary" Baillière, Tindall & Cox., London, 1919.

24. " " "The Sex Complex", Baillière, Tindall & Cox, London, 1920.

25. " " Lancet, 1913, i, 809, 937, 986. (Arris & Gale lectures 1913).

26. Bellerby, C.W., Lancet 1928, i, 1168.

27. Berblinger, W., Verhandl d. Deutsch. Path. Ges., 1914, xv.

Mitt, a.D. Grengeb.d.Med.u.Chir.,1921, xxxiii,456.

28. Blair, E., Amer.Journ.Physiol., 1923, lxv, 223.

29. Böhne, C., Klin. Woch., 1931, x, 210.

30. Bourg, R., Compt.Rend.Soc. de Biol., 1930, cv, 466.

31. Bourg, R., Rev.Franc. de Gynéc. et d'Obst., 1931, xxvi, 65.

32. Bourne, A.W. and J.H. Burn, Lancet, 1928, ii, 1020.

33. Brambell,F.W.R., A.S.Parkes and U.Fielding, Proc.Roy.Soc.Med., 1927, B. ci, 95.

Proc.Roy.Soc.Med.,1928,B. cii,385.

34. Brouha, L. and H.Simonnet, Compt.Rend.Soc.de Biol., 1928, xcix, 759.

35. Brühl, Klin.Woch., 1929, viii, 1716.

36. Brühl, Zentralbl. f. Gynäk., 1930, liv, 192.

37. Bryant, A.R., Anat. Record, 1930, xlvii, 131.

38. Bugbee, E.P., A.E.Simond and H.M.Grimes, Endocrinology,1931, xv, 41.

39. Butenandt, A. and E.von Zeigner, Ztschr.f.Physiol.Chem., 1930,clxxxviii, 1.

40. Carnot, P and Bouttier, Clin.Journ., 1930, lix, 288.

41. Clauberg, C., Zentralbl. f. Gynak., 1929, liv, 7.

42. Collip. J.B., Canad. Med. Assoc. Journ., 1930, xxii, 761.

43. " Brit.Med.Journ., 1930, i, 552 (review)

44. " Brit.Med.Journ., 1930,ii, 1080.

45. Comte, L. Beitr. z. Path. Anat. u.z.Allg.Path.,1898,xxiii, 90.

46. Cordua, R., Zentralbl. f. Gynäk., 1930, liv, 211.

47. Corner, G.W., Physiol. Rev., 1923, iii, 457.

48. " Amer.Journ.Physiol., 1930, xcv, 43.

49. Coward, Kath.H. and J.H.Burn, Journ.Physiol.,1927,lxiii,270

50. Cole, H.H. and G.H.Hart, Amer. Journ.Physiol.,1930,xciii,52
" " " 1930,xciv.597

51. Crowe, S.J., H. Cushing and J.Hormans, Bull.Johns Hopkins Hosp.,1910,xxi,217.

52. Cushing, H., "The Pituitary Body and its Disorders" , Philadelphia 1912.

53. Cutler, M., Journ.Amer.Med.Assoc., 1931, xcvi, 1201.

54. de Snoo, Zentralbl. f. Gynäk., 1928, lii, 2703.

55. Dingemanse, E., E.J.Freud, S.E.deJongh and E.Laquer, Arch.f.Gynäkol, 1930,cxli, 225.

56. Dixon, W.E. and F.H.A.Marshall, Journ.Physiol.,1924,lix,276.

57. Dodds, E.C. and F.Dickens, Journ.Obst. and Gyn.Brit.Emp., 1929,xxxvi, 92.

58. Dodds, E.C., A.W.Greenwood and E.J.Gallimore, Lancet, 1930, 1, 683.

59. Doisy, E.A., Amer.Journ.Physiol., 1929, xc, 320.

60. Eberson, F. and M.H.Silverberg, Journ.Amer.Med.Assoc.,1931, xcvi, 2176.

61. Ehrhardt, K., Zentralbl. f. Gynäk., 1930, liv, 1538.

62. " Klin. Woch., 1929, viii, 2044, 2330.

63. Engle, E.T., Journ.Amer.Med.Assoc., 1929, xciii, 276.

64. Engle, E.T. and C. Mermod, Amer.Journ.Physiol.,1928,lxxxv, 518.

65. Erdheim, J. and E.Stumme, Beitr. z. Path.Anat.u.Z.Allg. Path., 1909, xlvi, 1.

66. Ettinger, G.H., G.L.M.Smith and E.W.McHenry, Canad.Med. Assoc.Journ.,1931,xxiv,491.

67. Evans, H.M. and J.A.Long, Anat.Record.,1921, xxi, 62.

68. Evans, H.M. Harvey Lectures, 1924, xix.

69. Evans, H.M. and M.E.Simpson, Journ.Amer.Med.Assoc.,1928, xci, 1337.

70. " " Amer.Journ.Physiol., 1929, lxxxix, 381.

71. Fanz, J.I. and E.J. Gault, Journ.Lab. & Clin. Med., 1930, xvi, 27.

72. Fee, A.R. and A.S.Parkes, Journ.Physiol., 1929,lxvii, 383.

73. Fellner, O.O., Centralb. Allg. Path. u. Path. Anat., 1912, xxiii, 673.

74. " " Arch. f. Gynäkol., 1913, c, 641.

75. " " Arch. f.d. Ges.Physiol., 1921, clxxxix, 199.

76. " " Zentralbl. f. Gynäk., 1929, liii, 1135.

77. Fels, E., Klin. Woch., 1926, v, 2349.

78. Fichera, G., Deutsche. Med. Woch., 1905, xxxi, 123.

79. Fluhmann, C.F., Amer. Journ. Obst. & Gyn., 1929, xviii, 740

80. " " Journ.Amer.Med.Assoc., 1929, xciii, 672.

81. " " and C.V. Kulchar, Proc.Soc.Exp.Biol. & Med., 1931, xxviii, 417.

82. " " Amer.Journ.Obst. & Gyn., 1930, xx, 1.

83. Ford, F. and D. Drips, Radiology, 1929, xii, 393.

84. Fraenkel, L., Arch. f. Gynäk., 1903, lxviii, 488.

85. " " " " 1910, xci, 705.

86. " Zeitschr. f. Geb. u. Gynäk., 1913, lxxiv,107.

87. Frank, R.T., R.G.Gustavson, C.D.Bonham, Amer.Journ.Physiol 1925, lxxiv, 395.

88. Frank, R.T. and M.A. Goldberger, Journ.Amer.Med.Assoc., 1930, xciv, 1197.

89. Frank, R.T. "The Female Sex Hormone", Baillière, Tindall & Cox, 1929.

90. Friedman, M.H. and M.E.Lapham, Amer.Journ.Obst. & Gyn., 1930, xxi, 405.

91. Friedman, M.H., Amer.Journ. Physiol., 1929, xc, 617.

92. Fröhlich, A., Wien. Klin. Rundschau, 1901, xv, 883.

93. Gander, G., Zeitschr. f.d.Ges.Exp.Med., 1930, lxxii, 44.

94. Gant, Madeline, P., Anat.Record, 1931, xlix, 373.

95. Genther, Ida, T., Amer.Journ.Anat., 1931, xlviii, 99.

96. Ginglinger, A., Bull.Soc. d'Obst. et de Gynéc., 1931,xx,99

97. Halban, J., Arch. f. Gynäkol., 1905, lxxv, 353.

98. Hannan, J.H., Brit.Med.Journ., 1930, i, 150.

99. Heape, W., Quart. Journ. Micro. Sci., 1900, xliv, 133.

100. Heller, J. and P. Holtze, Brit.Med.Journ., 1931, i, 1023.

101. Herrmann, E., Monatschr. f. Geb. u. Gynäk., 1915, xli, 1.

102. " " " 1921, liv,152.

103. Herrmann, E. and M. Stein, Wien. Klin.Wooh.,1916,xxix,177, 178.

104. Hill, Margt. and A.S.Parkes, Journ.Physiol.,1931,lxxi, 40.

105. Hirose, T., Kinki Fujinka-Gakkai-Zasshe, 1920, xvi (quoted by Collip (42)).

106. Hisaw, F.L.,Physiol. Zool., 1929, ii, 59.

107. Hitschmann, F. and L.Adler, Monat. f. Geb. u. Gynäk.,1908, xxvii, 1.

108. Jares, J.J., Anat. Rec., 1931, xlix, 185.

109. Knauer, E., Arch. f. Gynäkol., 1900, lx, 322.

110. Knaus, H., Klin. Wooh., 1930, ix, 961.

111. Kosaka, J., Japan Journ. Obst. & Gyn., 1930, xiii, 238.

112. Kraul, L., Amer.Journ. Obst. & Gyn., 1931, xxi, 301.

113. Lane-Claypon, J.E. and E.H.Starling, Proc.Roy.Soc.,1906, B., lxxvii,505.

114. Laqueur, E., P.C.Hart, S.E. deJongh and I.A.Wijsenbeck, Versl,d.K. Akad.v.Wetensch,Amsterdam 1925, xxviii, 890.

115. Laqueur, E., P.C.Hart and S.E. deJongh, Lancet,1927,i,1126.

116. Lehmann, J., Virchow's.Arch.Path.Anat.u.Physiol.,1928,ccixviii, 346.

117. Loeb, L., Centralb. f. Allg. Path., 1907, xviii, 563.

118. Loeser, A., Klin.Woch., 1930, ix, 1855.

119. Magrath, T.B. and L.M.Randall, Journ.Amer.Med.Assoc.,1931, xcvi, 1933.

120. Mahnert, A., Zentralbl. f. Gynäk., 1930, liv, 2883.

121. Mahnert, A. and H. Siegmund, Zentralbl.f.Gynäk.,1927, li, 1626.

122. Maino, M. and B. Frattini, Boll. d. Soc.Ital.di Biol.Sper., 1930, v, 1065.

123. Marrian, C.F. and A.S.Parkes, Journ.Physiol.,1929,lxvii,389

124. Mauriac, P., Journ. de Méd. de Bordeaux, June 20,1931,p.539.

125. Mazer, C. and J.Hoffmann, Journ.Amer.Med.Assoc.,1931,xcvi,19

126. Miklos, L., Zentralbl. f. Gynäk., 1930, liv, 1755.

127. Murata, M. and K.Adachi, Zeitschr.f.Geb.u Gynäk.,1927,xcii, 45.

128. Novak, E. and A.K.Koff, Amer.Journ.Obst. & Gyn., 1930,xx, 481.

129. Nürnberger, L., Med.Klin. Berlin 1931, xxvii, 343.

130. Parkes,A.S. "The Internal Secretions of Ovary", Longmans, Green & Co. 1929.

131. Parkes, A.S. and C.W.Bellerby, Journ.Physiol.,1926,lxi,562.

132. " " " " " 1926,lxii,145.

133. Parkes, A.S. and C.W.Bellerby, Journ.Physiol.,1927,lxii, 301, 385.

134. Perez, M.L., Zentralbl. f. Gynäk., 1930, liv, 1937.

135. Philipp, E., Zentralbl. f. Gynäk., 1928, lii, 416.

136. " " " 1929, liii, 2386.

137. " " " 1930, liv, 2754.

138. " " " 1930, liv, 3076.

139. " " " 1930, liv, 450.

140. Raisz, D., Zentralbl. f. Gynäk., 1930, liv, 2887.

141. Reeb, M., Bull. Soc. d'Obst. et de Gynéc., 1931, xx, 94.

142. Reynolds, S.R.M. and M.H. Friedman, Amer.Journ.Physiol., 1930, xciv, 696, & 705.

143. Reynolds, S.R.M., Amer.Journ.Physiol.,1931, xcvii, 706.

144. Reinhart, H.L. and E.Scott, Journ.Amer.Med.Assoc.,1931, xlvi, 1565.

145. Riddle, O., Endocrinologie, 1929, xiii, 311.

146. " Physiol. Rev., 1931, xi, 91.

147. Rosenburg, A., Frankfurt Ztschr. f. Path., 1922,xxvii,466.

148. Rössle, R., Virchow's Arch. f. Path.Anat. u. Physiol., 1914, ccvi, 248.

149. Schilder, P. " " f. Path.Anat. u. Physiol., 1911, cciii, 246.

150. Schneider, P.F., Surg. Gyn. & Obstet., 1931, lii, 56.

151. Schught, P., Strahlentherapie, Bd.27, 5605. (Quoted by Genther(94))

152. Schuschania, P., Zentralb. f. Gynäk., 1930, liv, 1924.

153. Schultze-Rhonhof, F., Zentralb. f. Gynäk.,1930, liv, 578.

155. Siddall, A.C., Journ. Amer. Med.Assoc., 1928,xc, 380.
" " " " 1928,xci, 779

156. Siegert, F. and Schmidt-Neumann, Zentrabl. f. Gynäk.,1930, liv, 1630.

157. Silverberg, M., Proc.Soc.Exp.Biol. & Med.,1929,xxvii, 166.

158. Simoes, F de Freitas, Compt.Rend.Soc.de Biol.,1930,cv,150.

159. Smith, P.E., Anat.Record, 1926, xxxii, 221.

160. Smith, P.E. and E.T.Engle, Amer.Journ.Anat.,1927,xci, 159.

161. Smith, P.E., Amer.Journ.Physiol., 1927, lxxx, 114.
" " " 1927, lxxxi, 20.

162. Smith, P.E., Anat.Record, 1923, xxv, 150.

163. Smith, M.G., Bull.Johns Hopkins Hosp., 1927, xli, 62.

164. Stewart, W., Lancet 1931, i, 1347.

165. Sticker, P. and F. Grueter, Presse Méd.,1929,xxxvii,1268.

166. Stockard, C.R. and G.N.Papanicolaou, Amer.Journ.Anat., 1917, xxii, 225.

167. Szarka, S., Zentralb. f. Gynäk., 1930, liv, 2211.

168. Teel, H.M., Amer. Journ. Physiol., 1926, lxxix, 170.

169. Vozza, F., Ann. di Ostet. e Ginecol., 1929, li, 1189.

170. " " " " 1931, liii, 267.

171. Wagenen, G.von, Anat. Record, 1925, xxix, 398.

172. Walker, B.S. and J.C.Janney, Endocrinology 1930,xiv, 389.

173. Wiesner, B.P., Brit.Med.Journ., 1931, i, 860.

174. " " Edin.Med.Journ., 1930, xxxvii, 73.

175. Wiesner, B.P. and F.A.E.Crew, Brit.Med.Journ.,1930,i,777.

176. Walter, A., Endokrinologie 1929, iv, 1.

177. Zondek, B., Klin.Woch., 1930, ix, 964.

178. " " " 1930, ix, 1207.

179. " Zentralbl. f. Gynäk., 1931, lv, 1.

180. " " " 1930, liv, 2306.

181. " Klin. Woch., 1929, viii, 1149.

182. " Endokrinologie, 1929, v, 425.

184. " Klin.Woch., 1930, ix, 679.