OBSERVATIONS ON THE ZONDEK-ASCHHEIM AND OTHER TESTS FOR PREGNANCY.

15th NOVEMBER, 1930.

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INTRODUCTION.

The diagnosis of pregnancy is not always an easy matter. It is desirable that a definite diagnosis should be made as early as possible, especially after the interruption of the menstrual cycle. It is equally desirable that the method employed should be unpleasant neither to patient nor to physician, and above all, that it should be reliable. Most of the laboratory methods introduced so far have been of a highly technical character and have given such indifferent results, that their success is usually confined to the original investigator who introduced the method.

In the Lancet of January 4th, 1930, a few details of another test named the Zondek-Aschheim reaction were published. It certainly seemed worthy of further investigation and suggested a theme for the following work.

Various attempts have been made to establish a biochemical diagnosis of pregnancy. Abderhalden's reaction - although good results were obtained by this worker himself - never received universal recognition and is now more or less accepted as being of only historical interest. It was slightly modified by Luttge and Mertz, but in the hands of Schultze it gave a forty per cent error. In the same category comes the procedure of Munter and Grafenberg (1) who mixed placenta

with the serum to be examined and then added Ninhydrin and observed the change of colour. In 53 pregnancies they obtained 48 positive reactions, and in 42 non-pregnant cases they obtained 40 negative results.

Another test founded on the disturbance of the sugar metabolism during pregnancy has given rise to much work with results often contradictory (levy-Solel, Laudat, and Mmlle Wolff), (2). Bèkelman and Rother discarded all tests and returned to clinical examination (3). However, Adlersberg and Porges (4) slightly modified this test by giving their cases a non-carbohydrate diet, and in 30 pregnancies they obtained 30 positive results, but in 24 controls 2 gave a positive reaction.

The sedimentation of the red cells which is accelerated during pregnancy has been used. Schwarez (5) obtained 75 positives from 100 pregnancies, the percentage error being rather high.

A method which received some support was that of Kamnitzer - Joseph, who injected phloridzin intramuscularly. Scharoff (6) tried it on 47 women; in all those who were only three months pregnant the result was positive, and in 9 non-pregnant cases it was negative. Diaz-Nielsen (7) in 100 pregnancies obtained positive results in 94, and in 100 controls found 84 negatives. K.Fink (8) had 96 positives in 100 pregnancies.

As for the intradermal injections of placental peptone of Wintz and Engelhorn, it is considered by Coudert and Daunay (9) as useless.

All these methods are, at least for the present, easily superseded by that of Zondek and Aschheim.

Heape, some 25 years ago, first thought of the existance of a sex hormone which influences the growth of the genital system. Research on the degeneration and atrophy of the testicles following the removal of the pituitary, drew attention to the manner in which sexual maturity is attained. More recently Smith has shown that after destruction of the pituitary in an immature animal sexual development is arrested. If grafts of anterior pituitary are transplanted, sexual development takes its normal course. Concurrently with this appeared the work of Allen, Doisy, and Frank, on the ovarian hormone (10).

Zondek and Brahan showed that in the immature animal this ovarian hormone induces rut - increase in size of uterus and cornification of vaginal epithelium.

In 150 mice Zondek and Aschheim implanted grafts of various ductless glands (thymus, thyroid, parathyroid, suprarenals) without any effect on the ovaries whatsoever. The anterior pituitary alone had the effect of stimulating the ovaries. From their work they concluded that the anterior pituitary acts on the ovary and the ovary acts secondarily on the uterus and vagina.

Steinach and Kuhn (11) working on immature rats, injected extracts of anterior pituitary and found signs of premature sexual development - descent and increase in size of the testicles with hyperaemia of the skin of the scrotum, development of the prepuce and of the corpora cavernosa. There is also an increase in the size of the seminal vesicles and prostate. The cessation of the injections brought about a retro-gression of these abnormally premature features.

Lowe and Voss (12) have tried, they claim not without success, implantation of anterior lobe of pituitary in cases of amenorrhoea.

Volpe, confirmed by Berblinger (13) has shown that there is a hypertrophy of the pituitary during pregnancy. This was also confirmed by the late Noel Paton. He states in his "Regulators of Metabolism" that the increase is due to the chief, or chromophobe cells. These undergo involution during lactation. The same result is obtained by castration and thyroidectomy.

Zondek and Aschheim were the first to point out that there is an excretion of anterior pituitary hormone in the urine during pregnancy. They believe that this over-action rapidly increases after conception and attains its maximum when menstruation ceases and that it remains at this point

till the eighth month after which it gradually diminishes, and disappears altogether at the eighth day following labour.

For the detection of this pituitary hormone they inject urine into immature female mice.

Approaching very near to this reaction was the method of Siddal (14), who injected the serum of a pregnant woman into a mouse and compared the weights of the injected mouse's uterus with that of a control. In 203 cases there were 9 errors - 5 positive in women not pregnant and 4 negative in women who were pregnant.

TECHNIQUE OF INJECTION.

Zondek (15) uses 5 mice, 1 of which is a control. they were all 3 to 4 weeks old and weighed 6 to 8 grammes. The urine is that of the first morning specimen. If the urine is infected or had to be kept for any length of time, a drop or two of pure cresol is added. He gave the injections at the following times:-

Monday - 12 midday, and 5.0 p.m.

Tuesday- 10.0 a.m; 1.0 p.m; and 6.0. p.m.

Wednesday 10.0 a.m.

In all he gave six subcutaneous injections, He gives different mice different quantities, but the quantity remains the same for the same mouse throughout the course, namely, six doses of 0.4 ccm; six doses of 0.3 ccm; six doses of

0.25 ccm; and six doses of 0.2 ccm. The mice, if started on the Monday, are killed on the following Friday morning and examined immediately. In the majority of cases he found macroscopic examination sufficient, but in a few cases he performed serial section of the ovaries. He attempted to accelerate proceedings by injecting a total of from 2 to 3 ccm. in the course of 36 hours, but the results were not found to be reliable. Examination showed:-

(1) Canalisation of vagina and appearance of oestrus as judged by the vaginal smear,

(2) Hyperaemia and increase in size of uterus,

(3) formation of dark spots - "Blutpunkte" - in the ovaries and also corpora lutea atretica.

He obtained similar results by implantation of anterior pituitary substance.

RESULTS OBTAINED BY OTHERS.

Zondek and Aschheim report 511 cases; of this number 37 specimens of urine killed the mice before results could be obtained. 253 were from pregnant cases, the others from controls and males. Their results are as follows:-

	Number of Cases	Positive	Negative
Menopause.....	6	0	6
Amenorrhoea.....	16	0	16
Uterine haemorrhage, non-pregnant	3	0	3
Males.....	15	1	14
Circulatory, Inspiratory & Infectious ...	15	0	15
Cystitis..... (On repeat gave a negative result)	1	1	0
Acromegaly..... (In two cases the mice died)	3	0	1
Hypophyseal Obesity.....	3	0	3
Pituitary Tumour.....	3	0	3
Myxoedema.....	5	2	3
After ovariectomy.....	3	0	3
Acute Pelvic Inflammation..... (Including two pelvic tumours)	12	2	10
Benign pelvic tumours.....	10	1-partial	9
Fibroids.....	18	3-partial	15
Genital carcinoma.....	29	2	27

Of the pregnant cases 68 were in the first 8 weeks of pregnancy. In 2 pregnancies negative results were obtained. In cases where the foetus was dead for three days

the reaction was positive, and the reaction was negative if foetus had been dead from 8 to 21 days. Two cases of hydatidiform mole gave a positive reaction. In cases of abortion a positive reaction disappeared at the 6th day. In 14 cases the pregnancy was tubal and 11 of these, the foetus being alive, gave a positive reaction. In 3 where the foetus was dead the reaction was negative. Of particular interest is a case of chorionephithelioma following a hydatidiform mole. The patient came under observation 21 months after hysterectomy had been performed. She gave a positive reaction and complained of a swelling in the renal area. Nephrectomy was performed and metastases were found. The reaction remained positive until death.

Allen and Dickens (16) working at the Middlesex Hospital slightly modified the test. They used mice 21 to 22 days old and of average weight 7.5 grammes. Five mice were used one of which was the control. Each mouse received six injections of 0.3 ccm. over a period of 45 hours, namely:-

First day	12 noon;	6.0 p.m.	
Second day	9.0 a.m.	3.0 p.m.	9.0 p.m.
Third day	9.0 a.m.		

Originally the doses recommended by Zondek were used. This necessitated the marking of individual mice and also those receiving the largest dose sometimes showed less response than those receiving a smaller dose. Therefore as a routine 0.3 ccm. was injected in each mouse. The mice were killed on the morning of the 5th day and their ovaries and uteri were examined. Their results are as follows:-

		Positive	Negative	Percentage error.
Cases of pregnancy	126.	122	4	3.2
Non-pregnant cases	82.	1	81	1.2
Males.....	6.	0	6	nil.

They report other conditions:-

Condition.	Number of cases.	Positive	Negative
24 hours after delivery.....	1	1	0
48 hours after delivery.....	1	0	1
Lactating.....	2	0	2
Menstruating.....	2	0	2
Infant female, three months.	1	0	1
Female child, aet 10 years..	1	0	1
Ovarian tumour (benign).....	2	0	2
After abortion, four days...	1	0	1
Menopause.....	5	1	4
Various gynaecological conditions...	5	0	5

Condition.	Number of cases.	Positive.	Negative.
Continued:-	21	2	19
Extreme obesity.....	1	0	1
Menorrhagia.....	1	0	1
Pituitary tumour.....	1	0	1
Uterine fibroid.....	1	0	1
	25	2	23
Normal female 1½ hours after delivery.....	1	1	0
Pulmonary T.B. & pregnancy.	1	1	0

In the hands of Frank (17) the mice died during the test but this has not been the general experience.

Hannan (18) after failure with mice had more success with the albino rat; he used the following method. One albino rat aged about 6 to 8 weeks was injected subcutaneously with 3 ccm. of urine in one dose. The animal was killed and examined 96 hours later. In his results he omits two types of cases:-

(1) Those which he did while he was gaining experience of the technique,

(2) Those women, who, after missing one or more periods, had a loss, since it is clear that unless something can be produced by these women for examination, it is impossible to estimate whether the test was accurate or not.

His results are:-

Normal pregnancy.	Cases.	Results.
Six weeks to three months.....	21	2 wrong
Three months to term.....	12	1 wrong
Total	33	3 wrong

Ectopic pregnancy,		
Tubal abortion.....	2	Both posit:
Tubal mole.....	1	Negative
Pelvic haematocele.....	2	Both neg.
Total	5	

A foetus was found only in the case of both tubal abortions.

Puerperium.

Three cases; daily tests. Two females ceased to give a positive reaction on the 5th day after delivery, one woman ceased on the 7th day.

Total of pregnancies.....41. Three wrong.....error 7.3%

Controls.	Cases.	Results.
Regular menstrual cycles.....	15	none wrong
Secondary amenorrhoea..... (Probably endocrine in origin)	8	2 wrong
Menopause, with amenorrhoea.....	12	4 wrong
Fibroids.....	8	2 wrong
Ovarian Cysts.....	3	none wrong
Genital carcinoma.....	7	2 wrong
Exophthalmic goitre.....	4	2 wrong
Males.....	5	2 positives.

Summary.

Pregnancy.....	41.	3 wrong.	error 7.3%
Normal women.....	15	0 wrong	error nil.
Women with disease.....	42	12 wrong	error 28%

He summarises the work of others:-

Louria and Rosenweig, Bruhl, Aschheim and Zondek, Vozza;

	Total cases 913.	Percentage error.
Pregnant women	452	1.8
Non-pregnant women	461	3.2

Hannan states that in non-pregnant cases his own figures and those of others are in agreement. In cases of pregnancy his margin of error is higher. He made use of different animals and different technique of injection and the test in his hands can hardly be described as the Zondek Aschheim reaction.

Professor Grew (19) reports the results of the first year's working in his department at Edinburgh. In his results macroscopic examination of the ovaries was relied upon. He found microscopic examination unnecessary.

Of his 830 cases, he was unable to obtain any result in 24 cases by reason of the toxicity of the urine, of the other 806 cases he was only able to obtain control information in 460, viz;

Positive	286
Negative	174

These had been controlled thus:-

Correct	446
Incorrect	14

In the incorrect series the results show an error of 3.04%. In all the control cases there is an error of 2.2%.

Mazer and Hoffman (20) used the method of Allen and Doisy (21) who demonstrated the succession of changes in cell types found in the vaginal lumen of mice during the oestrual cycle. As these cyclic phenomena do not appear after castration, the injection of urine followed by their appearance, demonstrates in their opinion, beyond doubt, that the urine contains the female sex hormone responsible for the positive Zondek-Aschheim reaction. They state that acute inflammation in the pelvis yields a positive result, and therefore they maintain it is of no use in ectopic pregnancy. In two of their cases reported, the positive reaction ante-dated the first missed menstrual period. Their mice were castrated after being tested as regards the regularity of their oestral cycle. Their results are founded on the following classification:-

Dioestrus. negative, if there is a preponderance of leucocytes, some mucus, and an occasional nucleated epithelial cell.

Pro-oestrus. weakly positive, smear contains only a few leucocytes, an excess of nucleated epithelium, and some non-nucleated or cornified cells.

Oestrus; Positive, if the smear contains no leucocytes, no mucus and a few nucleated epithelial cells with a marked preponderance of cornified epithelial cells.

Strongly positive if the smear shows only non-nucleated or cornified cells.

Their results are:-

	Positive	Negative.
Few days before first missed period....	2	2
1 week after first missed period.....	8	2
2 weeks after.....	7	1
3 -"-	6	2
4 -"-	6	0
5 -"-	6	1
3rd month pregnancy.....	8	0
4th -"-	8	0
5th -"-	10	0
Total	61	6
Non-pregnant cases.		
1st week of menstrual cycle.....	1	10
2nd -"-	2	24
3rd -"-	2	28
4th -"-	2	22
Menorrhagia due to hyperplasia	0	6
Puerperium.....	2	2
Lactation period.....	0	7

Non-pregnant cases (Continued)	Positives	Negatives
Menopause.....	0	11
Uterine fibroids.....	0	6
Ovarian cyst.....	1	0
Amenorrhoea of unknown cause.....	1	7
Acute inflammatory pelvic disease..	4	4

In Mazer and Hoffman's cases of pregnancy there is a percentage error of approximately 10. In their control cases there is a percentage error of approximately 11. These results rather discredit the good results of other workers. Their technique differs essentially from that introduced by Zondek and Aschheim and, as I hope to show later, their results should, in my opinion, be discarded. The only surprising feature is, as far as I can see, that their percentage error should be so small.

Kraul and Rippel (22) report 13 very early pregnancies; one had 8 days amenorrhoea and eight of the others were afterwards shown to be pregnancies of four week's duration; all gave positive results. In 2 cases, pregnancy was in a myomatous uterus in which amenorrhoea could quite well have been due to either pregnancy or to the menopause.

Odescalchi (23) obtained good results in 36 cases of pregnancy in the later months.

Louria and Rosenweig (24) used female white mice varying in age from 3 to 6 weeks and in weight from 10 to 15 grammes; 0.3 ccm. was injected subcutaneously three times each day for 3 days. The mice were killed and examined on the 5th day. In all, 155 cases were done, but in 23 cases no results were obtained owing to the death of the mice.
Of cases of pregnancy there was a correct result of 98%.
The earliest case was one in which the menstrual period was 7 days overdue. The results are tabulated thus:-

Cases of pregnancy.	No. of cases.	Positive.	Negative.
From 1 to 3 months.....	31	30	1
From 3 to 6 months.....	26	26	0
From 6 to 9 months.....	30	30	0
Non-pregnant cases.			
Males.....	5	0	5
Fibroids and Ovarian cyst..	5	0	5
Functional amenorrhoea.....	18	0	18
Miscellaneous.....	17	1	13 (3 doubtful)

THE OVARIAN HORMONE.

For a considerable time now it has been known that the ovary is the seat of some internal secretion and in recent times experimental work on this hormone has been intensified. It has been isolated and prepared in a water-soluble form, and it has been used therepeutically in various disorders of the female sex. This hormone has been termed "Oestrin", which term is used throughout this work as meaning the internal secretion of the ovary.

For the recognition of the presence of this hormone in solution, much credit must be given to Allen and Doisy who elaborated the vaginal smear technique in mature and immature rodents and proved its origin from the growing Graafian Follicle. This work has now received universal recognition and I have found the method reliable in the course of my work. The vaginal changes during the oestrus cycle in the mouse are very pronounced. During the pro-estrus the vaginal mucosa becomes many layers thick, and this is followed by keratinisation of the superficial cells. These are thrown off into the lumen of the vagina and may be collected for examination. This stage is accompanied by infiltration of vast numbers of leucocytes from the Stroma.

These cyclic changes can be summarised thus:-

Di-oestrus. Vaginal smear shows epithelial cells, mostly nucleated, and large numbers of leucocytes.

Pro-oestrus. Leucocytes disappear and nucleated cells are fewer and stain lightly.

Oestrus. Nucleated cells replaced by cornified cells with complete absence of polymorphonuclears (Fig. 1.)

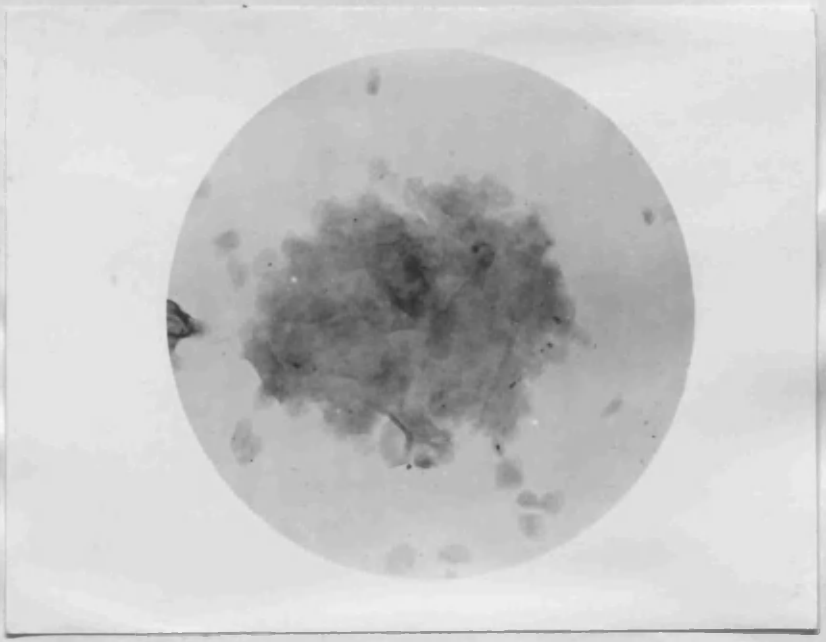

Fig 1.

Vaginal smear. Note keratinisation of cells with absence of Polymorphs; magnified 100 diameters. Effect of oestrin only.

Met-oestrus. Return to di-oestrus where the cornified cells are replaced in increasing numbers by polymorphonuclears and nucleated epithelium.

If injected into immature mice, oestrin brings about the appearance of precocious oestrus, namely, increase in size of the uterus and opening of the vagina, accompanied by cornification of the superficial cells. Recent work seems to suggest some close relationship between the anterior pituitary body and the ovary. Disease of pituitary often results in marked aberration of the sexual organs and the general configuration. Some experimental work has been done with extracts of pituitary and their effect on the ovary. Evans (26) with a saline extract produced in rats the cessation of the oestrus cycle and extensive formation of corpora lutea atretica in the ovaries. The amount of evidence that the ovary does not regulate its own periodicity led Zondek and Aschheim to carry out research on the effect of implantations of the other endocrine glands. As a result of their work they came to two definite conclusions.

(1) Implantation of male or female anterior pituitary into immature mice brings about precocious oestrus including the ovarian changes.

(2) The effect is exerted solely through the ovary, which is caused to undergo premature maturation and elaborates its own internal secretion "oestrin".

To corroborate this feature several experiments have been performed.

(a) Serum was obtained from a patient aged 18, one day after commencement of menstruation. 0.3 ccm. was injected as each dose twice on 3 successive days. The mice were examined 5 days later. These showed the typical uterine and vaginal changes of Oestrin (Fig. 2. (b).

(b) The urine from this case was sent by Professor Beckwith Whitehouse for oestrin investigation. The chief points in her history are - 18 months previously patient had had sub-total hysterectomy, double salpingectomy, and left oophorectomy performed. After operation menopausal symptoms appeared. These symptoms gradually disappeared. 16 months later she again came under observation with symptoms arising from the remaining right ovary. Laparotomy revealed a diseased but functionating right ovary which was removed. 3 weeks after operation, typical menopausal symptoms again appeared in a most pronounced fashion. Four to five flushes per diem occurring three to four times a week. The urine was obtained shortly after three of these pronounced flushes. The injected mice showed no response to oestrin.

(c) A commercial preparation of oestrin was used and showed the same reactions (d). Fig. 2.

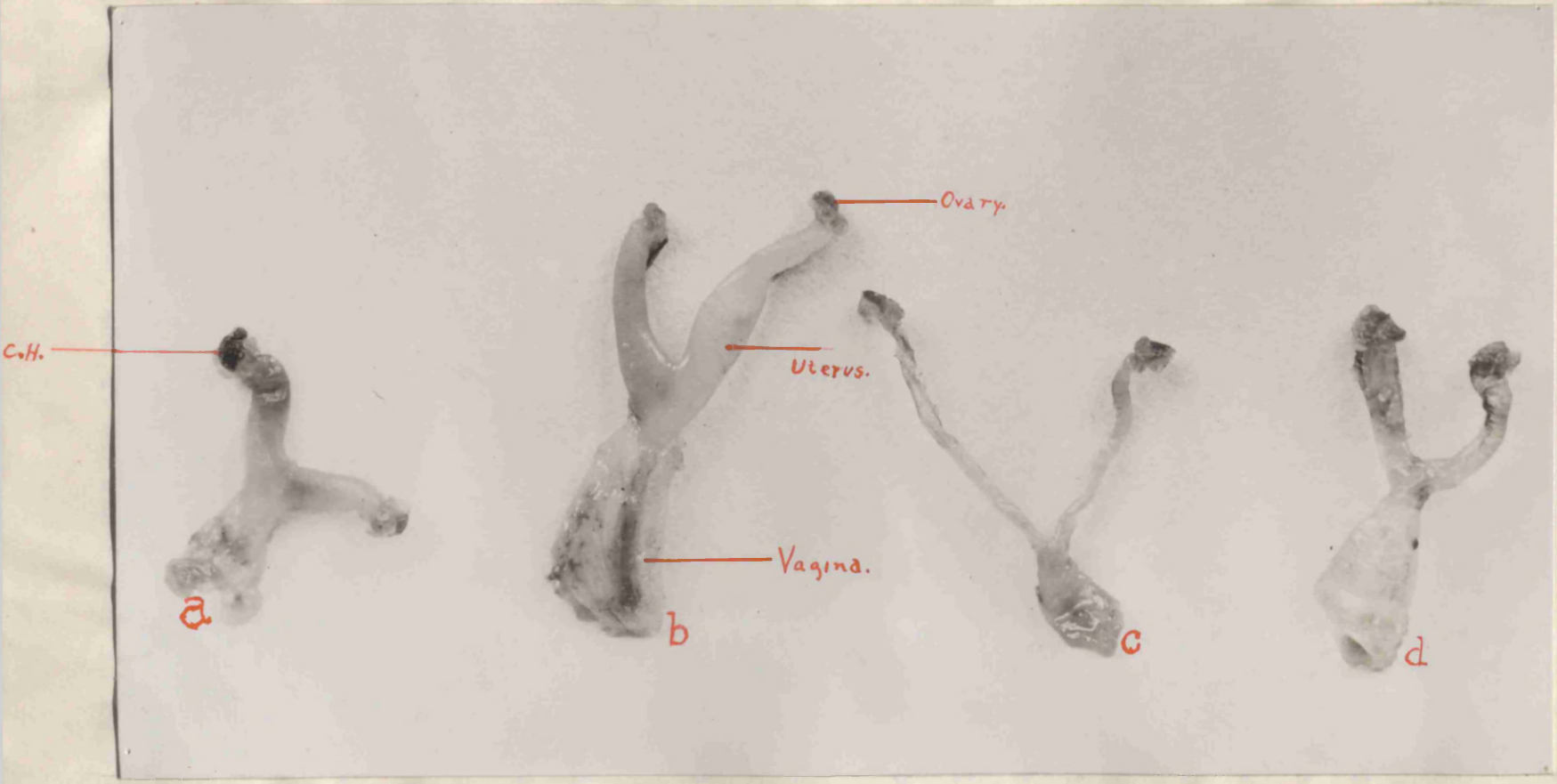

Fig.2.

(a) genital tract showing changes in vagina, uterus and ovaries.
(c.h. corpora haemorrhagica), in positive Z.A. reaction.
(b) oestrin reaction from serum obtained during menstruation.
(c) genital tract removed from control mouse.
(d) oestrin reaction (commercial preparation). All magnified 3 diameters.
In (a) note both oestrin and anterior pituitary effect.
Dark spots in ovaries of (c) and (d) due to shadows while photographing.

In none of these 3 cases were there any ovarian changes beyond slight congestion.

It may not be out of place here to interpolate the results on the specimens of urine which have been examined in which the Zondek Aschheim reaction was negative, but in which there was a response in the injected mice due to oestrin which was present in the urine.

No. of specimens.	Oestrin present.	Oestrin absent.
63.	27	36.

In these 27, although the Zondek-Aschheim reaction was negative, Mazer and Hoffman by their method should have obtained a positive Zondek-Aschheim reaction.

METHOD EMPLOYED, in the course of this investigation.

As a routine method, that of Allen and Dickens has been followed. 0.3 ccm. is injected twice each day over a period of three days. The time selected has been morning and evening and I have found it is of little importance to keep to the exact time each day.. If this should interfere with the laboratory routine work, the intervals between each injection may be diminished or increased. The mice were killed and examined on the morning of the 5th day.

The mice used were 21 days old or as near it as were procurable. According to Kirkham (27) the mouse attains puberty at 6 weeks, and it is essential to keep well within this time.

To distinguish males from females at this age is not easy. In the female, immediately posterior to the clitoris is a pale blue line - the momologue of the hymen in the genus homo.

They were injected subcutaneously on the right side near the root of the tail. As small a needle as possible should be used and a 1 ccm. graduated syringe. To make sure that the needle point is subcutaneous the hair in this region may be removed by depilating powder or as an alternative the hair may be parted after moistening with damp cotton wool. Introducing the needle too far is a very easy procedure and is usually immediately fatal. The mice are killed by enclosing them in some small receptacle with a lid, (as a cigarette tin) with a sponge saturated with chloroform. This kills them within a few seconds. They are then pinned out in a plaque of Columbia paste or solid paraffin and vagina examined. If patent, a smear is taken from the lumen with a platinum loop and is mixed with a drop of distilled water on a numbered microscopic slide. The slide is then put on the microscopic lamp to dry after which it is fixed with mercuric chloride and stained with haematoxylin. In taking the smear one has to be careful that none of the superficial cornified cells of the skin are carried into the lumen on the platinum loop.

The abdomen is next opened and the uterus and ovaries examined. A positive case shows:-

(1) Opening of vagina.)
(2) Increase in size of uterus,) Oestrin effect

(3) Increase in size of ovaries with the formation of corpora haemorrhagica and corpora lutea atretica. (Fig. 3 and Fig. 4.). = Anterior Pituitary effect.

The vagina in the control mouse is not open. The lumen is shut off by a thin wall of cells which is ruptured at the first oestrus, but there is distinct canalisation of the anterior or uterine end.

The vagina is composed of the three layers found in the genital tract, mucosa, muscular, and peritoneal. In the mucosa there are a large number of mucus-secreting glands, and it is lined with squamous epithelium which is usually a few cells deep. In a positive case there is -

(a) Distension of the vagina with increase and hypertrophy of the squamous epithelium,
(b) Increase in the sub-epithelial connective tissue and
(c) great activity on the part of the mucus-secreting glands.

The squamous epithelium undergoes very considerable proliferation.

Fig. 3.

Genital tract, natural size.

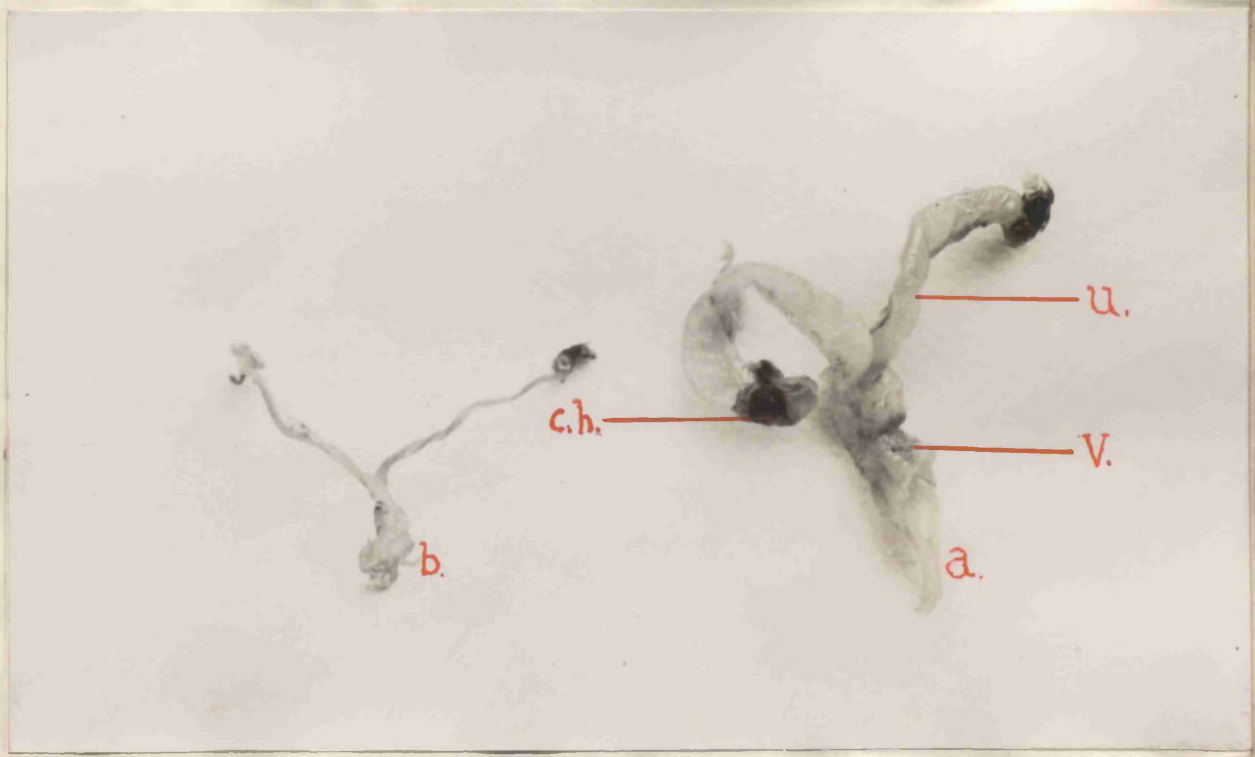

Fig. 4.

Magnified 3 diameters.
(a) is genital tract from positive case. (b) is that from control. In (a) note increase in size of vagina (v), in uterus (u), and in Ovaries (o) within the latter, corpora haemorrhagica (c.h.)

The surface cells lose their nuclei, and are shed off into the lumen as cornified cells (Fig. 5.) This opening of the vagina usually occurs on the 4th day.

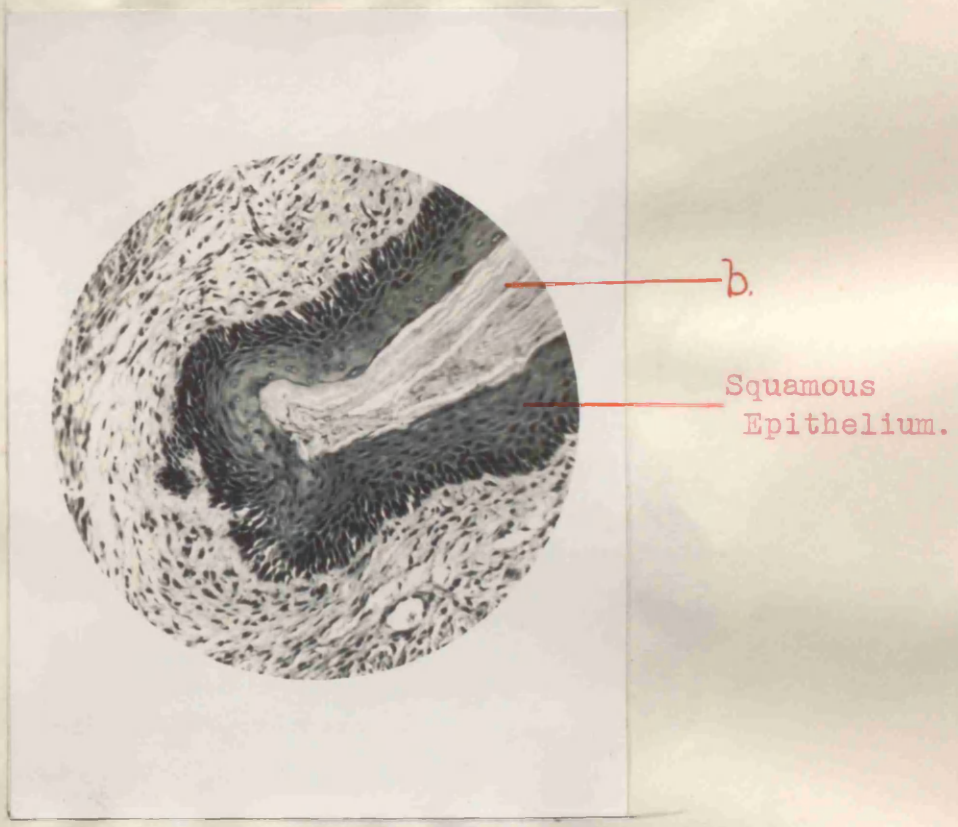

Fig.5.

Transverse section of vagina in a positive case.
Note proliferation and keratinisation of the squamous epithelium which is cast off into the lumen as cornified cells (b) Compare with Fig.6.
Magnified 150 diameters.

Occasionally in a mouse with a good positive response it may open on the evening of the 3rd day. In only one case have I seen a non-patent vagina in a positive reaction.

THE VAGINAL SMEAR.

In a negative case there is no smear. In a positive case after staining one sees most of the cells have undergone keratinisation (Fig. 6.)

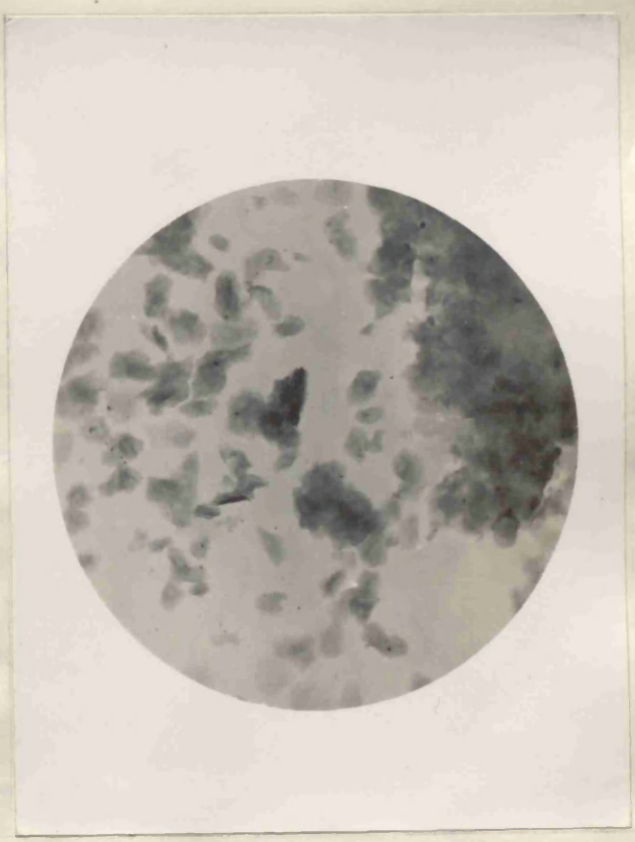

Fig. 6.

Cornified cells obtained by vaginal smear in a positive reaction. Magnified 100 diameters.

Usually there are also some cells which have retained nuclei, and there are also some polymorphonuclears. The cornified cells may be relatively few and the smear obtained is usually of a mixed type, and in some there is a relatively large number of polymorphs. In more than 500 smears which I have now examined there were very few in which nucleated cells and polymorphs were absent, and to attempt a diagnosis on this smear examination alone would not, in my opinion, give very reliable results.

The keratinisation of the superficial cells was used by Allen and Doisy to demonstrate the presence of "Oestrin" in the injected fluid and although this "oestrin" effect (Fig. 7.) is produced in a positive reaction to the anterior pituitary hormone it is not proof positive of the presence of this anterior pituitary hormone as "oestrin" may be present alone.

The smear containing the large number of polymorphs and nucleated epithelium is, as I have pointed out before, characteristic of the pro-oestrus stage. Blair Bell has demonstrated the emigration of leucocytes between the epithelium of the glands in the uterus of the rabbit.

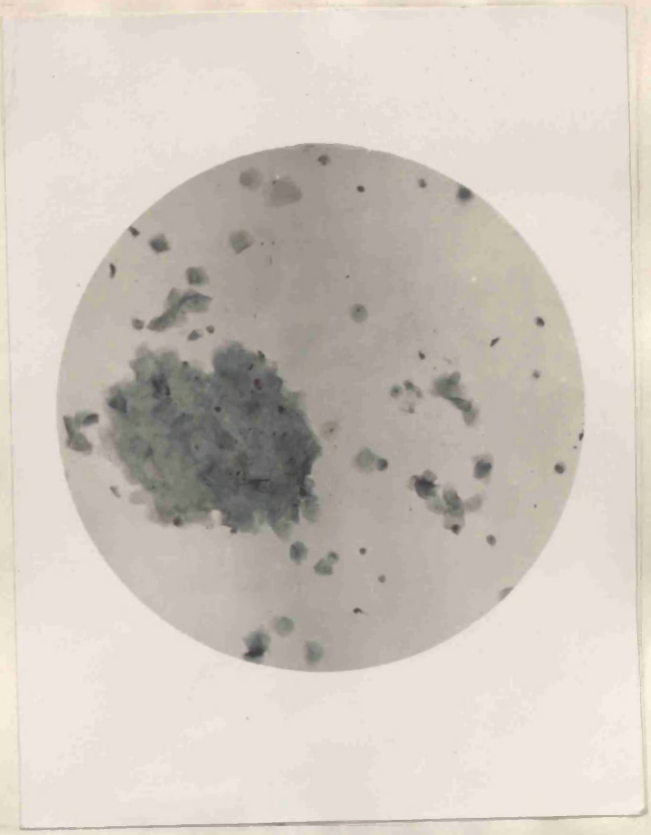

Fig. 7.

Cornified cells in a negative Zondek-Aschheim reaction in which oestrin was present in the urine. Magnified 90 diameters.

UTERINE CHANGES.

The uterus in the mouse is bi-cornuate. In the control (Fig 3 and Fig.4.) it is thread-like and lies in intimate relation with the ureter. It is composed of three layers (Fig.8.)

(1) Mucosa.

(2) Muscle,

(3) Peritoneum.

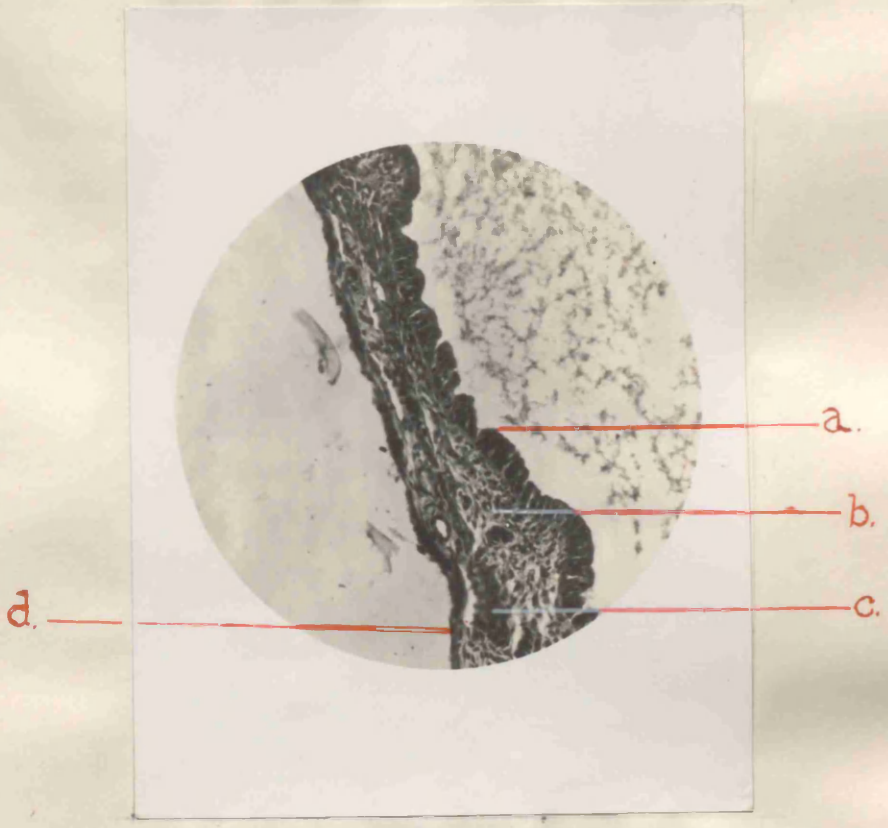

Fig. 8.

Longtitudinal section of uterus in control mouse. (a) columnar epithelium (b) trophospongia (c) muscle layers, (d) peritoneal layer.
Magnified 150 diameters.

The mucosa is lined with columnar epithelium, into it there open glands which, according to Marshall (28) secrete protein material. Underneath the columnar epithelium is a loose connective tissue which has been very aptly termed by Hubrecht the "tropho spongia". Outside this is the muscle coat which consists of two layers, circular and longtitudinal, with a peritoneal covering on the outer side. In a positive reaction (Fig.9.) the uterus is ballooned-out and increased in size.

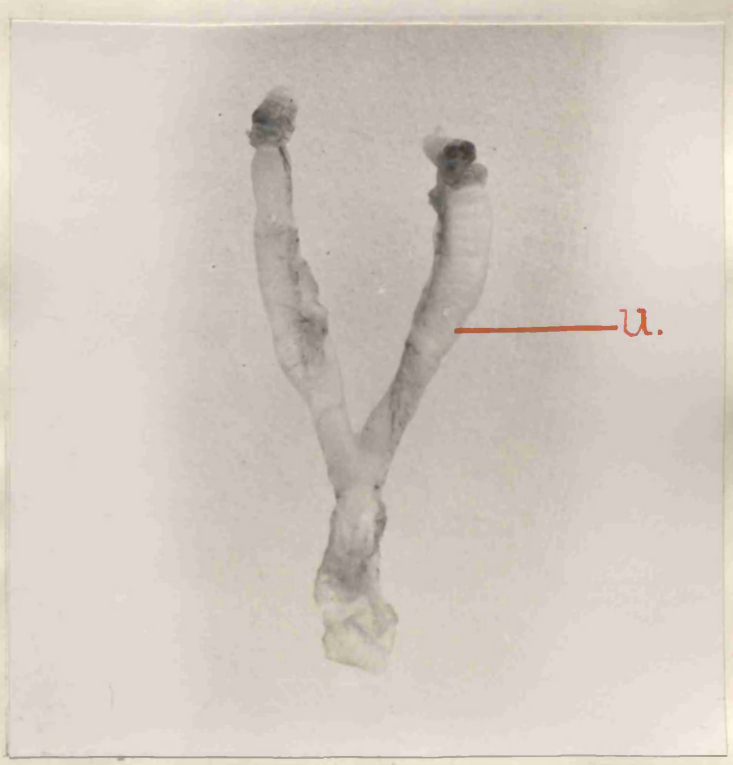

Fig. 9.

Genital tract from positive case.
Note ballooning of uterus (u).
Magnified 3 diameters.

From actual measurements it is often 8 to 10 times larger in diameter than the control. This increase on section is seen to be due to:- (Fig. 10)

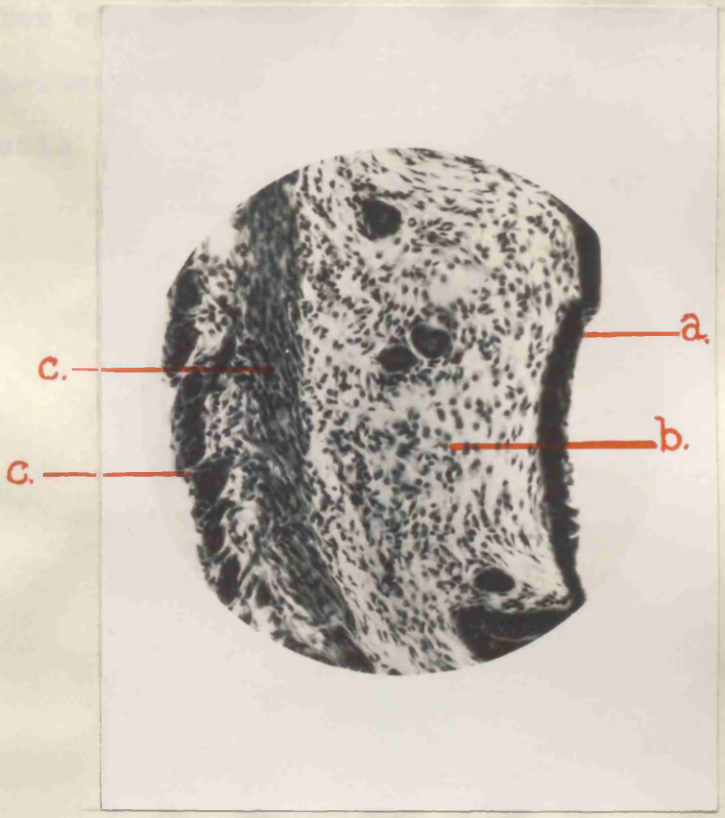

Fig. 10.

Transverse section of uterus in a positive case. (a) columnar epithelium, (b) trophospongia, (c) muscle layers. Magnified 150 diameters. Compare with Fig 8. and note increase in trophospongia and in muscle layers.

(1) Oedema.

(2) hypertrophy and proliferation of the cells of the tropho spongia.

(3) hypertrophy and increase in number of the muscle fibres of both layers,

(4) Pronounced engorgement and increase in the number of vessels.

The engorgement and congestion is very pronounced and a large artery running along the inner border of the uterus is often prominent.

The macroscopic and microscopic appearances so far described, are all typical of the reaction due to "oestrin".

THE OVARIES.

The ovaries lie underneath the lower pole of each kidney. They are of about the size of a pin's head and are pale grey in colour. In close relation with them are the Fallopian tubes which lie in a whorled arrangement. The Fallopian tubes are not of importance in this test.

MICROSCOPIC APPEARANCES OF OVARIES.

In the control the ovary is seen to be made up of numerous primordial follicles and small Graafian follicles without cavities. In most cases the epithelial cells of the interior of these follicles have not differentiated themselves into the membrana granulosa and the discus proligerus. The ovum usually occupies a central position. There are no corpora lutea to be seen (Fig.11.)

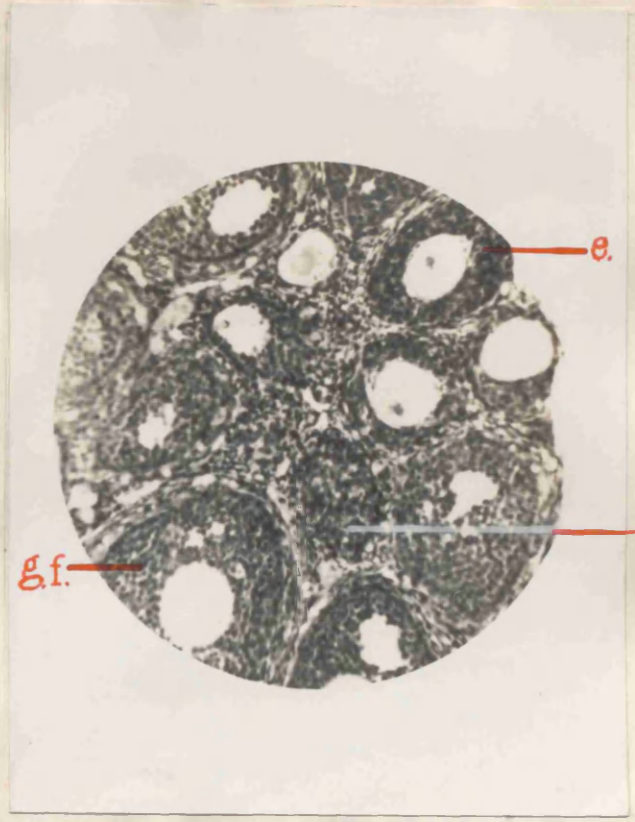

Fig. 11.

Transverse section of ovary in control mouse.
(p.f.) primordial follicles,
(g.f.) immature graafian follicle,
(e) lining epithelium of follicle.
Magnified 150 diameters.

In the positive case the ovaries are increased in size (Fig.4.) This increase in size is due to :-

(1) maturation of Graafian follicles

(2) formation of corpora haemorrhagica

(3) formation of corpora lutea

(4) congestion.

The corpora haemorrhagica are easily seen. (Fig 12)

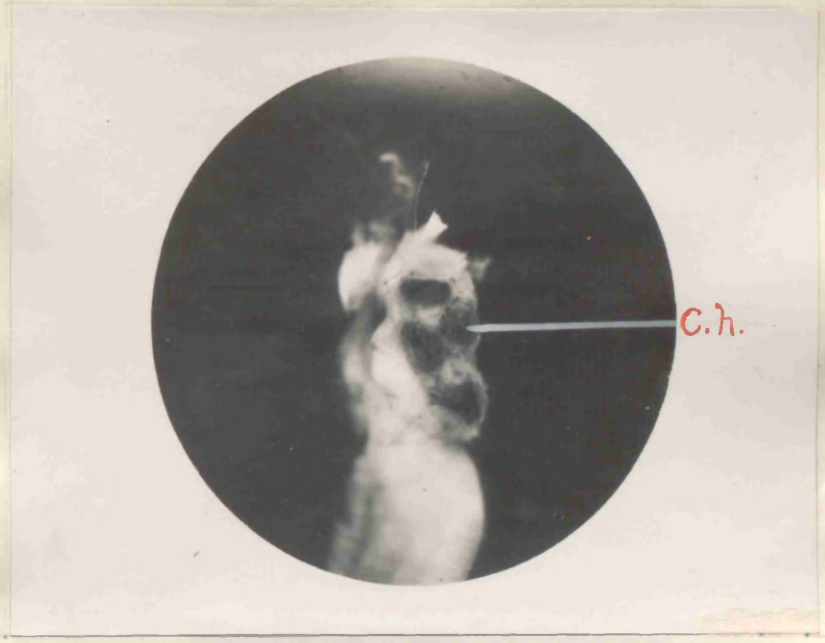

Fig. 12.

Ovary - showing corpora haemorrhagica (c.h.).
Magnified 10 diameters.

They are of about the size of a pin head and deep red in colour, and on section of the ovary they are seen to be composed of an enormously enlarged Graafian follicle in

the cavity of which are red blood corpuscles. (Fig 13). The theca externa is usually intact but the theca interna and the epithelial lining cells are usually partly or completely destroyed, which probably occurs at the time of rupture.

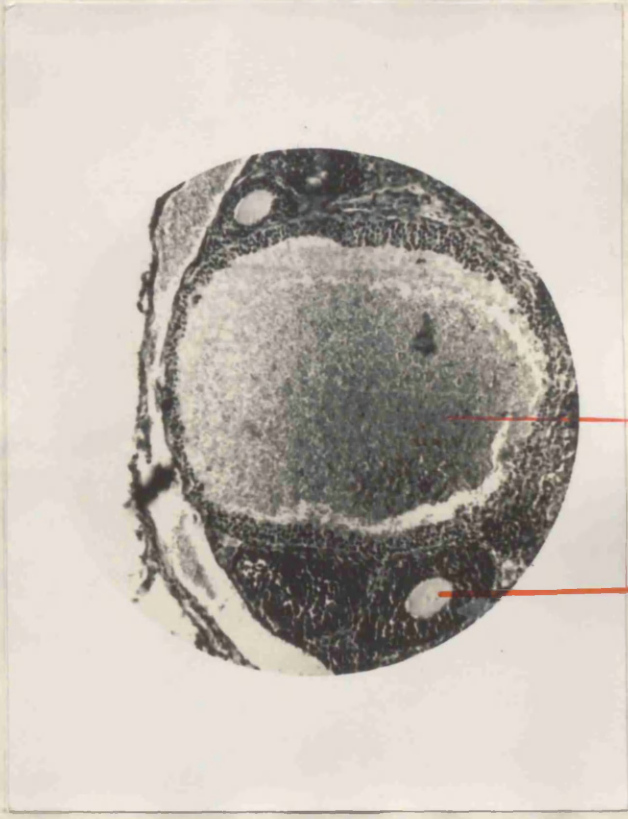

Fig. 13.

Transverse section of corpus haemorrhagicum. Interior composed of red cells; epithelial lining partially destroyed but fibrous theca intact.
Magnified 150 diameters.

Other follicles are seen to have matured and show the formation of the membrana granulosa and of the discus proligerus around the ovum and a cavity containing the liquor folliculi. So far, most workers maintain that there is rupture of the follicle with discharge of the ovum into the Fallopian tube followed by the haemorrhage to form the corpora haemorrhagica. In most cases which I have examined by section, I could find no evidence of rupture nor have I seen a single ovum in the Fallopian tube. If the corpora haemorrhagica be situated in the depths of the ovary as they sometimes are, it is difficult to see how the ovum could have been discharged. On the other hand, I have not seen any ova in the corpora haemorrhagica. In only two of the mice examined have I seen distinct evidence of rupture. In both there was a gelatinous exudate around the ovary and Fallopian tube. This exudate was bloodstained, the blood coming from a ruptured Graafian follicle or corpus haemorrhagicum. If one kills the mice on the 4th day instead of the 5th, the corpora haemorrhagica are seen sometimes very small in size and almost indistinguishable. Probably these would be the normal size on the following day, so that they do not form suddenly within a few seconds, but go on increasing in size over many hours.

If one examines a corpus haemorrhagicum immediately after removal, a small capillary can be seen running into its centre from the ovarian stroma.

THE CORPORA LUTEA ATRETICA.

These are usually slightly smaller than the corpora haemorrhagica. They are quite distinct and of a pale greyish-yellow tinge. They were, in my series of cases, more frequently seen than the corpora haemorrhagica. If examined in the fresh specimen under the low power, they are circular in shape, of a glistening character, and very vascular. A central artery can be seen with numerous radiating branches and anastomosing capillaries. On section they are bound by the fibrous theca and there is a distinct network of connective tissue among the luteal cells.

The ovum usually lies shrivelled up towards the centre. In the centre of some may be seen a blood clot which the luteal cells are apparently invading (Fig. 14). This suggests to me that the formation of the corpus luteum atreticum is sometimes preceded by haemorrhage into the follicle. In most cases no central haemorrhage is seen.

Fig. 14.

Transverse section of corpus luteum atreticum. Note central haemorrhage (c.h.) Magnified 150 diameters.

As regards the structure of the corpus luteum atreticum the cells are roughly arranged in strands radiating from the centre and in some are seen arising from the epithelial-like cells of the trus interstitial tissue of the ovarian stroma. In some sections there is a partially formed

corpus luteum atreticum. This shows that the epithelial cells which line the inner wall of the follicle, namely those which go to form the membrana granulosa and discus proligerus, are pushed inwards. They are mostly in process of disintegration. It is quite obvious that it is not from proliferation of these epithelial cells that the corpus luteum atreticum is formed.

The practical importance of these changes is, in showing that, under the influence of the anterior pituitary hormone there are brought about these follicular changes which characteristically only occur in the mouse at puberty. The corpus luteum atreticum is analogous to the true corpus luteum of oestrus or pregnancy.

These changes are said to be found in the immature rat normally. It is therefore inadvisable to use this animal for this test. Such has not, however, been definitely proven.

All the changes in this description, apart from the ovarian changes, viz, those of uterus and vagina, may be found after injection of "oestrin" or fluid containing this hormone. In such there are no ovarian changes; there may be slight congestion (Fig.2) To use these "oestrin" changes as characteristic of pregnancy as Mazer and Hoffman have done must fail to lead to accurate results.

"Oestrin" is normally secreted in the urine during pregnancy, in many cases of non-pregnancy amenorrhoea, and at the menopause at the cessation of menstruation. It is somewhat remarkable that "oestrin" should be present in these cases in the urine even in the absence of the menstrual flow. "oestrin" is now recognised to be the hormone of the ovary. According to most workers in this field the anterior pituitary stimulates the ovaries. The ovarian hormone arising therefrom stimulates the uterus and vagina. I believe that the uterine and vaginal changes are, at least, partly due to the "oestrin" already present in the urine.

As a routine procedure I have relied upon the macroscopic examination of the genital tract alone. Microscopic examination was performed to study the minute structure, and the histological changes brought about by this reaction. If sections had to be cut, the results would be delayed and the cost of such a procedure would certainly negative it as a routine in the majority of laboratories. In doubtful cases section of the ovaries may be performed. Zondek and Aschheim performed serial-section, in a large number of cases to control their macroscopic findings.

RESULTS OBTAINED IN THIS INVESTIGATION.

At the beginning of this series a fresh specimen of urine was obtained each morning during the course of injections. Only one specimen of urine is now used throughout the test. It is all that is necessary. It is placed in the ice chest over night. This was a point which had not been clear in the work already published.

In the course of this work a series of 100 cases are discussed and verification of the result has been obtained in all cases. In 57 cases the products of conception were present either in a physiological or pathological state; 43 were non-pregnancy cases. The latter group contains cases which simulated pregnancy. The additional cases were taken in the course of an investigation into any possible fallacies. Fallacies in this test must be regarded as of extreme importance. These cases may be divided as follows:-

A. 39 in which the pregnancy was physiological, some being complicated by tumours, others by constitutional disease.

B. 15 are definite pathological abnormalities of pregnancy,

C. 22 in which pregnancy was suspected or possible, including a few other cases of interest.

D. 25 of various gynaecological conditions, some of which are liable to simulate pregnancy and others are not: these were investigated for possible fallacies in the test.

As regards A, these are made up of the following:-

Normal uncomplicated pregnancies.....18

Hyperemesis.....4

Pregnancy and morbus cordis..... 1

Pregnancy, Pyelitis, and Albuminuria..... 6

Pregnancy and Epilepsy..... 2

Pregnancy and Syphilis..... 2

Pregnancy and Gonorrhoea..... 1

Pregnancy and pulmonary tuberculosis..... 2
(Including 1 of hydatidiform mole)

Pregnancy and excessive obesity..... 2

Pregnancy and uterine fibroids..... 2

Total 39.

All gave a positive Zondek Aschheim reaction.

B. Abnormal pregnancies.	Cases.	Positive.	Negative.
Hydatidiform mole.....	9	8	1
Carneous mole.....	2	2	0
Dead foetus.....	1	1	0
Ectopic pregnancy.....	3	2	1
Total	15	13	2

C.	Cases.	Positive.	Negative.
Amenorrhoes	9	0	9
Menstruation	1	0	1
Menopause	2	0	2
Rape	1	0	1
Abortion	3	0	3
Abnormalities of the thyroid.	4	1	3
Tabes dorsalis	1	0	1
Amenorrhoea and gonorrhoea...	1	0	1
Total	22	1	21

D.

Fibroids and fibrosis uteri..... 4

Uterine polypi (malignant)..... 1

Menorrhagia..... 2

Salpingitis (acute)..... 1

Carcinoma of cervix..... 3

Carcinoma of body of uterus..... 1

Ovarian tumours..... 9

Pituitary tumours..... 1

Chorionepithelioma..... 1

Unclassified..... 2

Total 25.

All gave negative results.

This makes a total of 101 since one of the patients had a four and a half months pregnancy. Syphilis (W.R.++), gonorrhoea and B. coli pyelitis. She deserves inclusion in two groups, pyelitis and syphilis.

GROUP "A". Normal uncomplicated pregnancies.

The days are numbered from the last menstrual period.

25 to 60 days amenorrhoea..... 10 cases.

60 to 100 days - " - ..... 10 cases

100 to 200 days - " - ..... 4 cases

200 to full time..... 11 cases

A few of these cases are important.

Case 2, Mrs. C., a school teacher by profession had obtained a position some distance from her home. She was extremely anxious to have a child, but there had been two miscarriages in the past. She was of an athletic nature and the miscarriages had been attributed to this. She visited her doctor to see if he thought she might be pregnant. If so, she was willing to forego her appointment in the hope that the pregnancy might terminate successfully. Her menstruation was of the 28 day type and was not due until 5 days after the visit to her medical adviser. There had been no interruption of menstruation. The urine was obtained on the Tuesday, Wednesday and Thursday, menstruation being due to commence on the Friday of the same week.

The mice were killed on the following Saturday and in only one of the four which had been injected was there a positive "oestrin" reaction without any ovarian changes. The reation was therefore negative. The menstruation period which was due to commence on the following Friday was missed. The test was therefore repeated. The urine was obtained on the Tuesday, Wednesday and Thursday of the following week, and on this occasion one of the mice revealed a positive reaction. (Fig. 15.)

As in this series the test is considered positive if one mouse from the batch shows a positive response, the urine was reported as giving a positive reaction. Menstruation on the previous occasion lasted five days. The positive result was therefore obtained within a period of 28 days at the outside. The result of the test was subsequently verified. The patient gave up her intended post and the pregnancy has continued normally. As three specimens of urine had been used in this case it was impossible to say which one was responsible for the positive reaction, but quite possibly the hormone responsible was only beginning to be secreted as otherwise it would have given a positive reaction in all the other injected mice.

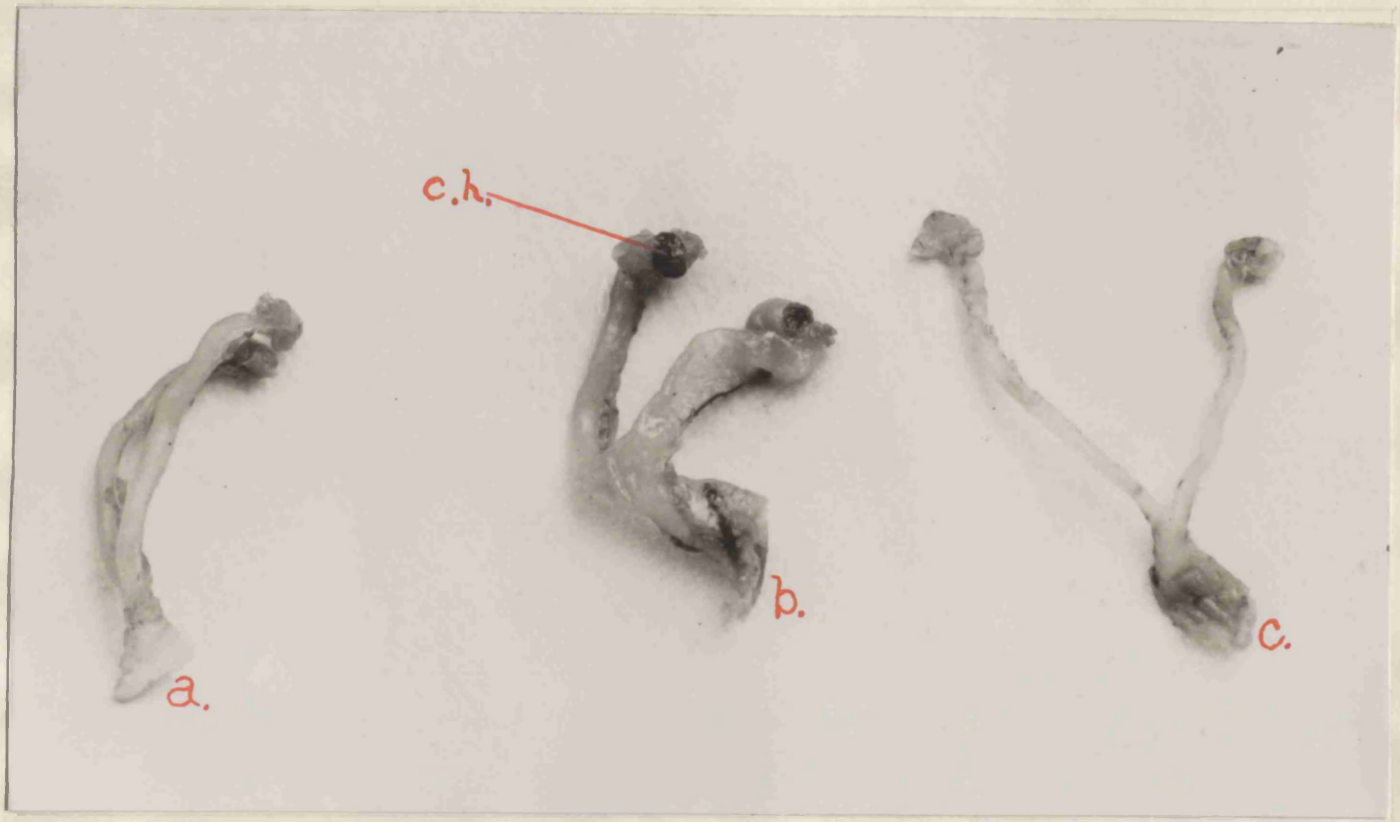

Fig. 15.

Genital tracts removed from mice in case 2, page 45.
(a) that before menstruation due, (b) after menstruation,- in this note corpora haemorrhagica (c.h.) (c) control mouse.
Magnified 3 diameters.

Case 97. The date of the last menstrual period was 14th August 1930. Coitus, which only occurred once, took place on the 1st September 1930. The menstruation which was due in the middle of September was missed. The urine was examined on the 22nd September 1930 and in all the mice injected there was a very strong positive "oestrin" reaction without ovarian changes as confirmed by microscopic examination. A second specimen of urine was obtained from that passed on the morning of 1st October 1930. This, in all the mice injected, gave a positive Z-A result. Therefore, in this case the transition from the negative to the positive phase was somewhere between the 23rd and 30th day from impregnation.

A few attempts have been made to obtain similar cases. Urines have been examined over intervals of 3 and 4 weeks with negative results and were followed in due course by normal menstruation.

Case 33, an unmarried woman aged 33; 77 days amenorrhoea. Report of a positive reaction followed by the publication of the banns within three days, and within a week of married life abortion took place.

This test is useful in cases of hyperemesis. It is often difficult for the practitioner to distinguish between that due

to pregnancy and that due to organic disease. Four cases are reported, in all of which there had been only one missed menstrual period.

In heart disease where the patient may have passed through a previous pregnancy with considerable danger to her own life, it may be advisable to advise against conception in the future. Such advice may not have been taken and the uterus may require to be quickly evacuated. Heart disease has apparently no effect on this reaction.

This reaction can also be used in cases of infected urine, the result being unaffected by bacterial invasion of the urinary tract. All other workers have failed to obtain results in such. In no case of urine received in the course of these investigations has the result been vitiated by reason of the death of the mice due to the toxicity of the urine. This difficulty has been overcome and details of the procedure appear later.

Neither venereal disease no tuberculosis have apparently any effect on this reaction.

It is useful in cases of excessive obesity. At a recent medical meeting where X-Rays and its application was being discussed, a case was mentioned of suspected pregnancy.

The weight of the patient was about 19 stone. Although she had been examined by various obstetrical experts, no definite decision was given. She was approaching the menopause and very nearly full-time if actually pregnant. The diagnosis was only arrived at after X-ray examination. I have had two similar cases:-

Case 103, aged 42, multiparous, weight 16 stone, menstruation very irregular usually twelve to sixteen months intervals, with only three menstruation periods in the last four years. Clinical examination was most indefinite. The Zondek Aschheim reaction was positive.

Case 110, aged 32, weight 15½ stone, with symptoms of hypethyroidism, amenorrhoea of 120 days. On clinical examination no definite opinion could be given. This case also gave a positive reaction.

It may be used in cases of pregnancy complicated by fibroids ~~or the pregnancy~~. The presence of fibroids in the uterus does not affect the accuracy of the Zondek Aschheim reaction.

GROUP "B". Abnormal pregnancies.

In the nine cases of hydatidiform mole here reported, only one gave a negative reaction. It is important to note

in this negative case that the urine was obtained five days after the uterus had been evacuated. The doctor who sent it unfortunately relied on the information I had given him, that the reaction remained positive for eight days. This information was obtained from the literature, and I do not think it can now be accepted. This would explain the negative result obtained.

Two cases of carneous mole are reported.

(Case 1.) there had been 178 days amenorrhoea, there was a history suggesting an incomplete abortion two months previously. The specimen of urine was obtained immediately before the patient was sent into the operating theatre. The specimen removed showed old placental tissue and some fresh placental tissue with blood clot.

(Case 2.) 95 days amenorrhoea. This patient was admitted to hospital and shortly after admission the contents of the uterus were expelled. The specimen passed was that of a carneous mole of a tuberous type. On section it showed active and degenerated villi embedded in blood-clot. There had been repeated slight haemorrhage for the past 3 weeks. The urine in this case was obtained half an hour after the uterus had been emptied. Both of these cases gave positive reactions.

Case of dead foetus.

Amenorrhoea of 233 days, before which, menstruation had been regular. The reaction was done because, on physical examination the clinical findings did not agree with the patient's history, but suggested a pregnancy of 3 months. She herself, thought there was something wrong - it was a second pregnancy. 27 days after a positive report had been sent, a macerated foetus was evacuated. The estimated age of the foetus was from two to three months. The placenta was fibrotic, and there was practically no haemorrhage during the confinement.

Case of ruptured ectopic gestation.

Case 83, was a patient admitted as a case of acute appendicitis; no menstrual period had been missed, and previously menstruation had been regular. As it was followed by a fatal termination, no further details could be obtained. Post-mortem examination showed the condition to have been a tubal abortion and the presence of pregnancy was confirmed by early decidual changes in the uterine mucosa. The specimen of tubal abortion could unfortunately not be obtained. The reaction in this case was negative.

Case 116.

32 days had elapsed since the last menstrual period. The time of rupture as judged by the onset of acute pain and abdominal symptoms as given by the patient preceded the

specimen of urine used by 31 hours. The urine was obtained before patient was sent into the theatre; it gave a positive reaction. The diagnosis was later confirmed by section of the foetal membranes.

Case 123.

58 days amenorrhoea, at operation a large quantity of clotted blood was found in the pelvis with the remains of the foetus and placental tissue. The urine gave a positive reaction.

GROUP "C".

Most of the cases in this section were of suspected pregnancy. Attempts were made to obtain very early cases, e.g., Case 63, where a further addition to the family was not desired and interrupted coitus had been employed with a recent lapse. The menstrual cycle was of the irregular type; the shortest interval being 3 weeks, and the longest usually twelve. The urine was examined 77 days from the last menstrual period for a suspected pregnancy of short duration, probably within 14 days. The mice used gave only a positive oestrin result. The examination was repeated during each of the 3 following weeks, and the result was consistently negative, to be followed 3 days later by normal menstruation.

Two cases were done at the menopause. It is noteworthy that in both there was a positive oestrin reaction, even after cessation of the menstrual flow.

The reaction was employed in a case of rape (29) for confirmation of a possible negative to a positive reaction. The rape had been committed during the menstrual period which was of the 3 week type. The urine was examined 10 and 17 days after the assault, with a negative result on each occasion. The following menstruation was missed and the examination repeated also with negative result. This was followed in due course by normal menstruation.

This was rather an interesting case, as sufficient time had elapsed between the assault and the trial of the prisoner to have the reaction done, and it would have been used in the criminal proceedings against the accused in the subsequent trial. I am not aware of any case in which the Zondek-Aschheim report has been incorporated in the evidence.

Abortion.

3 cases were investigated.

1. 6 weeks amenorrhoea, uterus completely evacuated 48 hours before specimen of urine obtained. Reaction negative.
2. Retained placenta, 4½ months pregnancy; urine collected 40 hours after evacuation of uterus. Reaction negative.
3. Was a specimen of urine obtained 16 hours after a complete abortion. Reaction positive.

Abnormalities of the Thyroid.

Case 1, aged 19, female, with very marked symptoms of exophthalmic goitre, both lobes of the thyroid being enlarged. Basal Metabolic Rate plus 43, menstruation regular.
The specimen of urine was obtained two days before menstruation This is the only case in which a definite fallacy of the reaction arose. Macroscopically it was quite definitely positive.

Case 2, female, aged 34, hyperthyroidism with uniform enlargement of thyroid gland, Basal Metabolic Rate plus 37, menstruation regular, Zondek-Aschheim reaction negative.

Case 3, female aged 46, not then arrived at the menopause; hyperthyroidism plus myocarditis, reaction negative.

Case 4, male aged 23, was a policeman with very marked symptoms of hyperthyroidism; Basal Metabolic Rate plus 136, both lobes of the thyroid involved, reaction negative.
This was the only case in which a male patient was investigated. It was done following the above positive case in the absence of pregnancy (1), merely as a matter of interest. Other workers record negative results in male urines.

One case of tabes dorsalis and one case of gonorrhoea, with amenorrhoea in both, the reaction was negative, and this was subsequently found correct.

GROUP "D", Gynaecological conditions.

As the work of most people has shown a higher percentage of error in these cases, more than in that of normal pregnancy, I thought it would be advisable to control the findings by a relatively large number of the various gynaecological conditions which might be mistaken for pregnancy. Most of the pathological specimens from the cases cited, have been personally examined. A large proportion have been submitted to microscopic examination.

Fibroids and fibrosis uteri have apparently no effect on the reaction, nor did I find any fallacy of the test in salpingitis. The cases of ovarian tumours are worthy of some detail.

(1) was a multilocular cystadenoma.

(2) was a rather interesting case. It was thought at first to be an ovarian pregnancy, the patient, aged 41, having had 110 days amenorrhoea. Laparotomy showed the ovary to be the size of a fairly large orange filled with blood, the wall being about 1 cm. thick. Section of this ovary showed it to be an infected endometrioma and not an ovarian pregnancy.

Two other cases of ovarian endo-metrioma were obtained. By endometrioma I mean those tumours of the ovary which are associated with fairly severe pain at each menstrual period. They are met with between puberty and menopause, the ages in my cases being 29, 33 and 41. In one case there was a fibroid on the posterior surface of the uterus, but unfortunately it was not sectioned to ascertain whether it was the endometriomyoma of Blair Bell or the adenomyoma of most other people.

(3) was a malignant papillomatous ovarian cystoma.

(4) was an ovarian multilocular cystadenoma.

(5) was one in which difficulty in diagnosis arose. Amenorrhoea 78 days. Laparotomy showed an enclarged ovary and a polypoid growth hanging down from the sigmoid colon,which on bi-manual examination resembled the pregnant uterus. Section of the growth showed it to be an adenocarcinoma of the bowel with secondary growths in the ovary.

(6) was an ovarian dermoid.

(7) was cyst of the left broad ligament about the size of a Jaffa orange with multiple cysts in the ovary. There were extensive adhesions in the pelvis. There had been a confinement six months previously. Section of the wall of the cyst showed only fibrous tissue.

Pituitary tumours.

One case was obtained, female aged 13, showed marked sexual development, hypertrophied mammae, and male distribution of pubic hairs. X-Ray showed enlarged sella turcica. The reaction was negative.

Chorionepithelioma.

One confinement 2 months previously. There was brisk post-partum haemorrhage. The uterus was explored, nothing being found. 4 weeks later a mass could be felt in the cervical canal about the size of a walnut. Wertheim's operation was performed and at the time of obtaining the specimen of urine there was no evidence of recurrence. Section of the tumour showed definite evidence of chorionepithelioma. The reaction was negative.

Unclassified Group.

Case 1. Amenorrhoea 42 days. Abortion 2 months previously. The following menstruation was missed and then slight haemorrhage commenced 14 days later. Curettage was performed. Microscopic examination of these curettings revealed them to be composed of blood clot and numerous oedematous villi. There was also considerable proliferation of Langhans cells to form structures resembling the oedematous villi of a hydatidiform mole. However, these showed in places considerable degeneration and there was no

invasion of the uterine wall. They were probably retained portions of the placenta which had been left behind at the previous abortion. Curettage was sufficient to clear up the symptoms. One could not place these curettings in either the hydatidiform mole or the chorionepithelioma class. The urine was obtained 5 days after the curettage and the reaction was negative.

Case 2. Confinement 6 months previously, miscarriage 6 days before test was done. The uterus on bi-manual examination resembled that of a 3 months pregnancy. Section of the curettings showed them to be composed of degenerative and active villi, with considerable blood clot. The villi were very oedematous. Langhans cells had also undergone considerable proliferation but these proliferated cells were in some places undergoing degeneration and in other places had completely degenerated. The cells in the latter were swollen and distended with rounded collections of fluid. There was also considerable round-celled infiltration but no invasion of the uterine wall. The specimen of urine was obtained in the morning prior to curettage. The reaction in this case was negative.

These last two cases are put in the unclassified group. They were probably both cases in which small portions of the foetal membrane had been retained in which degenerative processes had already occurred and somewhat masked the picture on section.

Summary of results obtained.

	No. of cases.	Positive.	Negative.
Group "A".....	39	39	0.
Group "B".....	15	13	2
Group "C".....	23	1	22
Group "D".....	24	0	24

On death of the mice during the test.

Various workers have had a relatively high mortality in the mice used. Some have had to abandon the test altogether on account of this factor. This has not been my experience. The mortality may be due to:-

(1) Operation

(2) Toxicity of the urine.

In previous pages I have stressed the importance of subcutaneous injection and only subcutaneous. Neither the peritoneal cavity nor the spinal cord is a suitable receptacle for the urine. This, I think, is responsible for some of the mortalities.

The urine may be infected. The mouse has a relatively high resistance to bacterial invasion. The urine of the patient may be infected when passed - pyelitis - it may require to be sent by post during which time the organisms multiply abundantly. To overcome this difficulty I have elaborated three methods all of which are equally successful.

(1) Using blood serum.

(2) Centrifugalisation of the urine.

(3) Treatment of the urine with ether.

As regards (1) it is obvious that any hormone which is excreted in the urine must be present in the blood serum. At the beginning I gave the mice 0.3 ccm. of serum at each dose. On further investigation I found it was active in

doses of 0.1 ccm. and in even slightly less quantities. I found in the use of the blood serum, as Allen and Dickens found in the urine, that mice receiving the larger dose sometimes showed less response than those receiving the smaller dose. I therefore used as a routine in after cases a dose of 0.1 ccm. at each injection.

As the blood serum is not always easy to obtain and causes distinct annoyance to the patient, other methods had to be devised.

(2) The centrifuge method. It is a well-known fact that organisms are thrown down by prolonged centrifugalisation. The urine was centrifuged at high speed for 15 minutes. The supernatant fluid alone was used, the sediment being destroyed. Centrifugalisation was performed before each injection. Although this does not give a urine which will be negative on culture it does yield a urine which may be used with success.

(3) Ether is known to have a distinct bactericidal effect. The urine is shaken up with ether and allowed to stand for some minutes. As the ether is of a lower specific gravity than the urine, it gradually rises to the top and evaporates. Evaporation may be hastened by incubation at 37°C. This temperature I have found has no effect on the hormone. Nothing is extracted from the urine and a urine which is sterile on culture is left behind. The urine is then used directly.

I have found this method serviceable where the specimens had been kept over an interval of 23 and 47 days.

Filtration was the first method tried. A Zeitz filter was used. The urine was cultured before and after filtration. This method, although it gives a sterile urine, unfortunately also extracts the active principle. The filtered urine is quite inert.

Boiling the urine was also tried. It was kept at 102°C. for 5 minutes. The active principle was also destroyed.

I now use the ether method as a routine and I have found it entirely satisfactory.

OESTRIN IN THE URINE DURING PREGNANCY.

Figures have already been given for specimens of urine examined in which the Zondek-Aschheim reaction was negative but in which there was a positive oestrin effect (page 24) Oestrin is also present in the urine of pregnancy. My reasons for believing that oestrin is present in the urine of pregnancy are founded on the results of three experiments:-

(1) In the attempt to sterilise the urine from cases of pyelitis it was boiled for 5 mins. After injection I found that although the anterior pituitary hormone had been destroyed the oestrin had apparently been left intact. All the injected mice gave a positive oestrin effect. This was repeated with similar results.

(2) In another attempt to obtain a sterile urine, the urine was shaken up with chloroform and the chloroform allowed to evaporate to leave behind a sterile urine. This urine when injected gave only a positive oestrin effect, the anterior pituitary hormone had apparently been destroyed. A part of the same specimen of urine was injected after being treated with ether and was found to give a positive Zondek-Aschheim result.

(3) Mice were taken at 3 weeks old and their ovaries were destroyed by X-Rays according to the method of Parkes (30). This entirely throws out of action any ovarian effect which

the ovaries may have in the detection of the anterior pituitary hormone. Urine from a case of pregnancy which was known to give a positive Zondek-Aschheim response was taken and injected. The mice in this case showed opening of vagina, oedema and distension of the uterus and the formation of corpora lutea atretica in the ovaries. There were no corpora haemorrhagica present. It is somewhat remarkable that after destruction of the follicular tissue of the ovary by X-Rays, that there should be the formation of multiple corpora lutea atretica in the ovarian tissue following the injection of fluid containing oestrin. This has also been noticed by Parkes. ("Internal Secretions of the Ovary").

From these results one must conclude that the urine of pregnancy also contains oestrin and that this oestrin may be responsible at least for part of the uterine and vaginal changes in the Zondek-Aschheim Reaction.

The detection of this oestrin alone, cannot be accepted as a test for pregnancy.

When does the reaction become positive.

Zondek and Aschheim from their results conclude that the anterior pituitary hormone is increased in secretion almost immediately after conception. I have not found it to appear at this early stage. In three cases described there was a distinct negative reaction at the commencement of pregnancy. The transition from negative to positive occurred in:-

(1) between the 21st and the 28th day (page 44)

(2) between the 22nd and 30th day (Page 48)

(3) in the case of ruptured ectopic gestation, no menstruation period had been missed and a tubal abortion was followed by a fatal termination (page 52). A negative reaction was obtained.

It could quite possibly have been that I was using too small a quantity of urine in these early cases to detect the anterior pituitary hormone. If larger quantities had been used a positive result might have been obtained. In order to test this, I did a repeat examination in cases 2 and 3 of the above, in which were used 0.6 ccs. at each injection instead of 0.3 ccs. The result was still negative. From this, one must conclude that, making use of the technique which is here described, the reaction is negative at the commencement of pregnancy.

This technique does not differ essentially from that of Zondek and Aschheim.

How long does the reaction remain positive in the pueperium.

Zondek and Aschheim maintain that there is a gradual decrease in this hormone until the 8th day. This has not been confirmed by other workers.

Authority.

Allen and Dickens.	Negative after 48 hours.
Hannen.	2 Cases. 1 became negative on the 5th day, and the other on the 7th day.

In investigating this point I did repeated examinations on several cases, thus:-

(1). In one case which was systematically followed out the results were as follows:-

10 hours after labour.....result positive.

20 hours after labour.....result positive.

34 hours after labour.....oestrin response only.

44 hours after labour.....result negative.

60 hours after labour.....result negative.

(2). Normal pregnancy. 54 hrs after labour, reaction negative.

(3). " " 77 hrs after labour, negative.

(4). Carneous mole. 3 days after, reaction negative.

(5). Hydatidiform mole. (36 hrs after, reaction positive.
(3 days after, positive.

(6). Hydatidiform mole and pulmonary tuberculosis -
18 days afterwards, reaction negative.

This seems to suggest that in cases of hydatidiform mole, the reaction remains positive longer than in a normal pregnancy. This is in agreement with the results of Zondek and Aschheim who found that there is a relatively increased quantity of the active principle in cases of hydatidiform mole.

In the cases of abortion above quoted, the reaction was positive 16 hours afterwards (page 54) but negative after 40 and 48 hours.

What is the reaction due to.

Zondek and Aschheim, as stated before, implanted various ductless glands subcutaneously and found that only one gave this response, viz:- the anterior pituitary. This I have personally confirmed. The other ductless glands I have not tried. I found that both the anterior lobe of the pituitary and extracts were active and produced the typical reaction in immature mouse. It is noteworthy that Weissner at Edinburgh claims to have isolated several hormones from the pituitary, but his results are as yet, only in the experimental stage.

Most workers have now agreed that the anterior pituitary is responsible.

What stimulates the anterior pituitary.

There is a positive reaction in pregnancy, hydatidiform mole, carneous mole, and dead foetus. Few examples of hydatidiform mole have been reported and these more or less isolated cases. I am not aware of any cases of carneous mole or dead foetus.

In view of the fact that the anterior pituitary hormone is present in the urine in all these cases, it cannot be the foetus per se which is responsible for the pituitary stimulation as it is absent in some and inert in others. It cannot be the endometrium as the reaction is negative during menstruation where the endometrium undergoes a certain amount of decidual change, and the reaction is also negative in the cases of endometrioma described. The only factor left is constituted by the foetal membranes and these are definitely present in all the conditions described and probably it is from these that the stimulus comes to give rise to the over-production of the anterior pituitary hormone. At present, work among these lines is being carried on. It is noteworthy that in the case of thyroid abnormality the reaction was positive(page 55); it is known that there is an increase in size of the anterior pituitary in such cases. This would explain the one fallacy which I have found in this test.

Observations on the hormone in this reaction.

The urine was treated with ether and then the urine and the ether were allowed to settle, and the ether separated in a separating-funnel. To the ether portion about half the amount of distilled water was added and the mixture shaken and the ether allowed to evaporate at 37°C. The remaining fluid was then injected and was found to give a similar reaction to the Zondek-Aschheim reaction. The active principle had passed into the water. This has been repeated and similar results have been obtained.

Following the work of Zondek and Aschheim, the effect of anterior pituitary implants has been investigated. Anterior pituitary glands were obtained from the slaughter-house and their anterior portion separated from the posterior, which in the Ox is quite easy. The anterior portion was then macerated in distilled water and injected subcutaneously. This was found to be quite active. The doses which I used was equivalent to 0.1 gram. There was opening of the vagina on the 3rd day and on the 5th day there were multiple corpora lutea and corpora haemorrhagica in each ovary.

Ether extracts of the anterior pituitary were then investigated. The anterior pituitary was separated and then ground in a mortar with some incinerated sand. This was then transferred to a stoppered flask and ether added. The mixture was then shaken and left over-night. The ether was then

filtered off; to this ether filtrate, half the amount of distilled water was added. The ether was then allowed to evaporate at 37°C, and the aqueous residue injected. This residue has a colour not unlike urine. On injection it was found to be definitely active in doses similar to those of the urine. This is quite conclusive evidence that the active principle can be extracted with ether,

The effect of placental injections of placenta was also investigated. A fresh human placenta was obtained and the amnion and the chorion were stripped off. The placenta was then thoroughly washed to remove the maternal blood. This is a necessary procedure. The placenta was then macerated in water and the pulp injected subcutaneously. The dose injected was equivalent to 0.04 gram of placental tissue. It gave the same changes in the immature mouse as those in a positive Zondek-Aschheim response. Ether extracts of the placenta were then tried with similar results.

Treatment of the chorion by the same method gave negative results.

Summary of results obtained.

(1) Implantation of anterior pituitary subcutaneously induces in the mouse of 3 weeks old, mature changes in the ovary.

(2) The same result can also be obtained by the subcutaneous injection of placenta.

(3) In both cases the active principle can be extracted with ether.

Properties of the anterior pituitary hormone as elucidated in the foregoing experiments.

It is soluble in water and in ether. It is destroyed by chloroform. It will not pass through a Zeitz filter, therefore it is in colloidal solution. It is thermo- labile. It is destroyed by heating to 100°C, but it is not destroyed by raising temperature to 37°C. It is not adsorbed by Keiselguhr. It is not affected by bacterial invasion of the genito-urinary system, and there is a definite increase in the excretion in the urine during pregnancy.

Is the Zondek-Aschheim reaction applicable to the lower animals.

A positive reaction has been obtained by other workers in the urine of the monkey and orang-outang during pregnancy. The urine of the cow, pig, rabbit and mouse and elephant have all been studied without satisfactory results.

Allen and Dickens (16) in the urine of the rabbit, cow and cat did not have much success. The urine of animals is usually toxic and strongly alkaline in reaction.

I have tried this reaction in the serum of a pregnant Cow (Cross bred Hereford) with a positive result. I have only been able to obtain one specimen, but I have no doubt that the serum of other animals would also give a positive and reliable reaction.

DISCUSSION.

It has been my endeavour in this work to investigate the Zondek-Aschheim reaction with an impartial mind. My results obtained clearly show that the reaction is a definite and reliable one. The urine in cases where the products of conception are present contains an active principle which acts as a specific stimulus to the ovary of the immature mouse causing it to assume a mature state.

As regards the time of onset of the reaction in pregnancy, my findings are not quite in agreement with those of Zondek and Aschheim. As regards the persistence of the reaction in the urine after parturition, my findings agree with those of Allen and Dickens, but not with those of Zondek and Aschheim, nor with those of Hannan. Hannan, however, made use of a different method.

The transition from the negative to the positive reaction appeared quite definitely to take place between the 22-28th day after conception. It will be remembered that about the same time as the reaction becomes positive the chorionic villi have almost completely formed and vascularisation has taken place. In my opinion it is from this chorionic development that the stimulus comes. I have been fortunate in obtaining a relatively large number of cases of hydatidiform mole. This is not a common condition. I was fortunate too, in having the co-operation of my colleagues who supplied me with clinical notes and specimens of urine from their cases,

I was unfortunate in not obtaining more cases of chorion-epithelioma.

I have no doubt that the foetus was dead in the case described (Page 52). The duration of pregnancy and the clinical findings definitely pointed to the death of the foetus at an early stage. My results in the puerperium seem to suggest that the reaction remains positive longer in cases of hydatidiform mole. It is interesting to note that Gynaecologists had noticed before the introduction of this reaction the presence of multiple lutein cysts in cases of hydatidiform mole and in abnormalities of the pituitary. The reason is not far to seek. Owing to the fact that similar results are obtained in injection of pituitary and placenta gland and since it is generally agreed that the pituitary is the gland responsible for this reaction, why should the placenta also give this response? Three reasons suggest themselves to me:-

(1) the absorption of the placental fluids may stimulate the pituitary which in turn stimulates the ovaries.

(2) the effect may come from the presence of maternal blood-serum in the placenta which has not been completely separated in the washing of the placenta with water.

(3) there may have been a storage of the anterior pituitary mormone in the placental tissues from the maternal blood during the months of pregnancy. This hormone presumably does not pass through the placenta into the foetal circulation in the same amount as it is in the maternal blood, or the foetus would be born at the stage of puberty.

This part of the work is at present far from complete but the investigation is being continued. Purification of the ether extract is being attempted and a definite conclusion as to where the stimulus comes from is being investigated.

From my results in the oestrin investigation I am thoroughly convinced that oestrin is also present in the drine of pregnancy. Fig.1, 6 and 7, are all vaginal smears; they show no difference as regards their cornification. Two of these were from negative cases. All these and similar cases ought to have given positive results by the method of Mazer and Hoffman, although there was no pregnancy present.

I have gone into the microscopic details both in the normal and positive cases. I have seen no detailed description published in the literature which was available to me. Microscopic section of the ovaries is not essential, in all cases I have found that the macroscopic findings were verified by microscopic appearance on section. The study of the anterior pituitary hormone or hormones is at this stage a very complex problem. Two hormones had been isolated in 1928, one oestrogenic and the other oestrus-inhibiting.

Weisner and Crew claim to have isolated 4 in all. It is quite definite that there is some relation between the internal secretion of the pituitary and that of the ovary and apparently both have mutual properties, so that the one is very easily mistaken for the other. The injection of placental tissue only produced numerous corpora lutea in each ovary, but no corpora haemorrhagica. The gelatinous exudate around the ovary and the fallopian tube in this case was very marked. this is a point I have not seen described before and it suggests to me one explanation as to how the ovum finds it's way into. the fallopian tube. The placenta has been recognised as having a high oestrin content for a considerable time. The extraction by ether has been described in detail. Ether has had the reputation of extracting oestrin only; curiosity on this point led me to investigate, and I found I had also extracted the anterior pituitary active principle; repeat examinations of different specimen od urine gave similar results. Commercial preparations of anterior pituitary preparations I have found to be quite inactive. At the beginning of this description the work of Siddal (page 5) was referred to. In his work he relied upon the relative weights of the uteri in the control and injected mouse. His results ought to have been 100% correct in cases which were actually pregnant, but in his control cases his percentage error ought to have been rather high in view of the fact that by his

method he was only testing for oestrin. His work deserves credit and he also deserves sympathy in not observing the prominent ovarian changes. On opening the abdomen of the mouse in a positive case, the ballooned-out uteri are the most prominent feature. His method approached very near the Zondek-Aschheim method.

Most of the gynaecological cases were obtained on the morning of the operation. This cleared up any doubt as to what the condition was. In cases of any doubt the specimen was submitted to section.

In a test of the description under investigation, it is essential that one should know exactly the condition from which the patient is suffering; otherwise the investigation is useless.

In the present investigation of the Zondek-Aschheim reaction, only one fallacy appears; this being demonstrated by the case of hyper-thyroidism described (page 55).

The Zondek-Aschheim reaction is the result of a careful and systematic investigation and in the hands of its discoverers yielded reliable results. This has been confirmed by all workers who have followed the original technique.

My own method, which only differs in detail from the original, has been described with the modifications introduced. The results of others have been gone into and my own results have been described with some clinical notes on the cases. This reaction is also apparently given by the pathological abnormalities of the products of conception, but this does not detract from it's reliability.

Due to the co-operation of my colleagues I have been able to investigate the onset of this reaction in pregnancy and it's duration in the puerperium.
The responses in the immature mouse after the injection of oestrin and after the injection of the anterior pituitary hormone have been sharply demarcated.

The mortality rate in the mice in the work of others has been reduced and the reaction has been extended to include all cases of infected urine.

The blood-serum of the lower animals has also been tried with success and the route along which further investigations will be carried out has been outlined.

The work of Mazer and Hoffman has been referred to in several places. This was essential in view of their results obtained and I hope I have clearly demonstrated why their method and results should be discarded.

CONCLUSIONS.

(1) All the biological tests for the diagnosis of early pregnancy can be dismissed as unreliable with the exception of the Zondek-Aschheim reaction.

(2) The method involved is neither distasteful to patient or physician; a specimen of urine is the only requirement.

(3) Those who have followed the technique of Zondek and Aschheim have obtained results which corroborate the excellent results obtained by these workers.

(4) Oestrin, the hormone of the ovary, in addition to the anterior pituitary hormone, is also present in the urine during pregnancy.

(5) Any method which detects this oestrin alone, cannot be accepted as a reliable test for pregnancy.

(6) The anterior pituitary hormone appears in the urine about the first missed menstruation period, and disappears from the urine about 30 hours after labour.

(7) The reliable detection of this anterior pituitary hormone at this early stage of pregnancy and it's use as a method of detecting pregnancy easily surpasses any other method, either clinical or biochemical, of diagnosing pregnancy.

(8) The excretion of the anterior pituitary hormone in the urine is also apparently increased in the pathological abnormalities of pregnancy, viz., dead foetus, hydatidiform mole and carneous mole.

(9) Specimens of urine from cases in which the urinary tract is infected, can be treated with ether and successfully used for the injection of mice in the Zondek-Aschheim reaction.

(10) This reaction in the urine during pregnancy clearly does not indicate whether the foetus is alive or otherwise.

(11) Similar changes in the ovaries of the immature mouse to those found in the Zondek-Aschheim reaction can be induced by injection subcutaneously of anterior oituitary gland and placental tissue.

(12) The active principle can be extracted by ether from the gland, placental tissue, and from the urine. This active principle can be made to pass easily into distilled water.

(13) The ovarian changes in the immature mouse are the only changes which can be relied upon in testing urine by the Zondek-Aschheim method.

(14) According to the work of others, the Zondek-Aschheim reaction represents a distinct advance in the early diagnosis of chorion epithelioma, a very malignant condition.

The Bromine-water test for pregnancy.

Voge (31) describes this test. He tests for the b-iminazolyl comounds since there has been demonstrated a connection between these and the products of the anterior pituitary. He uses the method of Knoop (32) for the detection of histidine, thus - 2.5 ccm. of urine are put in a test tube and 1 ccm. bromine-water is added. The mixture is boiled. If positive a pink coloration develops. In a negative, the original yellow colour is maintained. He corroborates his findings with the results of the Zondek-Aschheim reactions done in Edinburgh. The bromine-water test agrees with the results of the Zondek-Aschheim in 57 out of 60 cases. He does not state if the Zondek-Aschheim reaction was correct in these cases. Gladys H. Dodds (33) uses the same method. In 171 ante-natal cases there was a percentage error of 25.7. In a non-pregnant group it gave an error of 13.1%. She concludes that the bromine-water test is not pathognomic of pregnancy.

Professor E. P. Cathcart kindly gave me the references to some work which had been done on histidine-monohydrochloride in his laboratories by G. Hunter, now Professor of Biochemistry in the University of Alberta. Hunter (34) isolated histidine from the urine of cases of measles. The amount he was able to isolate did not agree with that estimated by the colorimeter, making use of a standard solution, and he concluded that the colour-production in Knoop's test is not entirely due to histidine mono-hydrochloride, but that it was probably due to some phenolic compound. He now believes that most of the

colour obtained in this test is due neither to histamine nor histidine. Several points from his work are important -

1. Histidine excretion is increased in measles and typhoid.
2. The colour produced is soluble in excess of bromine.
3. By repeated washings with chloroform this excess of bromine can be extracted - this was a definite advance in this test.
4. The maximum colour is produced when the histidine absorbs three molecules of bromine.

Various fallacies are described by Hunter. If the specimen to be tested be alkaline - carnosine will give the typical colour; if the solution be acid - tryptophane will give the colour prodection. Histamine was also believed by Knoop to give a positive reaction. I have found histamine give a negative reaction. In order to attain the correct amount of bromine, Hunter adds definite excess, and then extracts the excess with chloroform. This is repeated until the chloroform remains clear, The solution is then boiled for 10 minutes in a water-bath, after which it is washed repeatedly with amyl alcohol which extracts the colour due to tryptophane. The result is then read. This considerably increases the sensitiveness of this test. I used Hunter's modification of Knoop's test on some of the specimens of urine on which I was doing the Zondek-Aschheim reaction.

To sumarise the method used,

(1) One-third fill a test tube with urine.

(2) Add excess of bromine-water until colour deepens.

(3) Extract excess of bromine-water with repeated washings of chloroform.

(4) Separate the urine.

(5) Boil in water-bath for 10 minutes.

(6) Wash repeatedly with amyl alcohol till no further extraction of colour occurs.

(7) Read the result.

Results obtained.	Test positive.	Test negative
Cases of pregnancy	30	22
Non-pregnancy	29	23

Percentage error approximately 50%.

The results are not flattering to the test; even though the method used is more accurate than that described in any of the hitherto published work. A positive reaction is also given in measles, typhoid, pernicious anaemia, nephritis, and I have no doubt that a considerable percentage of the whole population, both male and female, would give a positive reaction.

It is noteworthy that no case of pyelitis of pregnancy is included in the results of Voge. In this it is consistently negative since histidine is broken up by removal of the carboxyl group with the formation of histamine. A considerable amount of work has been done on this subject by Ackermann (35)

Mallanby and Twort (36) Koessler and Hanke (37) and others; recently work on histamine has been intensified owing to the discovery that injections of histamine either subcutaneously or intramuscularly will cause a definite increase in the hydrochloric acid secretion of the gastric mucosa, even in cases of complete achlorhydria.

To conclude, the discovery of Voge is merely an interesting, physiological observation; as a means of diagnosing pregnancy it is of no value whatsoever.

This work has been entirely carried out in the Pathological Department of the Royal Hospital, Wolverhampton. I am much indebted to Dr. S.C.Dyke, Director of this department for originally suggesting this subject and for the admirable facilities which he has afforded me, also to the Board of Governors of the Royal Hospital, Wolverhampton. I am also indebted to many medical colleagues for assistance in providing specimens of urine and clinical notes on the cases. Special cases were sent to me by the following:- S.W.Maslen Jones Esq., F.R.C.S; M.S; and Dr. N. Wilkes of the Staff of the Women's Hospital, Wolverhampton; Professor Beckwith Whitehouse and Dr. A.P.Thomson of Birmingham; R. Stanley Lee Esq, F.R.C.S, Medical Superintendent, Municipal Institution, New Cross, Wolverhampton; Dr. Hart and Dr. Gilchrist of the Resident Staff of the Royal Maternity and Women's Hospital, Glasgow. I am also indebted to

G. E. Dyas Esq., M.C; M.B; D.M.R.E, for the facilities afforted in the X-Ray Department, Royal Hospital, Wolverhampton.

The micro-photographs were taken for me by B. Cranfield Esq., of Birmingham, and the photographs by Bennett Clark Esq., of Wolverhampton. All the photographs are from my own specimens.

Throughout this work I have strictly adhered to the favourite advice of Lord Lister:-

"Segnius irritant animos demissa per aurem
Quam quae sunt oculis subjecta fidelibus."

I am also indebted to George Mitchell, M.D., D.P.H., Medical Officer in charge of Venereal Disease Department, Royal Hospital, Wolverhampton, for the cases supplied from his department.

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