DISSERTATION

on

THE ENDOCRINE FUNCTIONS OF THE OVARY.

By

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With

Preface

General Historical Introduction

PREFACE.

Investigation of the ovarian function is not of recent origin, early evidences of this line of thought were shown in the days of Hippocrates (Evans)[1].

With the onrush of time investigators became more numerous and work was published in relative profusion.

Autoplastic transplantations of the ovaries was first carried out by Knauer[2] in 1896. Grigorieff[3] in 1897 reported pregnancy in four rabbits from whom the ovaries were removed and subsequently replaced. The following year attention was given to the corpus luteum, Prenant[4] being the first to suggest that it furnished an internal secretion. This theory was supported by Regaud and Policard[5] in 1901, and Sandes[6] in 1903. All this work was done without satisfactory perspective, but Hitschmann and Adler[7] in 1906 revolutionised the whole situation when they published their work on the endometrial changes during the menstrual cycle; this work being correlated with the original work of Adler[8,9,10] on the physiology of the ovarian function. After this came ardent scientific investigators such as Frank[11], Ancel and Bouin[12], Frankel[13] and others. These workers cemented the foundations laid for them and upon these foundations all the more recent superstructure has been built.

Villemin[14] provided the greatest advances since the earlier work when he provided a new conception of the ovarian relationship to menstruation. He was the first to indicate that the Graafian follicle ruptures at least twelve days before menstruation, this being a contrecoup to the view of the follicular rupture being coincident with menstruation.

The present work was commenced under the ardent enthusiasm

of Adler in Vienna in 1923. Adler, who is probably the greatest medical gynaecologist and whose work - in conjunction with Hitschmann- produced such a remarkable knowledge of the menstrual cycle, by his enthusiasm created a desire to investigate and learn more of the nature of the hormone basis for diagnosis and treatment in general gynaecological practice, i.e. both surgical and medical.

It is considered that an addition to existing knowledge on the subject is as follows:-

(a)   The blood calcium changes in the menstrual cycle and in pregnancy.

(b)   Some evidence for the complex nature of the endocrine balance in the menstrual cycle and pregnancy as evidenced by metabolic changes and clinical pharmacology of adrenalin and pituitrin.   (The results are, of necessity, incomplete, since a vast amount of work will be necessary to establish the final causes).

(c)   Clinical contribution to the evidence existing for luteal relationship to the menstrual cycle and pregnancy.

(d)   Basal metabolic variation in the menstrual cycle and in pregnancy.

The material for this thesis has been collected during the past five years, and it is particularly emphasised that its present presentation does not imply its completion, since it is proposed to continue the investigations for some considerable time.

It was considered, however, that its interim presentation would serve as the basis for a thesis for the degree of M.D.

The major portion of this work was done in the departments of Physiology and Biochemistry, and when these departments were divided, the work was continued in the department of Physiology and Pharmacology in the University of Adelaide.

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Part I. THE FOLLICULAR HORMONE.

GENERAL

HISTORICAL INTRODUCTION.

INTRODUCTION.

It has been shown at operation in primates and laboratory animals, in these and other investigations, that the changes in the endometrium coincide with the variations of the ovarian condition, the ovary undergoing regular anatomical and physiological changes. Immediately after menstruation the endometrium enters upon a resting or quiescent phase - a phase of reparation. The ovary at this time is apparently inactive. Nevertheless the thecal cells become active immediately after the corpus luteum becomes atrophic and these cells produce small amounts of oestrin which precedes the ripening of the next follicle. (Brambell and Parks[1]).

The follicle itself commences to distend with fluid and it migrates towards the periphery of the ovary which bulges the tunica albuginea. Dixon and Marshall[2] consider that this is concerned with secretion of the posterior lobe of the pituitary gland, the action of which is to contract the unstriped muscle of the ovary. At this stage the endometrium becomes congested and the glands extend into the musculature and form the corpus spongiosum, and at the same time the stratified squamous epithelium of the vagina thickens and the layers increase from four to twenty layers deep. Evans summarises these relationships by saying:- "Changes in the ovary and essentially the growth and transformation of follicles provoke changes in the entire reproductive tube from the fimbriated end of the oviduct to the external vaginal orifice".

At a phase of the ovarian cycle, before the follicle ruptures, oestrus occurs and at this time sheets of squamous epithelial cells are cast off into the vaginal lumen as cornified cells. When the follicle ruptures the endothelium still continues to build up a

pregravid decidua (Whitehouse[4]) until the ovum dies or is fertilised. In the former case the influence on the corpus luteum is lost and this means that this organ becomes atrophic and its effect on the endometrium ceasing, the endometrium is shed in the form of the menstrual flow.

It is thought that the ovarian secretions which occur during the phases of the ovario-uterine cycle are probably three in number. These are the Follicular Hormone, the Corpus Luteum Hormone, and possibly a secretion from what Frankel[5] termed the Interstitial Gland of the Ovary.

O'Donoghue and Rasmussen[6] in 1916 described the interstitial cells in the ovaries of marsupials, and Athias[7] published a careful study of the interstitial gland of the Cheiroptera. This gland is thought to maintain the female characteristics until puberty, or until the thymus degenerates and the ovarian follicles commence to mature. Paton[8] found that thymectomy, performed before puberty, causes rapid development of the genital gland. It is thought that the thymus checks development of the ovary until the thymic function begins to wane.

In the course of these researches no obvious evidence of the secretion of the interstitial gland has been found. Evidence of luteal influence however seems to become apparent. When the Graafian follicle ruptures the cavity fills with blood clot which is rapidly organised and luteal cells migrate from the theca vasculosa and invade the organised clot, and when it is finally replaced by these lipoid cells the yellow body is complete.

The follicular hormone is thought to be the instigator of the endometrial hypertrophy, but the corpus luteum appears to take

over the control when the follicle ruptures (vide ante). It con-
tinues to build up the endometrium for the embedding of the ovum
which it subserves, and it is only on the death of this controlling
body that infertile abortion (Whitehouse) occurs. Cotte of Lyons
reports that in fifteen out of sixteen cases ablation of the ovary
containing either a mature follicle or a corpus luteum in full de-
velopment, has provoked the anticipated appearance of the next mens-
truation. He states further that - "the cases in which I have
interfered have been young women menstruating regularly at the time
of operation". Cotte concludes by saying that "in each of these
observations we have not seen, on the ovary which was left intact,
another ripening follicle". The operation results are given in the
appendix.

In those cases where the ovum is fertilised the corpus
luteum remains and maintains an engorged endometrium by an hormonic
action which we think is similar to that exerted upon the mammae.
These conclusions are deduced from the fact that where the corpus
luteum is removed during the first three months of pregnancy abortion
occurs, and further, that removal of this organ in the last two weeks
of the intermenstrual interval, causes menstruation to occur soon
after.

Matsumo[9], Whitehouse[4] and others including ourselves, have
shown this in many cases. We have demonstrated it both in the humans
and in laboratory animals. Some of our cases are reported in the
appendix. Halban and Kohler[10] did considerable operative work on
the corpus luteum and their results were corroborated by Reusch[11],
Seitz and Wintz[12]. Halban demonstrated that the persistence of the
corpus luteum as a corpus luteum cyst is typified by a condition of

amenorrhoea which ceases on resection of this body. Rubin[13] and Frankel[14] confirmed this and we had exactly these results. Matsumo[9] records several cases in detail which with our results are given in the appendix.

In 1926 a monograph[15] was published in which we indicated that disorder of the luteal functions was succeeded by disorder of endometrial conditions as first suggested by Rokitansky[16]. Cases have been cited where removal of the corpus luteum after three months' gestation does not cause abortion, one such case being operated on by us. The reason which we suggested for this was the development of the placenta as an endocrine organ (Matters[17]); in fact, the hormone circulates in the systemic blood in considerably greater amounts (Siddall[18]).

The ovarian secretion with which most of the experimental work has been done in these researches is that of the Liquor Folliculi. The history of this hormone is not very long although Marshall and Jolly[19] in 1905 produced oestrus in rodents by injecting ovarian extracts. Alder[20] in 1912 produced artificial oestrus by injection of aqueous extracts of whole ovaries. Harmann[21] also made exhaustive efforts to produce a pure oestrin, but it was due to the investigations of Allen and Doisy[22] and their co-workers that the isolation of oestrin has been brought to its present state of efficiency.

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Section a.

ISOLATION AND STANDARDISATION.

The procedure adopted in these researches was based entirely on the isolation as originated by Allen and Doisy. This work was carried on by these two investigators assisted by Ralls and Jordan in a later work and subsequently by many other workers.

Ovaries were difficult to obtain as it takes roughly about one hundred sows to provide ovaries enough to turn the balance at half a kilogram. The great co-operation of the officers of the Abattoirs was the factor in enabling us to procure an adequate supply of material.

The ovaries were collected from time to time and kept on ice until a sufficient number were in hand to commence extraction. On two occasions the refrigerator of the Abattoirs was closed for repairs, and in these cases the ovaries on hand were weighed, minced and placed in three volumes of 90% alcohol. When a quantity which was large enough to extract had been collected, the proteins were filtered off and extracted with alcohol in a Soxhlet apparatus for eight hours. The filtrate and the extract from the Soxhlet were evaporated to dryness in the same dish.

The dry residue was taken up with water and centrifuged. The aqueous solution was then made alkaline with sodium hydroxide and extracted several times with ether. The extracts were combined, washed with dilute alkali and water, after which they were distilled. The dry residue was taken up in 10% alcohol and the cholesterol removed by five extractions of the alcoholic solution with petroleum ether. By these measures the hormone, which is soluble in alcohol, remains, while only traces pass over into the petroleum ether, and is retained while the cholesterol passes into the petroleum ether. This, then, removes the cholesterol and most of the solids leaving practically only the hormone in the alcoholic solution. This solution was then distilled to dryness and the residue taken up in sterile olive oil.

The hormone was thus provided in an oily suspension in which it might be injected for purposes of the experiments. Before this hormone could be scientifically used, however, it was necessary to standardise the solution.

The method used was the vaginal smear test of oestrus first introduced by Stockard and Papanicolou[1] in their work on guinea pigs, and subsequently developed by Evans and Long[2] in the rat.

This method is dependent on the fact that for the purposes of these investigations the conception of oestrus is that it is due to the action of the follicular hormone and with the advance towards oestrus the whole of the sexual organs become congested and the epithelial lining of both uterus and vagina become thickened. In the vagina the squamous epithelium develops so that the three or four normal strata proliferate until as many as fifteen to twenty layers of cells line the vaginal lumen.

When the lining is merely four cells thick leucocytes find

Microphotographs of Vaginal Smears of rats. These smears were taken from
castrated rats and were artificially produced by hormone injections.
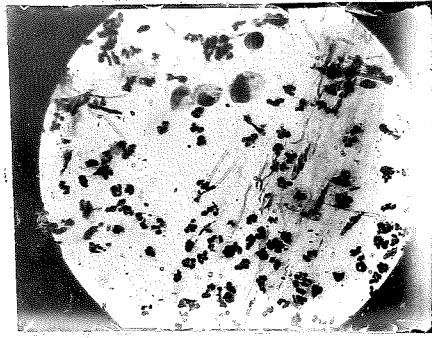
No I. Smear taken during anoestrus shews many leucocytes and few nucleated
epithelial cells.
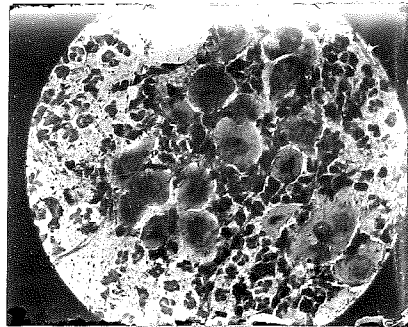
No II. Smear taken during pro oestrum. Relatively fewer leucocytes and
more nucleated epithelial cells.
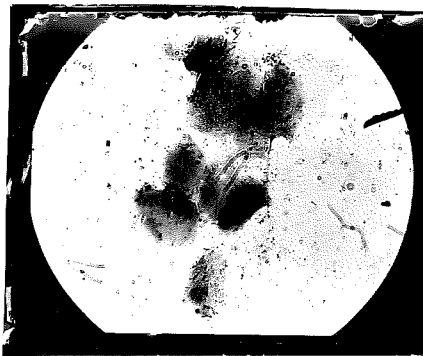
No III. Oestrum. In this phase only non nucleated cornified epithelial
cells are found in the smear.

their way through into the vagina and a few nucleated epithelial cells become part of the debris.   If at this time a smear is taken and stained the result is as shown in Fig.1.

With the thickening of the epithelium a smaller number of leucocytes find their way through and also a lowering of the blood supply of the more superficial cells causes more to desquamate and many of these are dead and present a cornified condition.   A smear taken during this phase presents many nucleated epithelial cells, fewer leucocytes and a few non-nucleated epithelial cells.

With the onset of oestrum, however, the epithelial cells are so thickened that practically no leucocytes attain the vaginal lumen and the superficial cells are so devitalised that sheets of cornified epithelial cells occur with a few nucleated cells as well.

The injections were made at zero, twelve and twenty-four hours and smears were taken at zero, twenty-four, thirty-six and forty-eight hours.   The smallest amount to produce a positive smear in a standard oophorectomised rat was regarded as a rat unit.   It was found also that a spayed rat who displayed considerable hauteur before injections, was prepared to receive the male under the stimulus of the hormone.

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Section b.

THE PHARMACOLOGICAL VALUE OF EXISTING COMMERCIAL

"EXTRACTS"

THE PHARMACOLOGICAL VALUE OF EXISTING COMMERCIAL "EXTRACTS".

The recent impulse to therapeutics given by Endocrinology has met with a response from commercial firms which is so great that various proprietary lines have been thrown on to the market in a profusion hitherto unparalleled. Each firm supplying alleged ovarian extracts provides therewith literature of a very specious character and some firms go so far as to indicate all the conditions which may be "cured" by their own "extracts".

During these investigations the available preparations have been assayed both by the vaginal smear method referred to earlier, and by taking sections of the uterus and adnexa.

The extracts for oral administration have in no case proved to be active when administered to rats, in fact, no gland which has not developed from the foregut appears to produce results when given by mouth.

In the present work the extracts stated to be for administration were manufactured by the following houses:-

      a.   Burroughs Wellcome
      b.   Carnricks
      c.   Harrower
      d.   Oppenheimer.

None of these extracts appeared to be active although the keratin coated extract of Oppenheimer might be active but owing to the size of the tablet they could not be fed to the experimental animals. This method of treatment, however, would appear to be more rational provided the hormone is present in the capsule, - supposition without much support from the results of these investigations.

Of the other extracts supplied in ampoule form for injection, three only have been tried. Of these the most active is

Systomensin, prepared and standardised according to the methods of Allen and Doisy.

The recent product of Parke Davis called Estrogen also appears to be active but not to the same degree as Systomensin.

The water soluble extract of Research Products Ltd. is very slightly active. The amount required is greatly in excess of the dosage indicated by this firm. In all these cases there appears to have been a decided tendency to follow a scientific course in the preparation of the hormone and these firms have provided active preparations although in the main they do not contain the number of rat units stated in the accompanying literature.

The use of the "extracts" for oral administration is to be deprecated because of the above-mentioned failure to demonstrate activity. They are marketed with literature which is liable to misguide the practitioner, with the result that poor response to treatment produces a retrograde effect upon the endocrinological outlook in therapeutics.

Part II.   THE MENSTRUAL CYCLE.

Part II.   THE MENSTRUAL CYCLE

INTRODUCTION.

PART II. THE MENSTRUAL CYCLE.

The menstrual cycle has been briefly described elsewhere and attention was drawn to the relation between these menstrual changes and the cyclic phases of the ovary. No reference, however, has been made to any relation between the ovary and any organ other than the genitalia.

Rogers[1], Simonton[2], Hoskins[3] and others have mentioned the ovarian disturbances connected with thyroid alterations. Rogers stresses the menstrual disturbances in Graves' Disease. It is in fact regarded by some as a sine qua non that alteration in either ovary or thyroid produces changes in the other gland. During these investigations the necks of pregnant women were measured at various stages of pregnancy and were found to become enlarged with the approach of full term.

The hypophysis also appears to be involved with the ovarian function. Cushing[4] indicates that cessation of menstruation is a recognised early sympton of acromegaly, and Erdheim[5] has reported striking morphological changes in the anterior lobe in pregnancy. Gentili[6] in a monograph on the secretory activity of the prehypophysis in pregnancy shows that the anterior pituitary lobe enlarges enormously during parturition, his work being done on both cows and humans.

We have examined five women over a long period of time and have presumed them to be partially acromegalic; their history in three cases dating from accouchement, the other two being nulliparae. The two groups are composed of very fat people with reduced menstruation either in time or regularity, and whose gaseous metabolism when estimated was not found to be far from normal. The symptoms were those of acromegaly with pituitary facies and that peculiar fatness

of "pituitary" people.   Further, the administration of thyroid appeared to be ineffective even after some weeks.

The interrelationship between the ovarian hormone and that of the parathyroid was shown by Collip[7], but the calcium metabolism has been considered with reference to the ovary for many years.

In 1908 Blair Bell[8] considered that there was some alteration in the calcium of the blood during the menstrual cycle, and he stated that he considered that a fall of blood calcium took place at menstruation and that calcium was lost from the human organism much as birds lose calcium by laying eggs.   His conclusions were largely speculative based upon insufficient evidence.

Erdheim[9] found that there was excessive ovarian activity in cases of Osteomalacia and he found concurrent changes in the parathyroids and also a great excess of mobilised calcium which caused the bone softening.   The treatment for osteomalacia is castration and also emptying the uterus - this latter because as stated elsewhere in this review, there appears to be considerable ovarian hormone in the placenta which, if left, would continue this calcium mobilisation.

Luckhardt and Goldberg[10] gave parathyroidectomised dogs sufficient calcium to prevent tetany, but during oestrus these dogs resumed the tetanic symptoms.

Mirvish and Bosman[11] using the ovarian hormone as prepared by Allen and Doisy[12], followed experiments of the type introduced by Dickens, Dodds and Wright[13] (who found that the injection of the ovarian hormone produced a lowering of blood pressure).   Mirvish and Bosman by injecting rabbits with the ovarian hormone produced a fall of blood calcium;   they further noted that when animals were spayed their blood calcium rose.   Elsie Watchorn[14] has investigated the

blood calcium in some phases of the menstrual cycle, but only as a subsidiary to her study of alterations in blood magnesium. Changes in the blood calcium content during pregnancy have been investigated in the course of the present work, and as will be shewn later there is in general a marked fall especially towards term.

In a recent publication (Matters[15]) of a case of puerperal tetany following a severe antepartum eclamptic state, it was indicated that the blood calcium was very low. In this case it is thought that there was either placental dysfunction or dysfunction of the ovaries with concomitant effect upon the general endocrine "balance".

Dulsace and Guillaumin[16] claim that lowering of blood calcium follows oophorectomy. It was probable in the above case that the placental secretion had ceased some time before delivery, as the foetus was very macerated.

In the light of the still obscure nature of blood calcium fluctuations as borne out by the above results of the existing literature, work was continued in this direction.

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Section a.

CALCIUM METABOLISM IN THE HUMAN SUBJECT.

CALCIUM METABOLISM.

It has already been noted that some relationship appears to exist between the blood-calcium content and the amount of the ovarian hormone present in the circulating blood.

The first man to investigate blood calcium during the menstrual cycle was Blair Bell[1], who published his findings in 1908. Bell, however, did not regard the alteration of the calcium as being of hormone origin but rather regarded the loss of calcium as analogous to the laying of an egg by a bird; he showed, in fact, that hens had lower blood calcium after laying an egg and the results obtained in these investigations show that the drop begins just prior to menstruation and continues to fall during that period.

Considerable work has been done on the blood calcium during pregnancy by Widdows[2], while the influence of diet on blood calcium was investigated by Dennis and Minot[3], and the result of injecting calcium into the blood stream has been ably discussed by Clark[4]

In 1924 Cook[5] published his findings concerning Blood Calcium and Toxaemias of Pregnancy, but was unable to find any correlation between the two.

The report of the work of Widdows was to the effect that there is a slight increase of blood calcium in the earlier months of pregnancy, while later the amount is considerably reduced. This latter is thought to be due to the foetal requirements making inroads on the mobilised calcium. Watchorn[6], in 1924, published the blood calcium estimations in pathological conditions, and two years later she[7] gave the results of blood calcium investigation during pregnancy, but attention was directed mainly towards the serum magnesium.

The year following this work Mirvish and Bosman[8] connected

the blood calcium content with the secretion of the ovarian hormone, and demonstrated that the introduction of follicular extract (prepared according to the method of Allen and Doisy[10]) into rabbits reduced the blood calcium. The same result was also found when the extract was injected into humans[9], either male or female.

The present work covers a period of nearly three years during which the blood calcium of over seventy different patients has been estimated in the varying phases of their menstruation. The blood of these patients could only be taken when they reported for treatment on certain specified days. The result of this has been that on several occasions the patient's blood has been taken in precisely the same phase, i.e. in relation to the menstrual flow, but these results agreed very nicely providing an unconscious check upon the analyses, while the blood calcium estimated during other phases of the cycle differed from these appreciably.

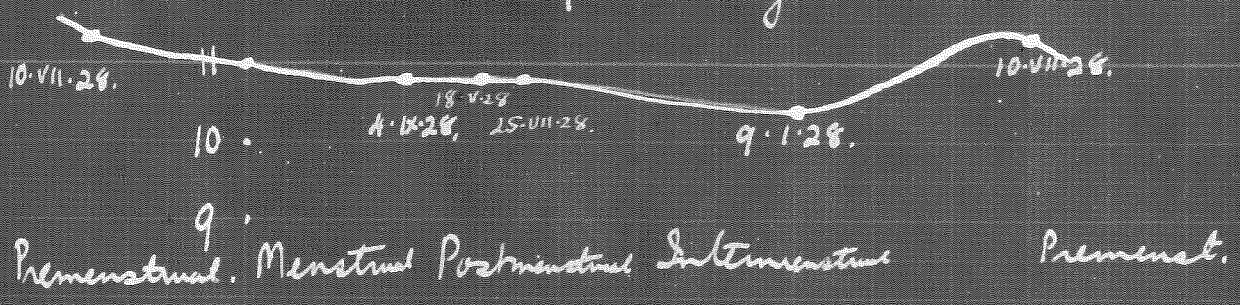As an instance of this type of case, we have:-

								Blood Calcium
lice M. et.28 yrs.	Blood taken 29/7/25 two days before menstruation.							11.5 mg.per 100 cc.
" "	" " 20/12/27 three " " "							11.6 " " "
" "	" " 10/7/28 two " " "							11.5 " " "

The same case, however, taken four days after the onset had blood calcium 10.3 mg. on 14/8/28, and on the fifth day which was 17/7/28, her estimated blood calcium was 10.5 mg. per 100 cc. of blood. Twelve days after the onset, 24/7/28, the amount was 11.0 mg.per 100 cc.
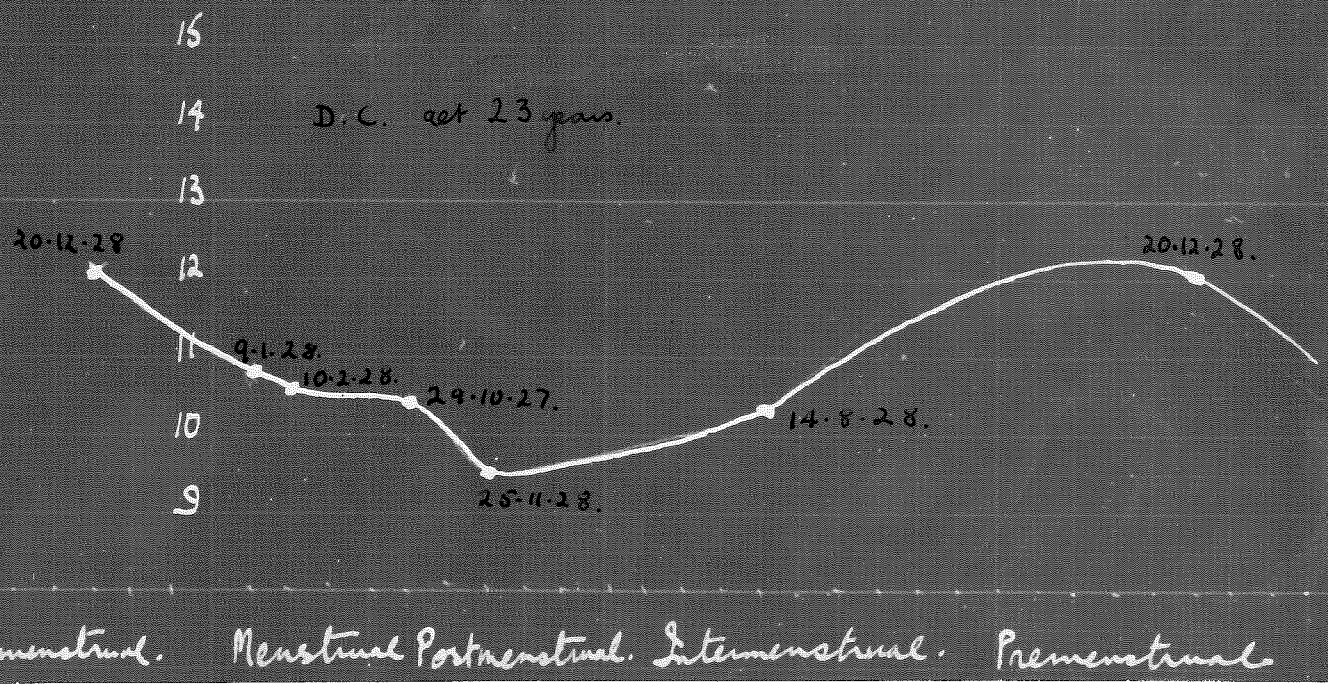
Several of these sets of results have been graphically expressed. Some of these are here shown.

Generally it is found that the highest point of the curve is about five days before the onset of menstruation. From this point
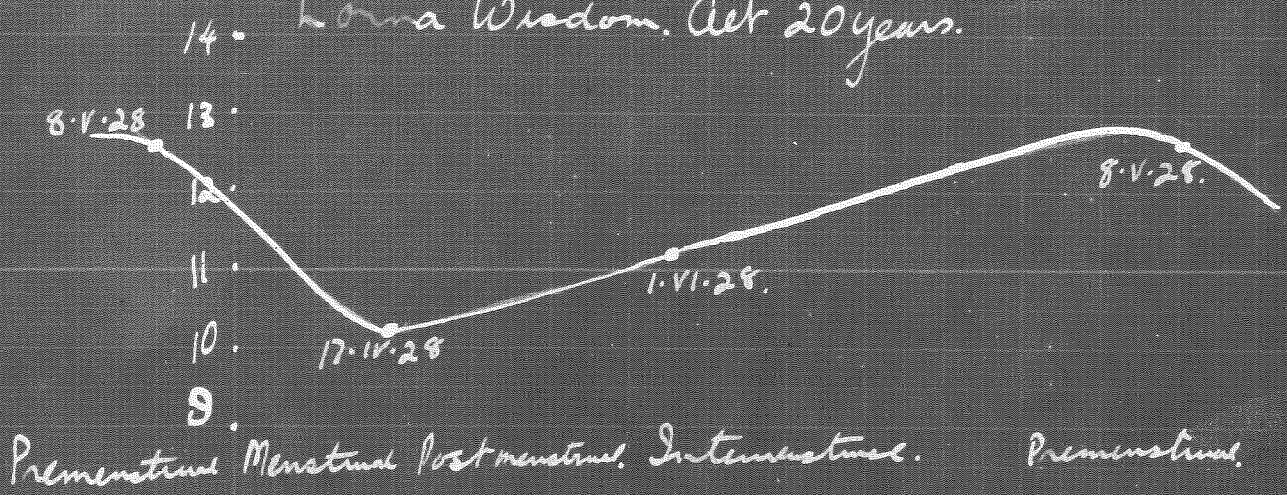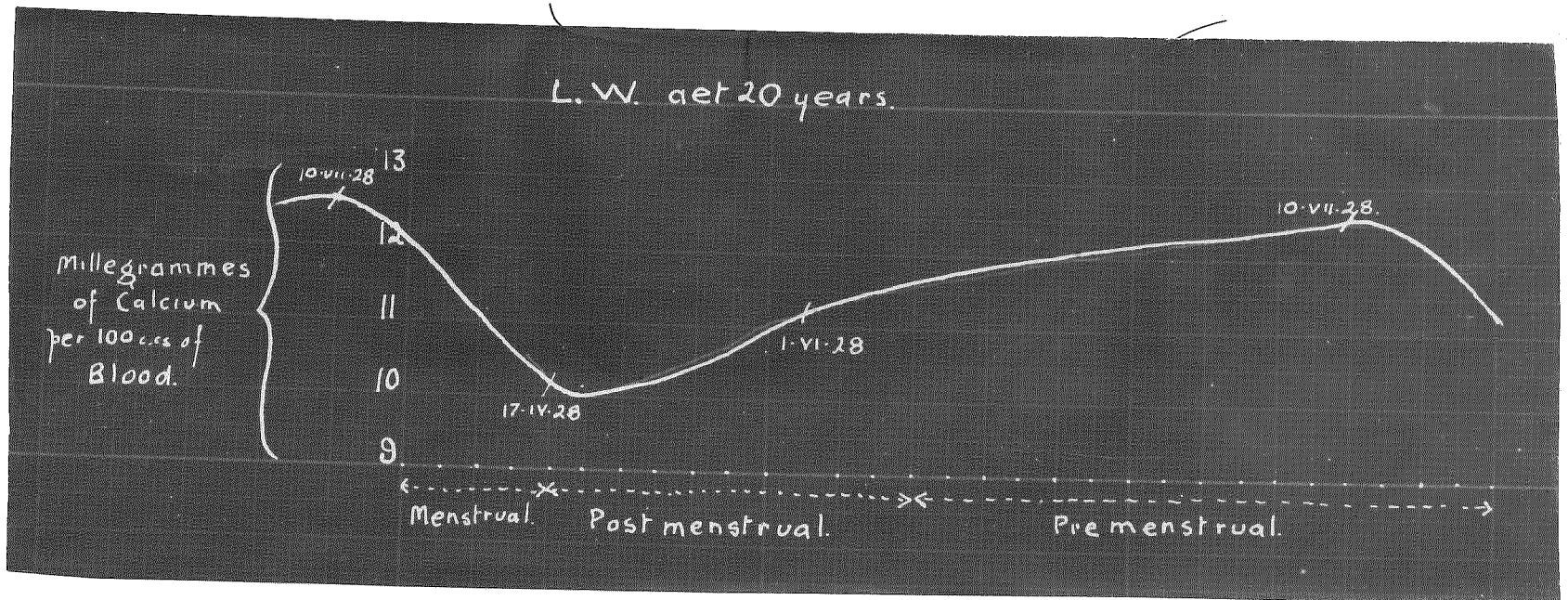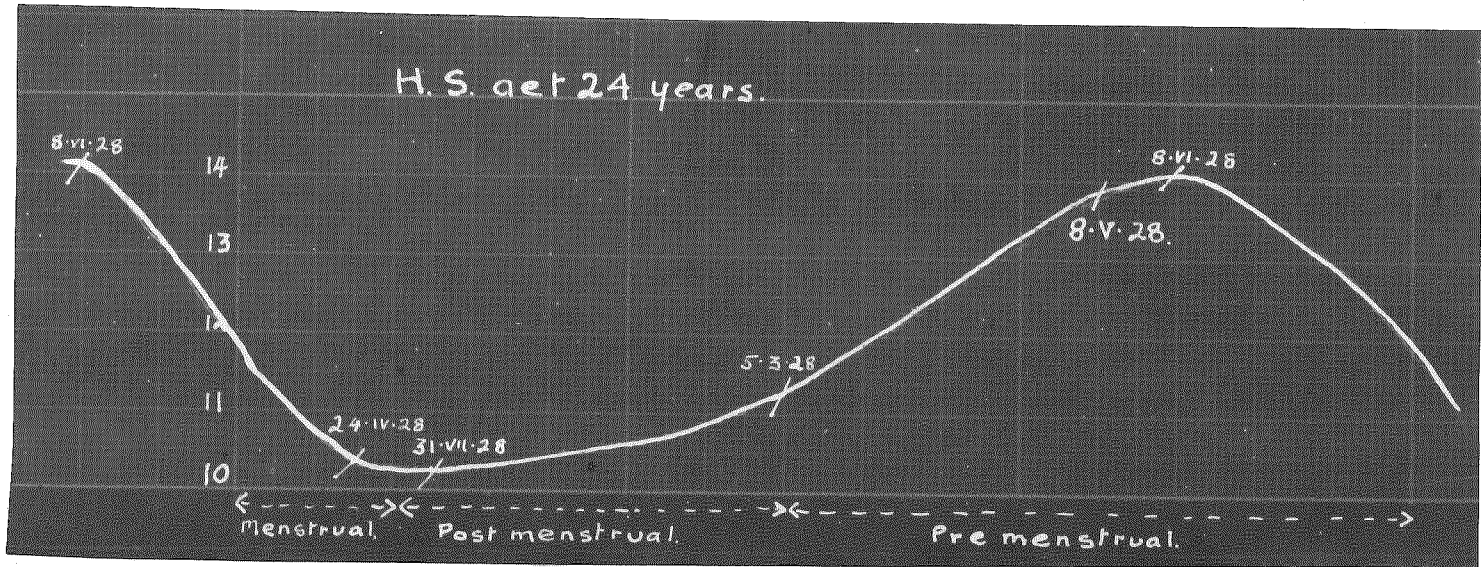
Cecelia Bray aet 26 years.

10.VII.28.   11   18.V.28   9.I.28.   10.VII.28.
10 .   4.IX.28.   15.VII.28.
9 .

Premenstrual.   Menstrual   Postmenstrual   Intermenstrual   Premenst.

15
14   D.C. aet 23 years.
13
20.12.28   12   20.12.28.
11   9.1.28.
10.2.28.   29.10.27.
10   14.8.28.
9   25.11.28.

Premenstrual.   Menstrual Postmenstrual. Intermenstrual.   Premenstrual

14   Lorna Wisdom. aet 20 years.
8.V.28   13
12.   8.V.28.
11   1.VI.28.
10   17.IV.28
9.

Premenstrual Menstrual Post menstrual. Intermenstrual.   Premenstrual.

H.S. aet 24 years.

8·vi·28
14
13
8·vi·28
8·v·28.
12
5·3·28
11
24·iv·28
31·vii·28
10

Menstrual. Post menstrual. Pre menstrual.

L.W. aet 20 years.

10·vii·28 13
12
10·vii·28
Millegrammes of Calcium per 100 c.cs of Blood.
11
1·vi·28
10
17·iv·28
9

Menstrual. Post menstrual. Pre menstrual.

27

Alice Martin. Aet 31 years.

Dates of various estimations are:-

3 days premenstrual	20.XII.27.	
2 " —	24.VII.28 }	Two years apart but same stage
	10.VII.28 }	and same result.
2 days menstrual	10.1.28.	
4 " "	14.8.28.	
2 " post —	11.IX.28.	
1 " " —	4.VI.28.	
7 — —	31.VII.28.	
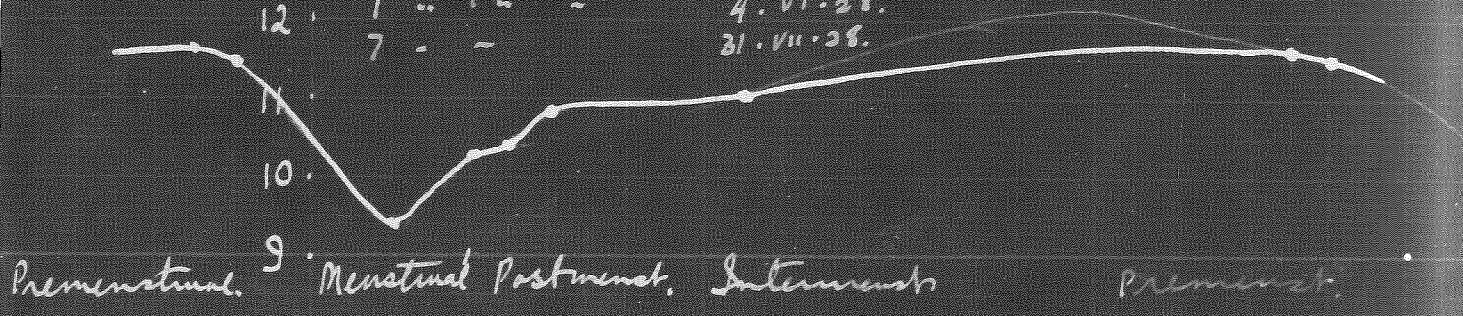
16 · 15 · 14 · 13 · 12 · 11 · 10 · 9 ·

Premenstrual.   Menstrual   Postmenst.   Intermenst.   Premenst.

Curves shewing blood cal-
cium estimations during
the menstrual cycle.
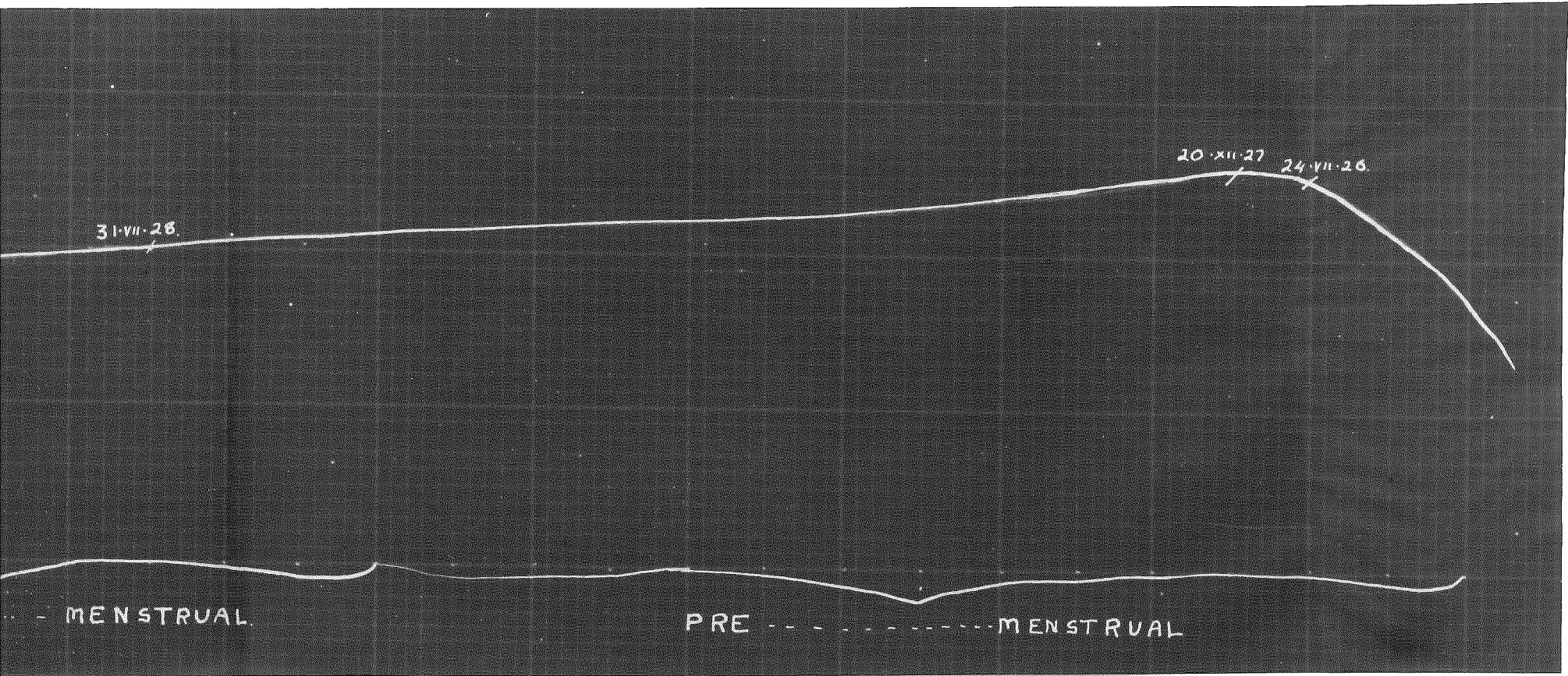
20·XII·27   24·VII·26.

31·VII·26.

- MENSTRUAL.   PRE - - - - - - - - - MENSTRUAL
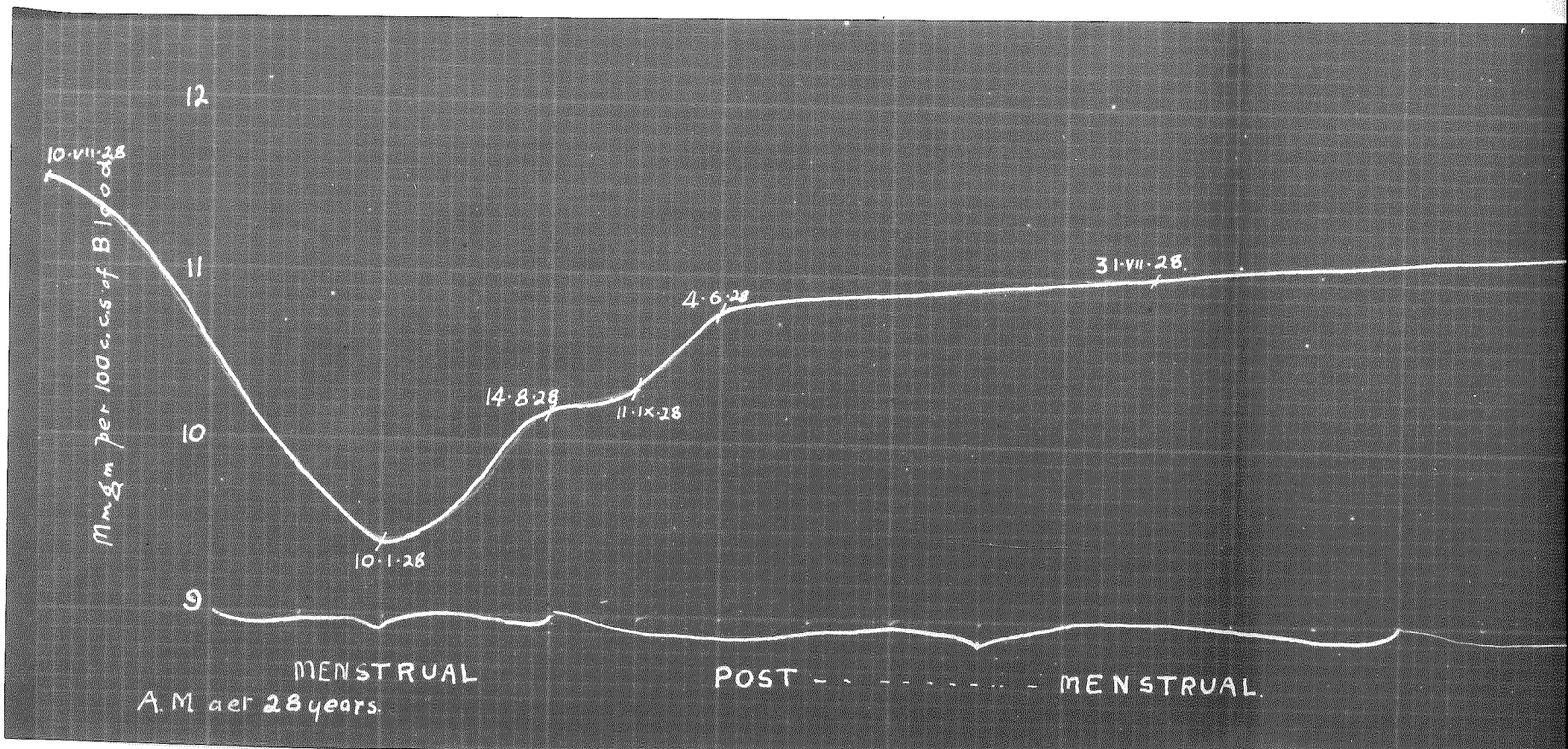
Curves showing blood cal-
cium estimations during
the menstrual cycle.

ST MENSTRUAL - - - - - - - - - X - - - - - - - - - - PREMENSTRUAL - - - - - - - - - - - - - - - - -

PRE MENSTRUAL ← - - - MENSTRUAL - - ✗ - - - - - - - - - - - POST MENSTRUAL- - -

C. Q. aet 32 years.
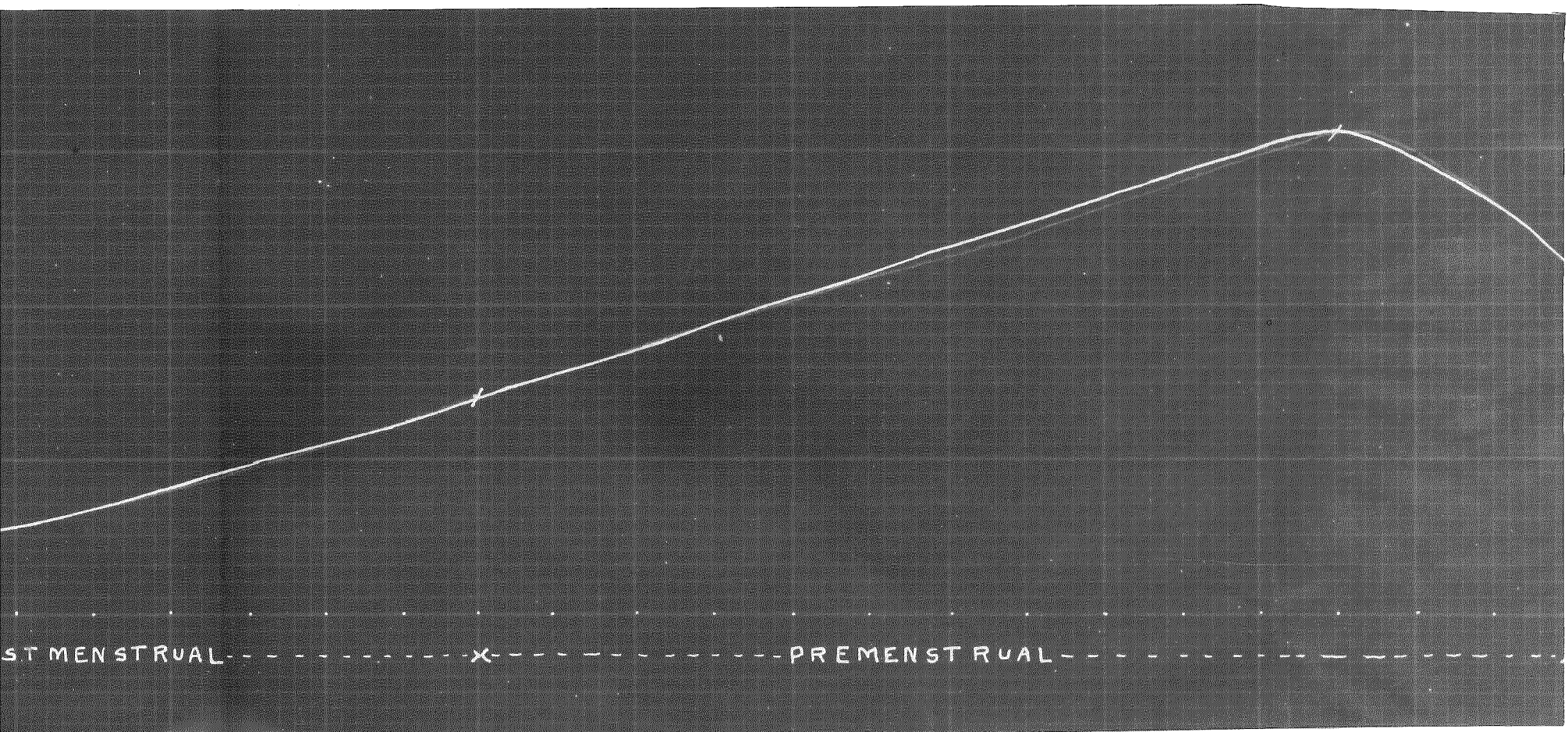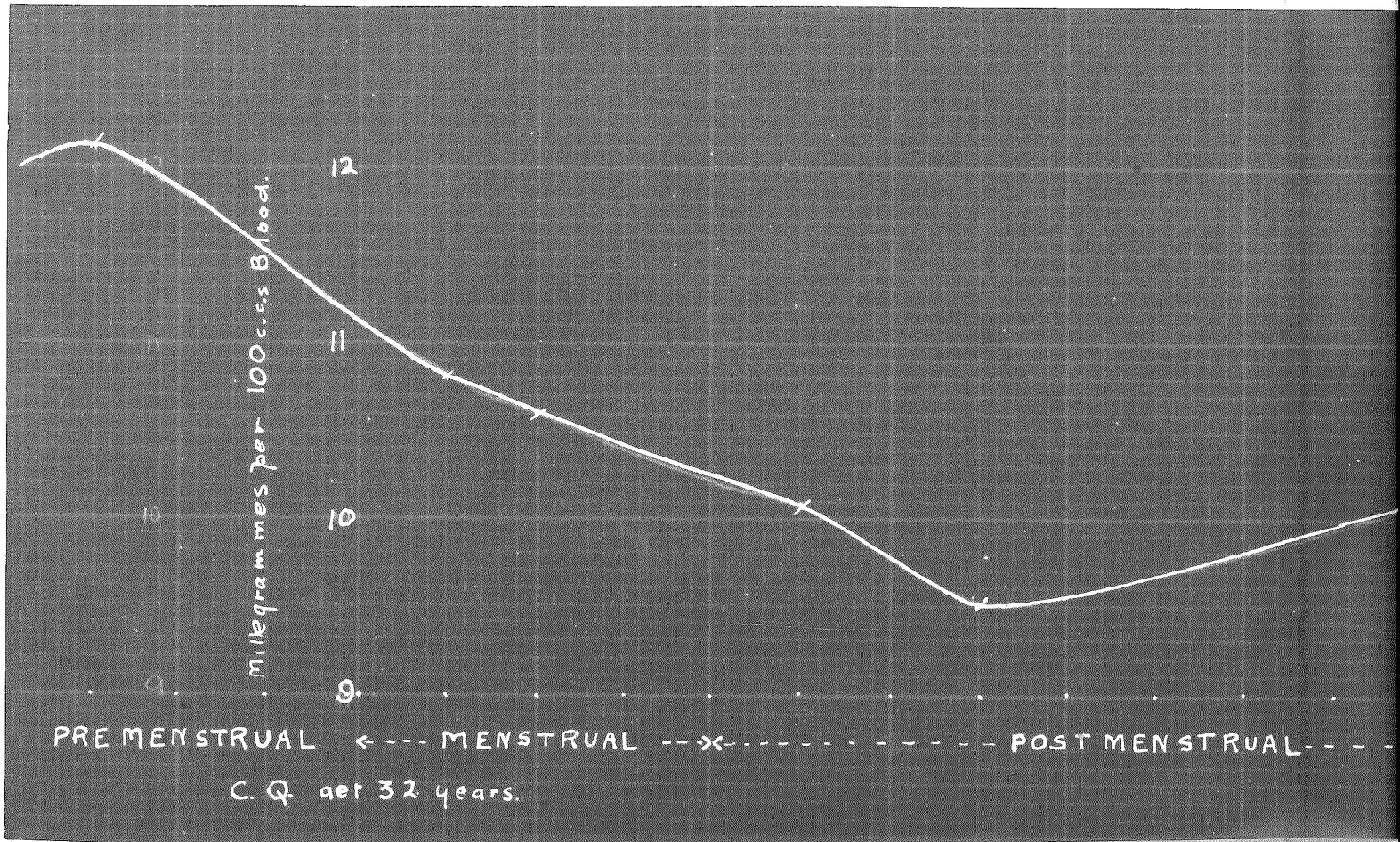
the blood calcium content rapidly falls, until about the third day
of menstruation.   After this there is a very slight fall until
about the seventh day after menstruation, when the blood calcium
fairly rapidly rises again to the acme about five days before mens-
truation.   The variations are not as a rule very marked, but average
between 9.5 mg. per 100 cc. of blood to 11.5 mg., or perhaps a little
more.   A statistical comparison of premenstrual blood calcium values
with corresponding menstrual and post-menstrual results has been pos-
sible in a series of eight determinations.   In the other cases, ow-
ing to the fact that the material was subject to ordinary clinical
availability, such an analysis was impossible.   Some idea of the
accuracy of the estimations and their value as a basis for deduction
can be gained from an analysis of these eight cases:-

Average difference between pre-menstrual and menstrual
blood calcium values .... =   7.7%

Mean error in the series .... = ± 5.8%

Average difference between pre-menstrual and post-
menstrual blood calcium values .... =   13.8%

Mean error in the series .... = ± 8.2%

Thus it can be seen that though the error is high it is not greater
than the amount to be measured, especially in the latter instance.
The error is a combination of (a) accidental variations among the
subjects, (b) accidental errors in calcium estimations.   It can be
safely asserted on the above findings that there is a difference of
at least 5% between the blood calcium of the pre and post-menstrual
phases.   When it is further appreciated that the method of analysis
is difficult in the extreme, and that the analyses in these cases
were done during routine work at the Adelaide Hospital Laboratory by
Miss Long B.Sc., the results are not unsatisfactory.   Slight varia-
tions, such as in the time of centrifuging or the rate of same, would

lead to errors quite as large as those indicated. The present re-
sults offer the only conclusive evidence yet adduced for blood
calcium variation in the normal menstruating human female.

The cases of pregnancy gave results which entirely conform
to those of Widdows, with the exception of a slight rise in these
cases during the last two months. The average of three sets of
cases taken at different times during parturition, were:-

1st month	Between 2nd and 5th month	5th to 7th months	7th to 9th months.
11.3 mg.	0.8 mg.	9.41 mg.	9.87 mg.

Blood drained from placenta contained 10.51 mg.per 100 cc.of blood.

Having in mind the results of Mirvish and Bosman, it is
thought that the reduction of blood calcium in the later months of
pregnancy is not entirely due to foetal requirements, but it is con-
sidered that the calcium is reduced as the ovarian hormone increases.
This increase has been indicated in the work on mice, where the serum
of pregnant women is injected and the ratio between total mouse weight
and uterine weight is estimated and the amount of hormone guaged ac-
cording to the ratio. Frank and Goldberger[11] noticed also that the
maximum ovarian hormone existed immediately before menstruation.

PROTOCOL

iect	Premenstrual Phase			Menstrual Phase		Postmenstrual Phase	
H.S.	14.0 (4)	14.0 (1)		10.1 (2)	10.35 (3)		11.6 (10)
M.S.		14.46 (1)		10.2 (2)		9.5 (2)	
C.Q.	12.1 (3)	10.1 (1)		10.42 (1)	10.6 (2)	10.5 (1)	10.4 (10)
D.C.	12.5 (10)	13.1 (4)		12.4 (3)			11.2 (8)
A.M.	11.6 (3)	11.5 (2)	11.5 (1)	10.3 (4)			11.0 (7)
M.B.	11.07 (8)	11.4 (7)	11.0 (6)	10.3 (2)			
L.S.	11.56 (5)			11.3 (2)	10.0 (4)	10.2 (1)	9.2 (4)
H.D.	11.4 (5)	11.01 (4)		10.9 (4)			
L.W.	12.6 (2)			10.22 (4)			11.2 (7)
C.B.	10.4 (10)	11.3 (6)		10.9 (2)	10.8 (4)	10.8 (2)	10.8 (3)
M.D.		11.4 (4)			10.2 (4)		11.4 (10)
P.N.		11.0 (6)		11.6 (1)		10.4 (2)	
I.E.		11.23 (2)					9.75 (8)
B.H.				10.6 (4)		9.0 (3)	
B.C.							11.2 (7)
N.B.				10.2 (4)		9.2 (4)	
L.S.		11.3 (2)		10.2 (1)		10.0 (3)	
M.J.						10.1 (2)	
M.P.		10.9 (4)		9.5 (1)			
N.W.						10.4 (5)	
R.S.	12.8 (10)			11.6 (1)			14.5 (10)
R.W.		11.7 (5)				11.2 (4)	
A.N.		11.6 (5)				11.0 (5)	
V.B.	11.7 (7)						
A.S.				11.2 (4)			
D.	11.4 (12)			10.2 (4)			
						10.6 (6)	

PROTOCOL CONTINUED.

ect	Premenstrual Phase	Menstrual Phase	Postmenstrual Phase	
..C.		10.6 (1)	10.0 (7)	10.1 (10)
1.C.			11.0 (7)	
P.G.			10.7 (7)	
K.H.	10.8 (11)			
I.S.	12.1 (6)	10.3 (3)		10.7 (9)
M.B.	11.1 (4)		11.2 (7)	
E.S.	12.4 (4)		10.9 (3)	
S.H.				
M.K.	11.4 (5)	10.8 (2)		11.2 (8)
A.F.		10.4 (2)		11.1 (7)
I.K.	11.3 (7)	10.2 (3)		
A.W.		11.2 (4)		9.9 (9)
L.B.				
E.G.	10.5 (12) 10.4 (1)	9.8 (4)		

In the above protocol the menstrual cycle has been dicided into three phases or groups for convenience; the menstrual phase consists of four days, while both premenstrual and postmenstrual phases comprise twelve days each, thus making a total of twenty-eight days for the complete cycle. The initials of the subject are given on the left-hand side, and the number of milligrammes per 100 cc. of blood removed from the subject are placed in the column according to the phase of the menstrual cycle when it was taken. The bracketed numeral after the calcium figure denotes the number of days of the phase either before, after the onset, or after the conclusion of menstruation.

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THE BASAL METABOLIC RATE IN THE FEMALE SUBJECT
DURING THE MENSTRUAL CYCLE.

During the menstrual cycle many metabolic changes have been demonstrated. Calcium metabolism and analagous conditions have been cited in the foregoing.

It was considered also that the energy metabolism might show alterations directly referable to the influence of the sex glands. In other countries workers have investigated the gaseous metabolism with regard to the menstrual cycle in a sporadic manner, but the present investigation of these basal metabolic changes is directed purely towards the elucidation of the ovarian and associated endocrine function.

Macleod[1] had indicated the scope for basal metabolic investigation and the admirable apparatus of Benedict[2] has supplied the means whereby results might be obtained. Another important factor for comparative purposes is the standard provided by DuBois[3].

In the course of these investigations it was found that the ovarian hormone had its maximum effect a short time before the onset of menstruation. It was therefore considered that there might be an increase in function of the thyroid at this time, and if so there would be a resulting elevation of the basal metabolic rate. This correlation between hormonic influences and the basal metabolic rate during the menstrual cycle has not, so far, been investigated. In this work an indication of some relationship was the observed increase in the size

of the thyroid during the premenstrual phase in a number of patients.
The possible increase of the basal metabolic rate at the time of ovu-
lation was also considered as a probability owing to the activation of
the thyroid by the ovarian hormone.

Rowe and Eakin[4] have arrived at the conclusion that there
is a premenstrual rise and a menstrual fall, but they supply no rea-
sonable explanation. Wakeham[5] also indicates this result and says
that "Blunt and Dye[6] (who thought that their results showed no con-
nection between basal metabolism and the menstrual cycle) demonstrated
that there is a distinct fall in the metabolic rate with the onset of
menstruation."

Benedict, in a private communication to Professor Hicks,
concerning the metabolism of the menstruating female subject, has
concluded that there is a definite fall in the metabolic rate during
the menstrual period. His communication reached us during our own
investigations in the same field. He had noted that in the case of
an artist's model, who had served intermittently as a subject for
respiration work at the Nutrition Laboratory, she had in a period of
twelve years been examined on some thirty-two menstrual days, and, on
examining the analytical figures he was led to conclude that the ap-
parent lowering of metabolism was more than could be accounted for by
accidental variation. He therefore followed this same subject closely
through daily measurement over a period of two months in 1928[7] with
results that our present work substantially corroborates. In this
work Benedict used the method of Hafkesbring and Collett[8] and so ob-
tained consecutive results.

In our work the Benedict portable Field Apparatus was used.
A large number of individual cases were examined in different phases

of the menstrual cycle, but they gave no definite indication of metabolic alteration, while the day-to-day examination of a set of subjects provides material for graphic determinations.

Several cases of irregular menstruation were examined, one particular case being a patient aet.24 years; the intermenstrual interval varied from three to seven months; she was thin and nervous. The basal metabolic rate at three consecutive readings gave a value of nineteen calories per square metre of body surface per hour. Administration of small doses of thyroid extract (.2 gram.per diem) improved her condition and her metabolic rate increased to forty-one calories per square metre per hour. The amount of thyroid extract was then reduced to .1 gram. Over a period of four weeks the normal metabolic rate has been maintained by this dosage, and, although the periods are not yet established there are symptoms and signs that a change is in progress in this direction.

In order to gather accurate information on the basal metabolic rate two women were enjoined to co-operate in this research. The first case, E.C.M., married woman aet.thirty-four years, multipara, had a regular menstrual cycle of twenty-eight days and the actual flow lasted for five days. The series of investigations covered three menstrual periods and included two complete cycles. The daily results have been calculated and plotted in such a way as to demonstrate graphically the variation of the metabolic rate. It might be mentioned, however, that some readings were not truly basal as a "late" preceding night complex with some nervous excitement increases the metabolisms to an astonishing degree.

For the purposes of these experiments the Benedict Portable Field-type Apparatus was used for E.C.M. This apparatus consists of

two chambers containing soda lime and surrounded by a rubber bathing cap which forms a roof to both chambers. Inspired air must pass through the soda lime in one chamber, thence to the rubber cap and out by the soda lime in the other chamber, a unidirectional flow being produced by the interpolation of two Sadd valves in the circuit connecting with the subject's mouth. The nose is clipped, and after this measured amounts of oxygen are introduced as required into the system. The rubber cap provides a means of variable volume without introducing abnormal pressures in the respiratory system.

The results are so plotted as to show at a glance whether there is any mechanical error or whether the subject is breathing regularly. Known volumes of oxygen introduced are plotted against time taken to absorb the same, and the closer the points approach to linear values the better are the conditions. Moreover, by plotting, small variations are balanced, and the final volume time projection is a corrected average of the result throughout the determination. Corrections for temperature and pressure are then applied to the projected result as indicated by reference to the adjoining graphs. The results are expressed finally in terms of calories used per square metre of body surface, per hour, using the usual factors for such conversion, i.e. 1 liter of oxygen at respiratory quotient of 0.8, which may be assumed in these cases without any likelihood of error, being associated with the production of 4.8 calories; the surface area being computed from the height-weight formula of Du Bois[9].

In the case of L.M., the so-called "British Benedict" apparatus, manufactured by Kendrick, was used. This needs no description, being merely a closed circuit gasometer method using soda lime as the CO_2 absorbent. It was selected both because it was necessary

to use more than one instrument on account of pressure of work,
and in order to test it out clinically. The Benedict method of
graphic plotting of successive volumes against time was applied as
above-mentioned, in order to check the respiration and also to secure
an accurate final figure for computing calorific requirements.

At intervals of approximately one minute the spirometer
reading was **taken**, and the results plotted graphically. As ordinarily
used the Kendrick apparatus would not be highly accurate owing to the
absence of such checks, while the spirometer inertia does not conduce
to the same ease of respiration as does the Benedict Field Apparatus.

Several cases were estimated by means of the Douglas Bag
and the Haldane Gas Analysis Apparatus. In this case there is no
check on the respiration, although the respiratory quotient is always
obtained. The method is tedious and was abandoned for a time. An
examination of the results indicates that there is some definite low-
ering of metabolism during the establishment of the menstrual flow,
and that immediately prior to menstruation there is a rise in the
metabolic rate.

As previously stated, it was anticipated on other grounds
that the reverse might be the case, so that for the moment the nature
of the correlation of this finding with clinical evidence of thyroid
disturbance in the menstrual period would appear obscure.

It is known that in regions where there is a tendency to
endemic goitre that menstruation causes a swelling of the neck which
may be more or less marked (private communication from Professor C.S.
Hicks who has worked among such cases), while the so-called adolescent
goitre which first becomes manifest during the onset of menstruation,
is looked upon as evidence of deficient thyroid activity, yielding as

it does to the administration of small doses of potassium iodide
and being associated with a subnormal metabolic rate. The signi-
ficance of the drop in metabolic rate must be sought in the effect
of the whole.glandular complex at this period, and more work will be
necessary to throw light upon the subject. Benedict in his study,
comes to no conclusion as to the cause of the drop in metabolism.
The association of thyroid function with activity of the female sex
organs is of course borne out in the higher percentage of thyroid
dystrophy in the female sex, while from the original investigations
of Marine and Marine and Lenhart it became clear that the adminis-
tration of iodides during pregnancy in cases which tended to develop
goitre at this time, effectively prevented the condition. Marine
himself considered that this was evidence of an extra call upon the
thyroid which under the conditions (i.e. anaemic goitre conditions)
was unable to fix sufficient iodine. In instituting this work,
therefore, it was confidently anticipated by Professor Hicks that
some metabolic evidence in support of their contentions would be
obtained. On our own part, however, for theoretical reasons only,
we had anticipated a fall. We had found (see adrenal investiga-
tion section) that the greatest response to suprarenal extract was
obtained in the premenstrual phase.

That this was to be correlated with the absorption of the
follicular hormone from the contemporaneously ruptured follicle,
seemed reasonable, and is further supported by the well-known hyper-
sensitivity to suprarenal extract existing during pregnancy. Since
the thyroid and adrenal glands both raise metabolism by their acti-
vity, and since both are supplied by the sympathetic nervous system,
it did not seem unreasonable to associate a rise in metabolism with

10 cxypo on time 1 min
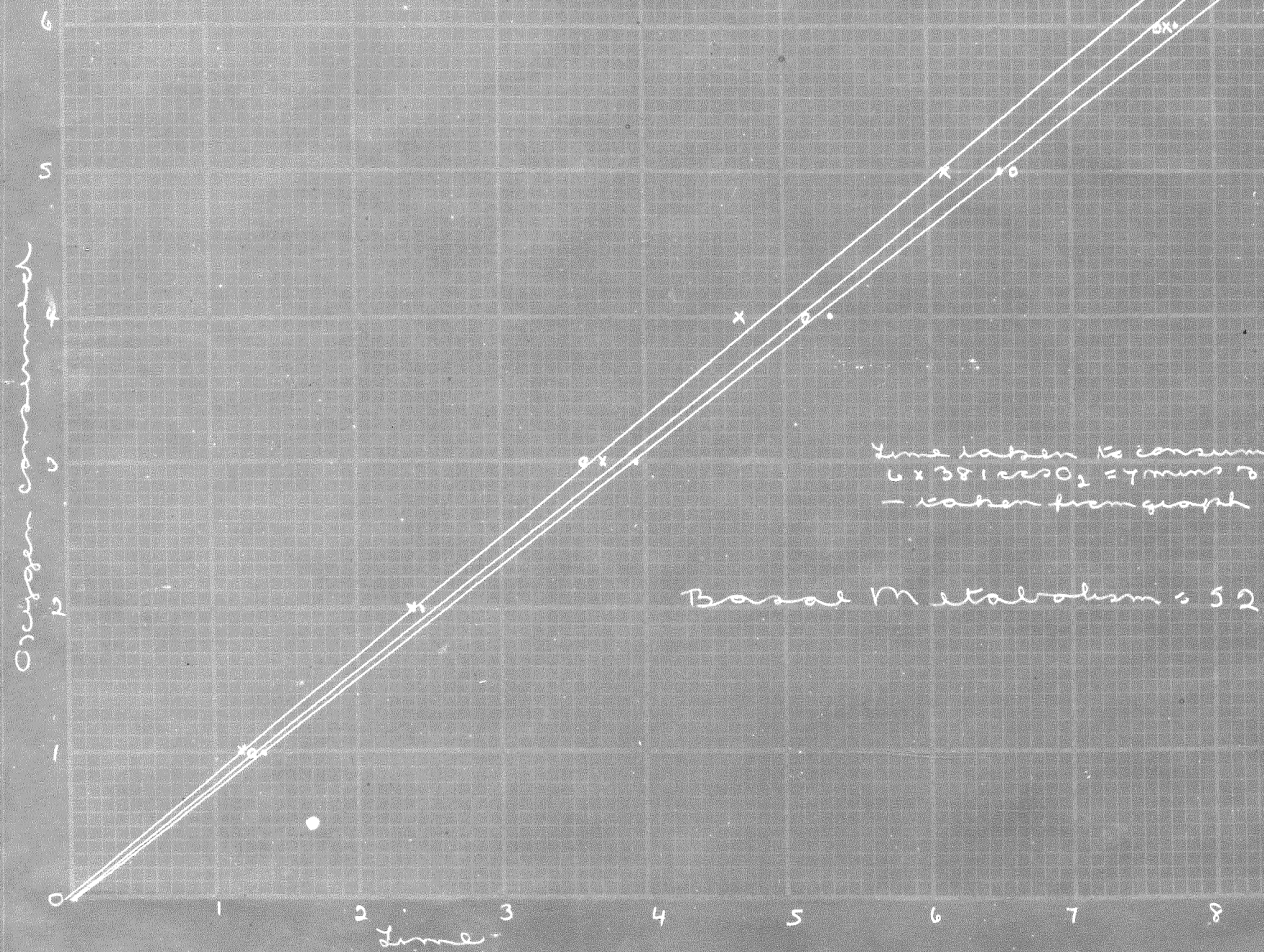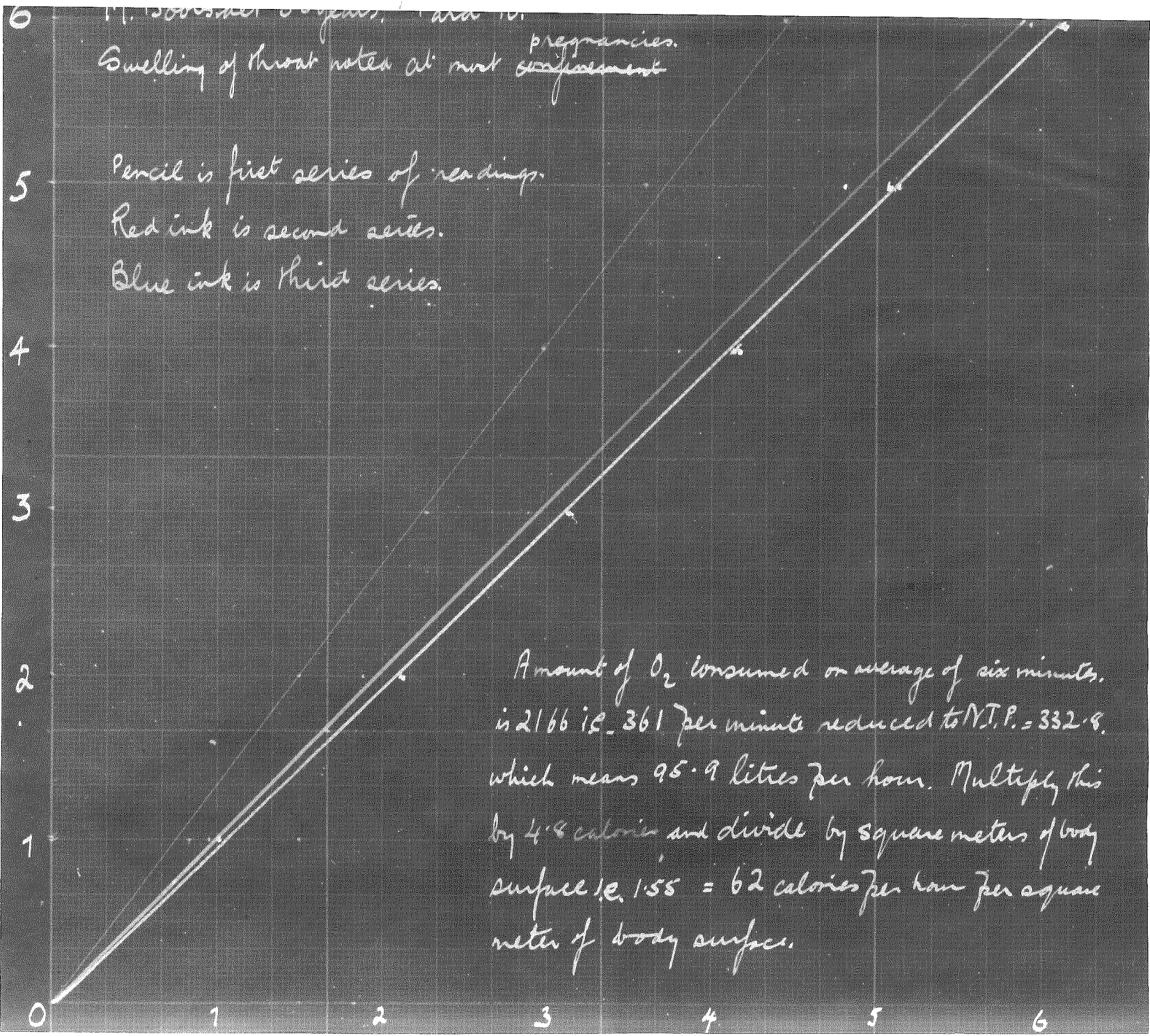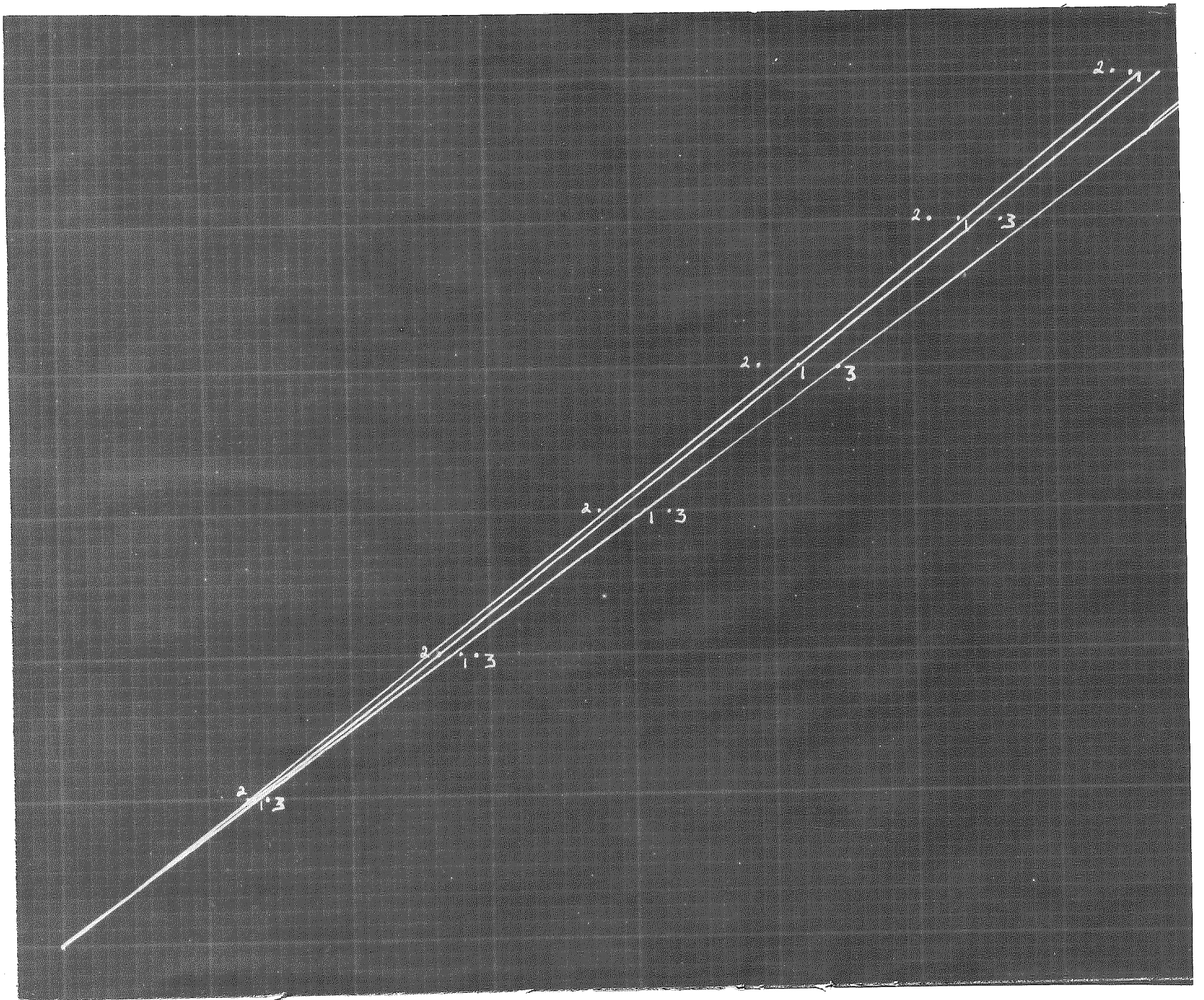
Oxygen consumed

Time taken to consume
6 × 381 ccs O₂ = 7 mins 36 secs
— taken from graph

Basal Metabolism = 52

Time
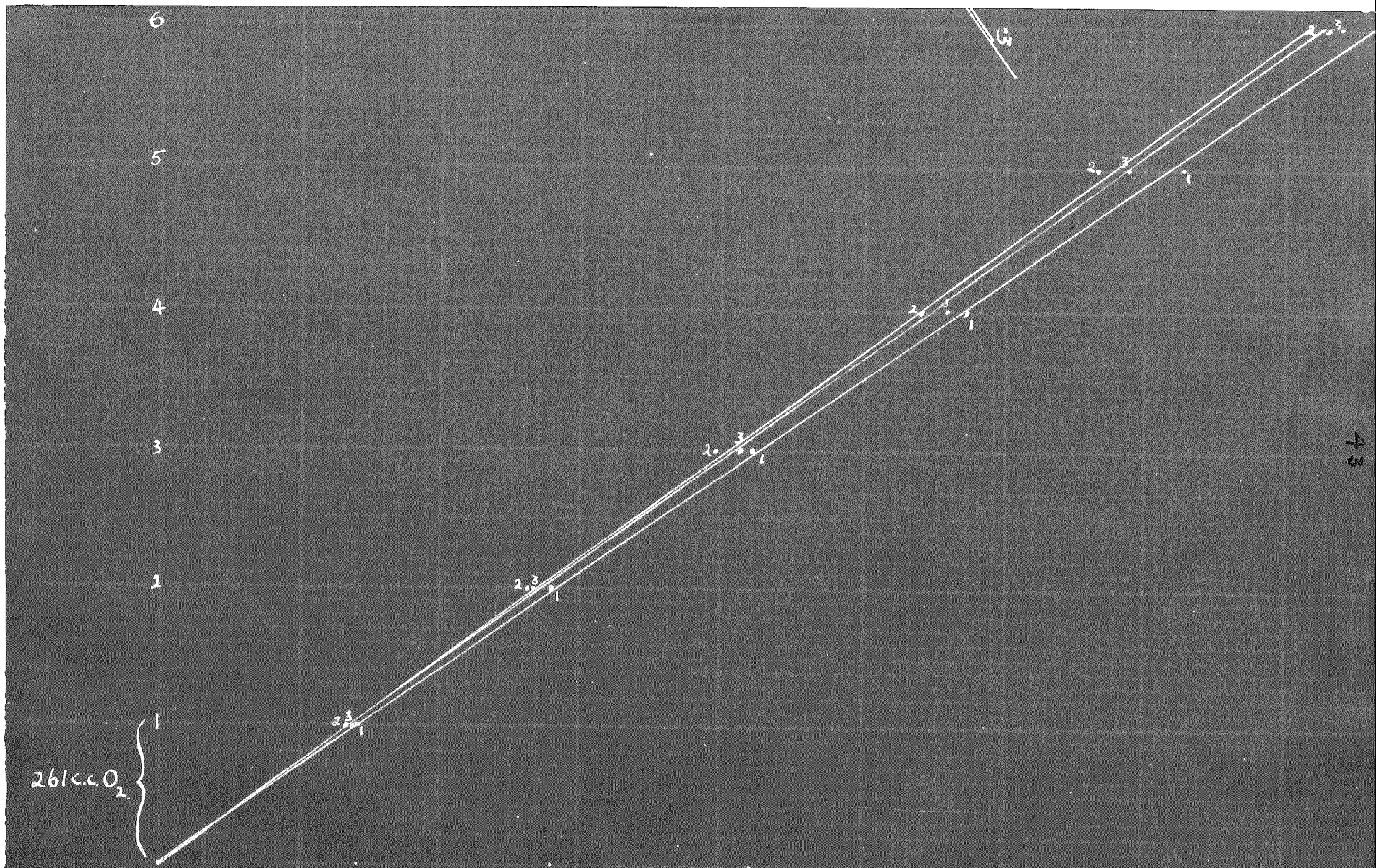
Dots along base line indicate regularity of Oxygen consumption.

October 31st. Temp 14. Barom 768. 15.84 litres per hour = 70.56 calories.

70.56 ÷ 1.83 (sq metres of body surface = 38.5 cal per sq. m. per hour.

M. .... 36 years, ... 1 and 10.

Swelling of throat noted at most ~~confinement~~ pregnancies.

Pencil is first series of readings.
Red ink is second series.
Blue ink is third series.

Amount of O_2 consumed on average of six minutes.
is 2166 i.e. 361 per minute reduced to N.T.P. = 332·8.
which means 95·9 litres per hour. Multiply this
by 4·8 calories and divide by square meters of body
surface i.e. 1·55 = 62 calories per hour per square
meter of body surface.

F. Diggle   Height 5ft 2 in. Weight 10st 4¾ lbs.

Six months pregnant. Barometer 752.
                    Temp.   20°C.

It is to be noted that the three sets of readings almost coincide. The average of the eighteen readings is 5 minutes and 20 seconds for 2166 c.c O_2. i.e. 408 c.c. per min. which is 376 c.c. at N.T.P. This means 22·5 litres per hour or 108 calories. Reduced to sq. metres the result is 65 calories per sq. metre body surface.

1   2   3   4   5   6
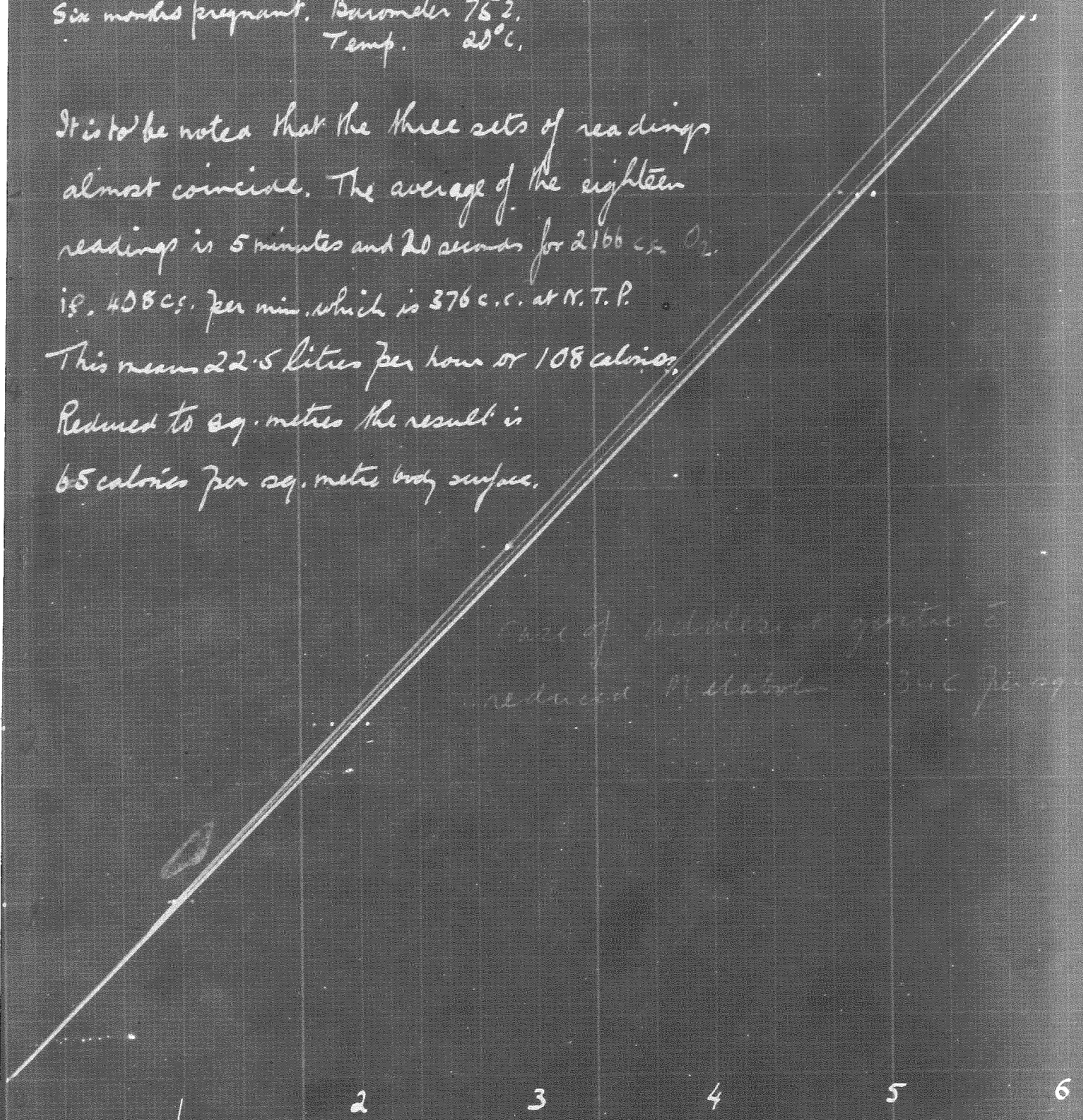
the premenstrual phase, and not with the period of catamenia itself.
In fact, one might have expected a drop below normal immediately
following the premenstrual rise, and such would appear to be the
case.   As previously stated, however, more work is necessary to
establish this theory, and there are many factors to be eliminated
so that single potent influences may be studied with accuracy. The
amount of work involved in these investigations and the// slow pro-
gress made might be appreciated when it is realised that each daily
estimation averages more than an hour to complete, this being merely
what might be termed the mechanical side.

Further work will be based upon the study of the follicular
hormone itself, the preparation of a sufficient quantity of which
requires much time and labour.   Material and apparatus for such in-
vestigation is at present to hand excepting ₣&₧ a completely adequate
supply of ovaries.   The ovaries are collected over a long period
from the Abattoirs and preserved until a large enough supply is ob-
tained.   The work is being continued at the present time.

The method of plotting the length of time taken for the
absorption of a known quantity is shown by the accompanying graphs.
One is taken from an experimental case of a student, the others were
taken during these investigations.   Three sets of six readings are
taken in each investigation and the average time is obtained in a
corrected form by the Benedict method of plotting.

It cannot be too strongly emphasised that the patient
must be in a really basal condition for obtaining correct comparable
results.

The following three sets of readings indicate the type
of result obtained by plotting oxygen consumption against time.   When

good standard conditions prevail the values obtained lie along a
straight line, and in these investigations measurements were carried
on until the above conditions were obtained:-

First set

Guns of O_2	Time		Time for each Gun	
1	81	seconds	81	seconds
2	157	"	76	"
3	237	"	80	"
4	319	"	82	"
5	389	"	70	"
6	464	"	75	"

Average time per gun = 77.6 secs.

Second set.

1	73	"	73	seconds
2	145	"	72	"
3	201	"	56	"
4	280	"	79	"
5	367	"	87	"
6	459	"	92	"

Average time per gun = 76.8 secs.

Third set

1	76	"	76	seconds
2	144	"	68	"
3	220	"	76	"
4	309	"	89	"
5	397	"	88	"
6	461	"	64	"

Average time per gun = 76.8 secs.

Amounts of Oxygen Consumed in Estimation of L.M.

(This Patient went away on November 12th).

Oct.13	224	c.c. per minute
14	226	" " "
15	208	" " "
18	191	" " "
20	222	" " "
21	203	" " "
23	192	" " "
25	196	" " "
26	201	" " "
28	205	" " "
31	208	" " "
Nov. 6	195	" " "
8	216	" " "
10	227	" " "
11	230	" " "
12	215	" " "

Amounts of Oxygen Consumed in Estimation of E.C.M.

Aug.	17	228	c.c. per minute	
	18	226	"	" "
	21	207	"	" "
	22	201	"	" "
	23	195	"	" "
	25	208	"	" "
	27	192	"	" "
	28	184	"	" "
	30	195	"	" "
Sep.	1	216	"	" "
	3	221	"	" "
	4	214	"	" "
	6	222	"	" "
	9	228	"	" "
	11	236	"	" "
	12	247	"	" "
	14	217	"	" "
	15	196	"	" "
	17	207	"	" "
	18	208	"	" "
	19	241	"	" "
	23	228	"	" "
	24	242	"	" "
	25	254	"	" "
	26	248	"	" "
	29	178	"	" "
	30	193	"	" "
Oct.	2	197	"	" "
	7	203	"	" "
	8	190	"	" "
	9	217	"	" "
	10	228	"	" "
	11	202	"	" "
	12	195	"	" "
	13	191	"	" "

Graphic representation of Basal Metabolic Rate of L. M. The figures at
the side denote calories per square metre per hour.
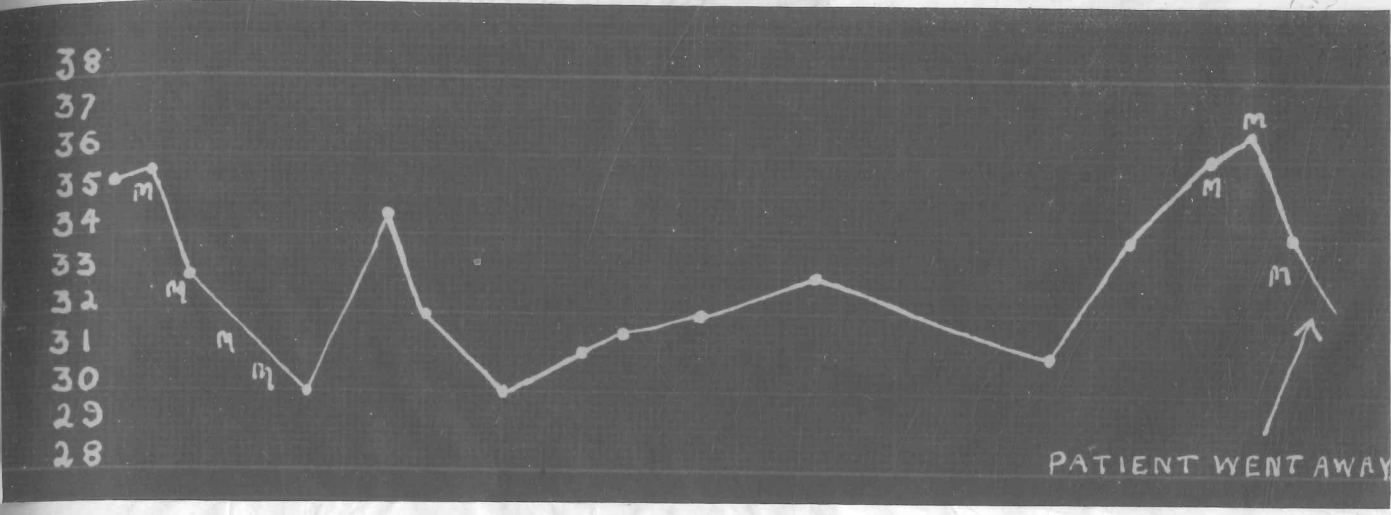
Below is graphic representation of Basal Metabolic Rate of E. C. M.

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SUPRARENAL ACTIVITY.

The Action of Adrenalin During the Menstrual Cycle. Its Significance at the Menopause.

Investigations of the circulating hormone, during pregnancy and the menstrual cycle, have been made. Some variations of the amount of the circulating hormone in the different phases of the menstrual cycle was found to be evident.

Consideration of this finding together with the changes of blood calcium content and the basal metabolism rate permits a view that a general endocrine variation might occur throughout the menstrual cycle. It was decided to investigate this hypothesis, as it affected the suprarenal gland, by injecting adrenalin hypodermically at different phases of the menstrual cycle, with a view to finding whether any demonstrable symptoms should occur. The injections were given to patients over a period varying from about two months to two years. Sometimes the interval between injections was as long as three months, but usually they were given at weekly intervals on the same day of the week. In every case a careful record was kept of the menstrual history, the exact relation of the time of injection to the onset of menstruation was always ascertained with the greatest possible accuracy. The results secured were not invariably constant, as unfortunately, the evidence, being largely subjective, was not always reliable. It was necessary also to avoid asking leading questions which might produce answers which would obscure the result.

A general survey of the whole situation, however, is productive of tentative conclusions which indicate an alteration of the endocrine "balance". The maximum effect was found to begin and to

continue until the fourth day prior to the menstrual flow. On some occasions, usually about the tenth day before the anticipated onset of menstruation, and therefore while the corpus luteum is very young, menstruation has ensued within a few hours despite the fact that the menstrual cycle had been most regular previously. The minimum response to adrenalin injection occurs during and immediately after menstruation. These facts are presented in the succeeding protocols. Some cases were not affected in any way by the injections. It is suggested that as these subjects were of a masculine type and of greater weight than the others, the reason for this non-conformity might have been either an inadequate amount of adrenalin per unit of body weight, or some fundamental difference in sexual characteristics. A standard amount of adrenalin was used, viz: ten minims.

Menopausal subjects were given adrenalin by injection, this administration of adrenalin being continued after the catamenia had ceased. In these cases the vertigo, headaches and flushes were more marked than during the menstrual cycle. Subsequently, however, the endocrine balance appeared to gradually readjust itself to the ovarian inactivity and the symptoms became less and less, until finally a new balance has been established and the symptoms disappear.

A recent paper on the response to adrenalin by subjects at the menopause draws attention to one aspect of this work[1]. It deals however entirely with that phase alone. The present work upon the menstrual cycle, so far as can be ascertained, has not been previously attempted.

It cannot be too strongly emphasised that throughout the whole of this work the only theory adopted has been that of general endocrine involvement in the menstrual function. It would be idle speculation to discuss the meaning of the interactions of one gland

upon the other because of the vast amount of detailed evidence yet to be collected. In this section of the thesis is presented what appears to be some definite evidence for the alteration in the response of the organism to the subcutaneous injection of the suprarenal extract. Stieglitz[2] attempted to demonstrate involvement of the suprarenal glands in menstrual function by a careful study of the systemic blood pressure in normal subjects over a considerable period of time. He was unable to draw any conclusions from the results obtained. The fact that no general change in blood pressure could be proved does not, however, mean that there is no vasomotor effect arising from the suprarenal activity during the menstrual cycle. The alteration may not only be insufficient to cause a rise of blood-pressure from splanchnic constriction, but also, owing to the superimposed effect of the pituitary hormone, the final result may be due to redistribution of the blood in local areas as well as its redistribution between capillaries and arterioles. The headaches may be due to such alterations as they affect the meningeal circulation. The work of Dale and others[3] has indicated how the apparently conflicting results of workers upon the blood pressure changes in the cat and rabbit, for some time past, can be reconciled. The reaction of the skin vessels in these phases may well be investigated in this connection along the lines of Sir T.Lewis[4]. So far as the vertigo is concerned the cause may be due to similar changes affecting the semicircular canals.

So far as it is permitted to discuss the direct effect of the administration of adrenalin upon menstruation, it is here suggested that this hormone depresses the effect of the corpus luteum with the result that its endometrial control is reduced and the endometrium is shed in the form of the menstrual flow, which thus appears earlier than normal. Owing to the fact that all the data here presented was col-

lected during clinical practice, it required some three years to se-
cure a satisfactory number of cases.

It is regretted that not until a deal of evidence had been
already collected, was the idea of a dosage based upon the body weight
considered.   Had this been done the different individuals would have
had a truer comparison in reference to the effects of hypodermic ad-
ministration of adrenalin.

Protocol of Adrenalin Injected Women.

Subject	Injection No.of Days	Phase	Result
H.S.	12	Premenstrual	Giddiness, headache & flushes.
	5	"	" " " period delayed
	4	"	" " "
	4 (different period)	"	" " "
	1	"	" Slight headache and flushes.
	3	Menstrual	Nil
	2	Postmenstrual	"
M.H.	14	Premenstrual	Vertigo, headache & Flushes
	7	"	Slight" " "
	3	"	Nil
	5	Postmenstrual	"
M.W.	7	Premenstrual	Vertigo, menstruation in ten hours
	4	Postmenstrual	Nil
O.M.W.	12	Premenstrual	Vertigo, headaches & flushes
	5	"	" " "
	1	"	" " "
	1	Postmenstrual	Nil
	7	"	Nil
	8 (different period)	"	Nil
D.C.	14	Premenstrual	Slight headaches and flushes
	5	"	Vertigo, headaches & flushes
	4 (different period)	"	" " "
	3 "	"	" " "
	2	Menstrual	Nil
	4	"	Nil
N.H.	12	Premenstrual	Headaches and flushes
	8	"	" "
	7 (different period)	"	" "
	6 "	"	" " Menstruated next day.
	3	Postmenstrual	Slight vertigo only
A.M.	10	Premenstrual	Vertigo, headaches & flushes
	5	"	" "
	4	Menstrual	Slight giddiness & headache
	6	Postmenstrual	Nil
	7 (different period)	"	Nil

Subject	Injection No. of Days	Phase	Result
H.M.	12	Premenstrual	Vertigo, flushes and epistaxis.
	1	"	Vertigo but no flushes.
E.L.	10	"	Vertigo, headache. Menstruated in fourteen hours.
	7	"	Vertigo, flushes & headaches. Since injections menstruated every two weeks for two months. Previously catamenia was 4-28.
	1	"	Vertigo etc.
I.E.	14	"	
	8	"	Nil. No reaction occurred
	7	"	at any time.
	3	"	
D.C.	14	"	Slight headaches & vertigo.
	12	"	Vertigo, headaches & flushes.
	4	"	" " "
	3 (different period)	"	" " "
	4	Menstrual	Nil
	6	Postmenstrual	Vertigo, headaches and flushes
M.N.	7	Premenstrual	Nil
	3	Postmenstrual	"
G.B.	Menstruation just beginning		Menstruation deferred for four days.
I.J.	2	Menstruation	Menstruation ceased instead of continuing usual five days.
V.B.	7	Premenstrual	Vertigo, headaches & flushes.
C.H.	8	"	Nil.
E.F.	8	"	"
H.M.M.	1	"	Headaches, vertigo & slight flushes.
M.N.W.	Three months pregnant		Vertigo, headaches & flushes
"	Four " "		Marked vertigo, headache and flushes.

Part III.   THE PARTURIENT WOMAN.

PART III.   THE PARTURIENT WOMAN.

Introduction.

An aphorism which came from Hippocrates[1] says:- "If you wish to stop the menses of a woman, apply as large a cupping instrument as possible to the breasts".   This indicates that some connection between the uterus and the mamma was probably recognised then, but the hormonic possibilities in these conditions is still far from solution. When the spermotozoon impregnates the ovum a change takes place and alterations of metabolism often occur.   It is found also that special metabolic processes may be so altered as to cause eclampsia, osteomalacia, hepatic atrophy, tetany, etc. (Matters[2]).

The causation of these changes is thought to be probably of endocrine origin.   It is thought that the ovarian sex hormone in the blood increases as pregnancy progresses, and, with the development of the placenta, more hormone appears to be secreted.   It has been suggested that the discus proligerous when expelled from the follicle bears granulosa cells which, when embedded in the decidua multiply and secrete greatly, thus producing the follicular hormone in the placenta.   This might account for the secretion of the follicular hormone by this organ.

From the placenta both luteal and follicular hormones are secreted (Frank and Goldberger[3]) and have been extracted.   By about six weeks some hormonic influence has produced an effect on the breasts which commence to enlarge and Montgomery's tubercles appear.   This condition is produced entirely by some blood hormone as was shown by Schanta and Brasch[4] in the case of the Blazek twins, that the lactation etc. is the result of some hormonic secretion.   These twins were pygopagus, having merely a cutaneous union along the thighs, and they were

both female.   One of them became pregnant while the other remained a virgin, and during the pregnancy each of the twins developed mammary enlargement, and later, when the pregnant one had been delivered, it was found that the baby could be suckled equally well by the mother or the nulliparous aunt.

When the ovarian hormone is secreted in a disorderly and excessive amount the results may be so serious as to endanger the life of the patient, the most disastrous result of this kind being osteomalacia.   Reasoning the condition as the effect of the hormonic influence Adler[5], by way of treatment, introduced removal of the source of the hormone as the method to be adopted in osteomalacia.   The method which was introduced was extirpation of the ovaries and emptying the uterus of foetus and placenta, the latter being most essential owing to its secretion of the hormone.   A study of the circulating hormone has been made in this work but pathological states have not been investigated in this respect.

Basal metabolic rates appear to be increased slightly in pregnancy, if the small number which we have estimated can be taken as an indication of the whole.   This is not quite unexpected as it has been shown by Erdheim and others that there is an enlargement of the thyroid during pregnancy, and the actual neck measurements in a number of cases have shown measurable increase in size during pregnancy.   This increase of metabolic rate is probably in part due to the dual metabolism of mother and foetus, at the same time the thyroid hypertrophy may be mainly due to the increased tax on limited iodine supply.   In spite of this possibility there were sufficiently numerous cases of toxic hyperthyroidism which began during pregnancy to warrant the idea of hyperthyroidism in many pregnant women.

It is considered that the alteration of endocrine balance

in pregnancy is responsible for many of these thyroid changes, and this might be illustrated by one outstanding case. This was a patient who had borne three children. During the first pregnancy signs of hyperthyroidism were noticed, during the second pregnancy the condition advanced so much that partial throidectomy was done. Following this operation the patient became relatively normal, but during the last pregnancy she showed all the signs of Graves disease. The basal metabolism was more than double the normal rate and we delivered her with forceps without anaesthesia in a state of utmost distress.

Evidence for the Existence of a Hormone in the Circulating Blood of Pregnant Women.

For some time the blood has been regarded as a possible source of information regarding the parturient state of woman, Abderhalden's test for pregnancy being most widely known. Smith and Shipley[6] extensively investigated this method and its modifications. DeLee[7] also cites the adrenalin-glycosuria test for pregnancy, in which case it was considered that the ovarian hormone reduced pancreatic activity, thereby giving the suprarenals a preponderating effect. The further addition of suprarenal extract by hypodermic administration may then so depress the Islets of Langerhans that glycosuria ensues. Ascheim and Zondek[8] claim that not only is the follicular hormone present in the blood of pregnant women, but that they have isolated it from the urine of women who are approaching full term.

M.G.Smith[9] working at the Johns Hopkins Hospital actually isolated the follicular hormone from the blood of pregnant women. Some of these women were in the early days of pregnancy when the blood was examined.

It has been shown by Steinach et alia[10] that there is pro-

duced in the placenta of a pregnant women, a hormone which circulates in the blood. This publication was made this year, and at this time it is estimated that the amount of follicular hormone circulating in the blood amounts to 50,000 mouse units. Shortly after this Siddall[11] published his results which were obtained from injecting virgin mice with blood sera from pregnant and non-pregnant women. The effect of serum from the parturient female was to produce uterine enlargement, but the injection of serum from the non-pregnant subject was without effect.

A peculiar case was observed by Binz[12]. A female child two years of age exhibited precocious puberty and at operation a large ovarian tumour was removed. Fluid when withdrawn from the tumour and injected into mice caused uterine enlargement. Similar results were obtained after injecting mice with fluid removed from a parovarian cystoma. A further observation by Binz was that blood serum of a pregnant woman if injected into mice produced uterine hypertrophy. Trevino[13] and Fels[14] observed the same effect of blood serum from pregnant women upon the uteri of mice; the observations however were purely qualitative and as such were not suitable for comparative purposes.

Several series of investigations of the ovarian sex hormone in the blood-serum of pregnant women were made by Frank et alia[15]. They usedfor these purposes the vaginal smear test of Evans and Long[16]. It would appear reasonable to assume that some activating agent produces effects upon the female genital and mammary organs, and in our investigations it has been found that not only are the uteri increased in size and weight, but that vessels of uterus and adnexa are very engorged. Mammary swelling also occurs in cases where the blood serum of pregnant women is injected into rodents. It is thought that the same blood born

hormone caused the breasts of the nulliparous member of the Blazek twins to develop and finally to secrete sufficient milk to feed the gestational product of her pygophagus sister.

In the present work we have repeated the uterine hypertrophy test using the empirical modification suggested by Siddall[11]. For this purpose we have used virgin mice of a standard type. Conditions were maintained which provided a continuity of similar conditions, so that as far as possible no extraneous factors were likely to affect the conclusions.

Each mouse was used for a specific serum and the blood serum of pregnant women was injected on five successive days, 1 cc. of serum being used for each injection. Controls were kept and these were injected with the sera of nulliparous and non-pregnant multiparous females, and a few mice were injected with the sera of males. The day following the final injection the mouse was killed and weighed, the weight being recorded in milligrammes. When this was completed the abdomen was opened and the uterus and adnexa were removed en bloc and weighed. The ratio between the weight of uterus plus adnexa and the total mouse weight was the result upon which conclusions were based. It was considered, as the result of experiment, that the ratio which was below 350 indicated the serum of a pregnant woman, while a figure above this was probably that of a non-pregnant person.

It was found that for several weeks post partum the test gave positive results and probably the same would be true of blood serum from a woman with luteal or certain ovarian cysts.

The serum was obtained by withdrawing the blood from the vein of the subject into a sterile test-tube. The tube was then incubated in order that the clot might retract from the serum, and then the tube was placed on ice against the time when the injection would

be made.   The results obtained from injecting mice with preparations of ovarian extract were similar to those of the pregnant women's serum.

Uteri of mice have been photographed both in situ and in the extirpated state, and these are shown in order that a comparison between the uteri of non-injected and injected mice of the same age might be appreciated.

In the earlier researches and prior to the publication of Siddall's work, we injected rabbits and rats with human blood serum, but owing to the greater weight of these animals the results obtained were more variable.   In addition also there was the difficulty of the larger amount of serum required for each injection.   Serum of pregnant women was injected into rabbits and oestrum was produced according to the vaginal smear test.   The number of results obtained was insufficient to admit of anything in the nature of finality.   It has therefore been decided to append only the protocol of results obtained from injecting mice with human blood serum.   Only the mice who received the four injections have been taken except in three cases where the mouse died or no injections were given.   Other cases have not been included, e.g. mice have been neglected over week-end, or larger number of injections have been made.

Photographs of rat uteri, three of the rats being previously injected with sera from pregnant women. One is normal.
All the rats were of a similar age and weight.
No. I. is the photograph of the uterus taken from a normal virgin rat.
No II. is the photograph of the uterus of a rat injected four successive days with serum from a pregnant woman. No 4 is the result of another rat similarly treated. No 3 is the photograph of the uterus of a rat which had injections on five successive days.

Below is the photograph of No2 rat with the uterus etc. in situ. The uterus is supported by two rods.
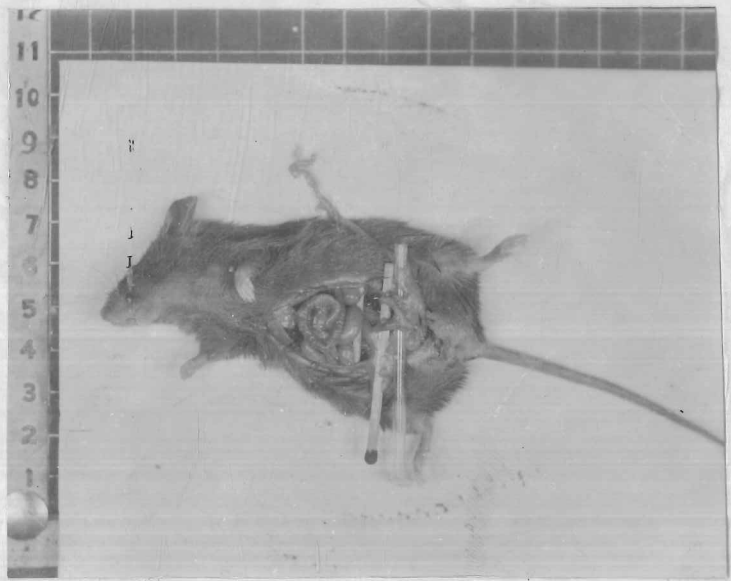
Three photographs of rats each having the extirpated uterus alongside.
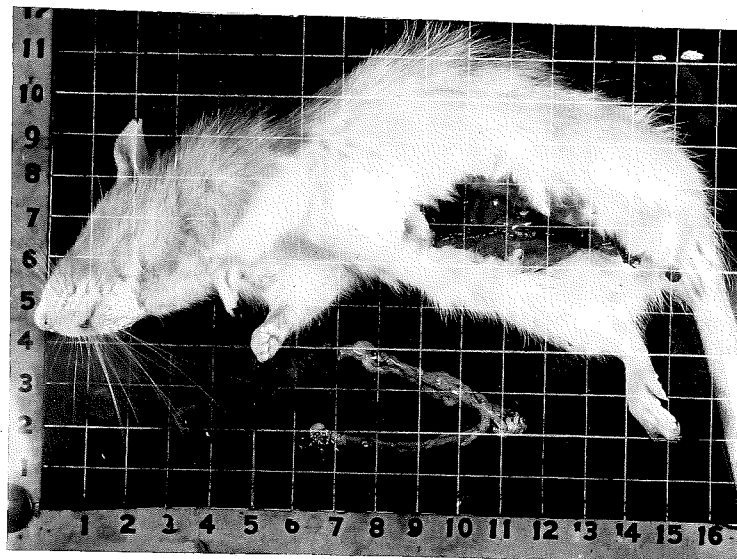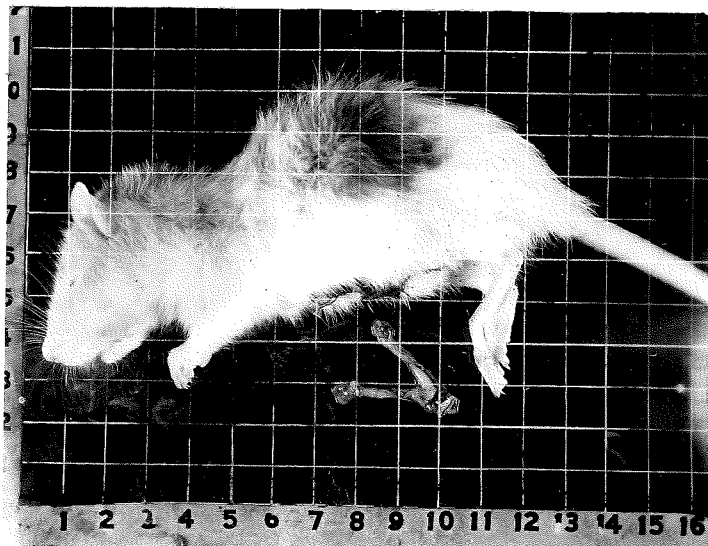
ject	Stage of Pregnancy	No.of daily Injections	Age	Parity	Wght.of Mouse	Wght.of Uterus etc.	Ratio and Results
E.	8 months	4	22	1	12520	120	105 + ve.
C.	7 "	4	36	2	17625	86	98 + "
C.	7 "	4	43	3	10800	92	116 + "
R.	5 "	4	20	1	11150	110	101 + "
S.	7 "	4	21	1	10625	80	157 + "
B.	8½ "	4	25	2	19420	105	185 + "
H.	7¾ "	4	23	2	19890	140	142 + "
O.	8½ "	4	24	1	15720	91	172 + "
C.	2½ "	4	24	2	10720	55	195 + "
L.	3½ "	4	20	1	26875	165	162 + "
O.	2 weeks puer-peral	4	22	1	26250	120	219 + "
M.	2 days premen-strual	4	25	0	14105	30	412 – "
H.	Intermenstrual	4 2	19	0	14250	21	688 – "
S.	Menopause	Mouse died second day.			14105	20	705 – "
L.	Male	4			14520	20	726 – "
ntrol	No injections given				15100	16	944 – "
varian Extract. Two injections). repared according to Allen &) oisy method)					12205	60	203 + "
stomensin"	3 injections						+
"	" "						+

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Section 2.

THE ADRENAL SECRETION.

The work that has been undertaken in this direction has been described under The Suprarenal Activity in Part II, and included in the protocol there given are cases of pregnancy which showed increased clinical response to the injections of "Adrenalin".

Section 3.

THE PITUITARY HORMONE.

It is only in comparatively recent years that the effects of the pituitary extract have been investigated, and it was in 1895 that Oliver and Schafer[1] discovered the effect of this secretion upon the blood pressure.   Later Howell[2] and Schafer and Vincent[3] demonstrated that the posterior pituitary lobe was the part which provided this pressor effect.

The effect of the suprarenal and pituitary extracts upon the blood-pressure were discovered at about the same time, yet the chemistry of the latter remained a controversial biochemical problem, while that of the former was soon established.

The different physiological properties of the posterior pituitary secretion have been discussed under various headings, each of which covers its own theory.   Four of these which were of marked prominence, were (a) The Epinephrine Theory, (b) The Histamine Theory, (c) The Unitarian Hormone Theory, and (d) The Multiple Hormone Theory.

The Epinephrine Theory.

Since epinephrine exerted the greatest effect upon the blood pressure of any known compound in the human organism, it was suspected that the pituitary extracted owed their pressor effects to a suprarenal cause, these glands being so activated as to discharge an increased amount of epinephrine into the circulation.   It was alternatively thought that a compound similar to epinephrine, or even epinephrine itself occurred in the pituitary gland.   Hening[4], Cramer[5] and

Bell and Hick[6] emphasised this relationship until in 1909 Dale's[7] classical article "The Action of Extracts of the Pituitary Body", clearly showed that the relationship of the pituitary extract and epinephrine were "superficial and illusory". Despite this work of Dale the epinephrine theory was still retained by many workers. As recently as 1916, Watanabe and Crawford[8] stated that "pituitary extracts, when prepared by certain methods, yield colour reactions which would suggest the presence of epinephrine or an epinephrine-like compound".

The Histamine Theory.

Even as late as the post-war period Abel and Kubota[9] in 1919 decided that "histamine is the plain-muscle-stimulating and depressor constituent of the posterior lobe of the pituitary gland. Their conclusions were based on their chemical and pharmacological studies after isolating histamine from a quantity of whole pituitary gland. The physiological and chemical evidence in favour of identity of the two principles coincide at every point.

Dudley[10] disproved the statements of Abel and Kubota with great dispatch in a piece of experimental work which left but little room for conjecture. Histamine is stable towards alkali, trypsin does not affect it, boiling chloroform dissolves histamine, but butyl alcohol does not readily extract it from acid solutions. Exactly the reverse situations occurred when the pituitary uterine stimulant was used in these tests. Subsequently Abel and Nagoyama[11] withdrew the Histamine Theory.

The Unitarian versus the Multiple Hormone Theories.

A detailed review of the voluminous literature derived from the attempts of biochemists, pharmacologists and physiologists is not justified as a means of answering the question "Is the post-pituitary

gland an exception to the rule that each endocrine structure contains one and only one hormone?" A long list of references is given in the bibliography (12 - 30) but no workers have adequately dealt with the question. The situation as it now exists has developed into two groups, both of which are supported by independent workers. Abel et alia[21] have stated that "all the evidence at hand is greatly in favour of our belief that the oxytocic pressor, diuretic and respiratory activities referred to are <u>properties of one and the same substance</u>".

Dudley, however, has been in the forefront of those who support the Multiple Hormones Theory. He concluded[31] "I am driven therefore to the conclusion that Abel and Roniller's preparation, like my own, contained at least three, and possibly more, active substances mixed with an unknown quantity of inert material". The physiological behaviour of each of the posterior lobe hormones is quite different from the others, but in their chemical reactions and also in many physical properties they resemble one another. When dyalised they migrate side by side, Smith and McClosky[22 & 23]; unusual similarities are exhibited when they are exposed to the action of enzymes. The products are not merely destroyed but appear to be inactivated at the same rate when subjected to drastic chemical reagents. Abel et alia[21]. These sets of facts were naturally most confusing to the workers in this field and the work of Dixon and Marshall[32] is the first satisfactory investigation. It is supported by the most satisfactory hypothesis yet advanced.

The oxytocic fraction produces very little change in the urinary output.

In non-anaesthetised rabbits pituitary extracts have a marked antidiuretic effect. This is the predominating renal effect on normal

2 c.c. serum
1st stage
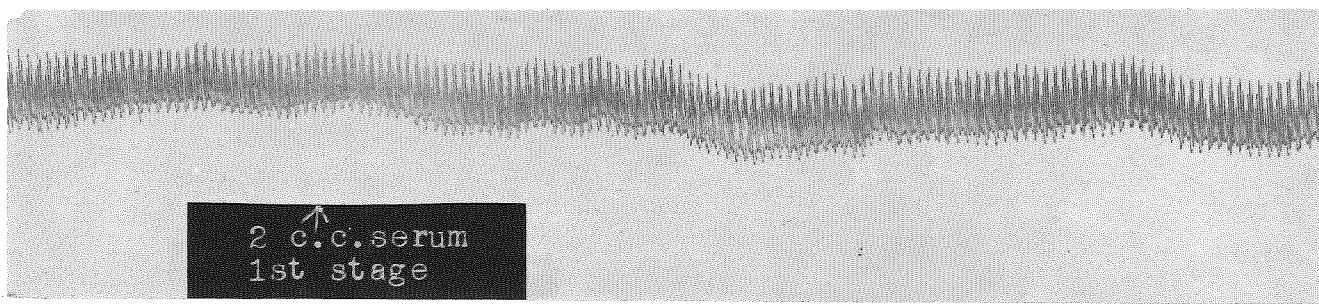
A. Carotid pulse tracing of rabbit. At the point marked 1 c.c. of serum (which showed oxytocic effect on the isolated rat's uterus) was injected into the internal jugular vein. No pressor effect resulted.

1 c.c. serum
1st stage
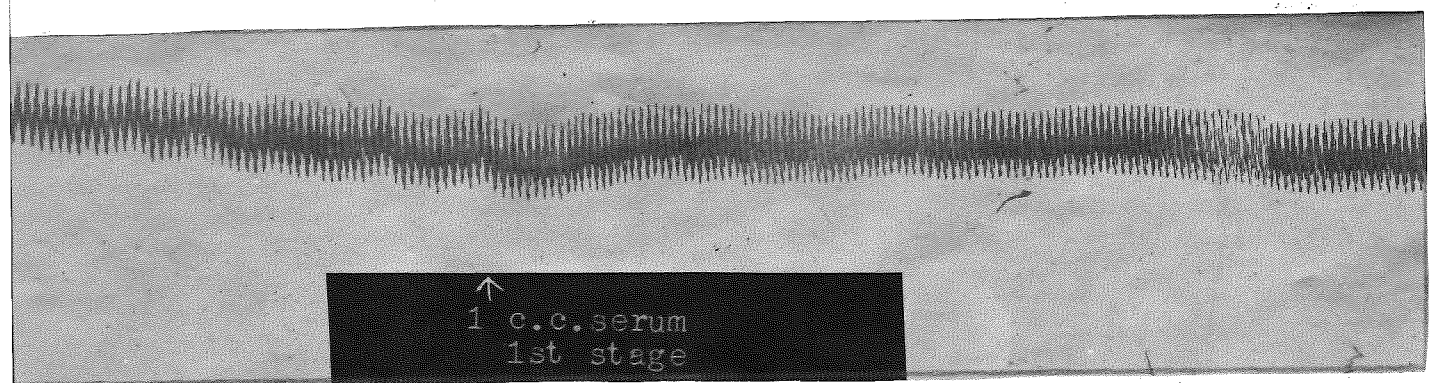
B. A second rabbit's carotid pulse tracing. Here 2 c.c. of serum taken in the first stage of labour showed no pressor effect.

1.c.c. Pituitrin
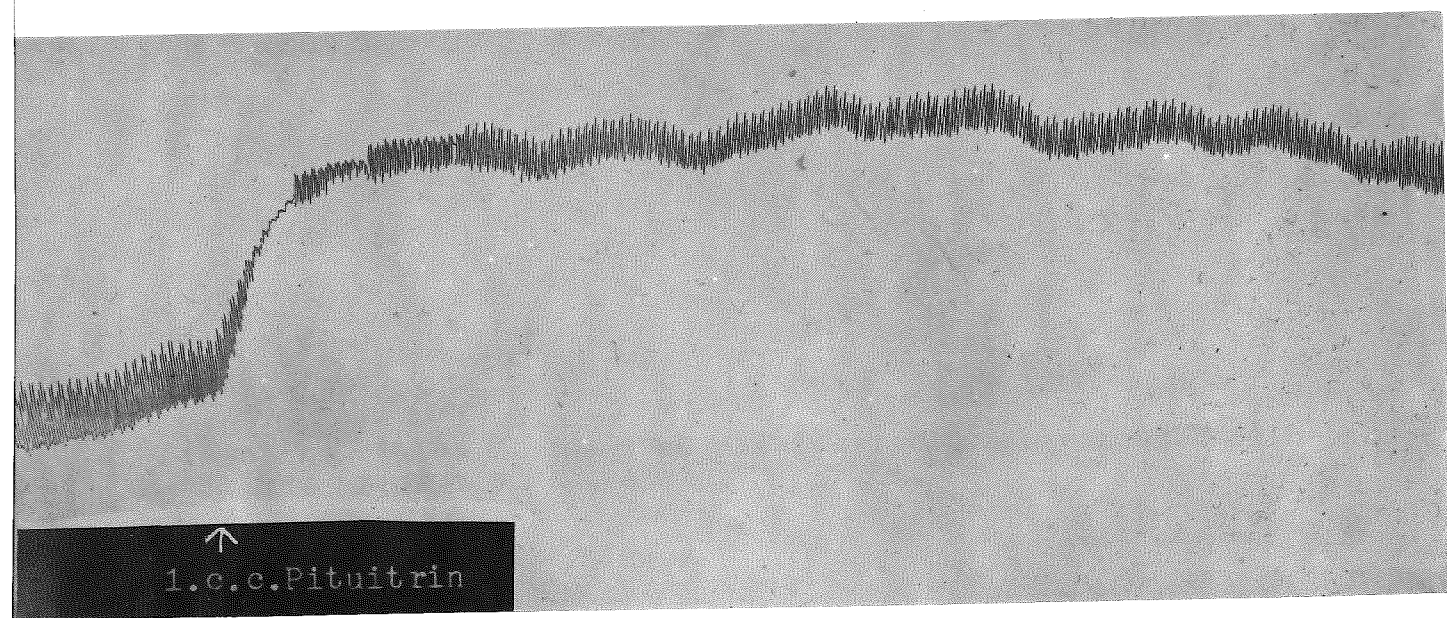
men and especially so in patients suffering from diabetes insipidus.
Smith & McClosky[23].   Recently Gargle, Gilligan and Blumgart[33] have
shown that the pressor fraction produces the full beneficial effect
of pituitary extract when administered to diabetes insipidus patients.
The oxytocic principle was found to be devoid of this effect.

The present contribution covers an assay of both pressor and
oxytocic properties which might exist in the blood sera of pregnant
women.

Assay of Pressor Effect.

This test is carried out upon a rabbit which is deeply anaes-
thetised with ether after an initial oral administration of paraldehyde.
When the details of technique are carefully carried out equal doses of
pituitary extract given at fifteen- minute intervals cause equal increa-
ses in blood pressure.   The blood pressure is taken from the carotid
artery to a tambour by means of a cannula and rubber tube.   The tam-
bour connects with a writing lever which records the pulse waves upon
a revolving drum.   Injections were made into the jugular vein.   This
method allows the unknown solution to be compared in pressor activity
with a standard pituitary solution by noting the relative amounts of
the two extracts required to cause the same rise in blood pressure in
several successive tests.   If small doses be used tolerance is not es-
tablished and a 10% accuracy of standardisation is achieved.

The Oxytocic Method of Assay.

This test is dependent upon the fact that part of the uterus
of a small animal if kept in suitable Ringer Locke solution will con-
tract if pituitary extract is added to the solution, as first shown by
Dale and Laidlaw[34] and later improved upon by Pittenger[35] and others,
[35 to 40]

A water bath maintained at constant temperature by thermosta-

tic control contains a vertical tube filled with Ringer Locke solution in which is suspended one horn of the bicornuate uterus of a rat. The lower end of the muscle is attached to a fixed point at the bottom of the tube, while the upper end is attached by means of a thread to a writing lever. The writing lever bears against the smoked paper of a kymograph. By this means the movements of the muscle are recorded on the smoked paper. The bath also contains a large reservoir of fresh Ringer Locke solution and by means of this it is possible to proceed with an investigation and wash out the uterus chamber with a minimum loss of time and minimum disturbance to the organ. At the points on the tracings marked "wash out" the chamber has been emptied and four successive flushings with Ringer Locke solution have been made to remove traces of the previous contents of the chamber. Before adding the serum the organ is allowed to settle down to regular contractile activity. The choice of the rat uterus instead of that of the guinea pig is made because the latter is susceptible to histamine which results from any cellular damage in the blood elements, while the former are insensitive.

Trials are made with definite quantities of the standard solution of pituitary extract and the solution under test, until the relationship between their activities has been determined. Although the two pituitary principles appear to be closely related, their separation from the large amount of foreign proteins with which they are associated and which mask their normal behaviour, it is found that they possess important physical dissimilarities. When 95% of the foreign proteins are removed from the posterior lobe fraction, the work becomes simplified and a gradual separation of the two principles may be made, the one from the other.

Very early in the present investigation and before we were

aware of the above trend of pituitary research, evidence was accumulated by us of an oxytocic effect as distinct from a vasomotor effect, when serum from subjects in the first stage of labour was investigated. At this stage it is desirable to explain the method adopted in this investigation. The author was in a position to secure blood from subjects in various stages of pregnancy, and it was pointed out by Professor Hicks that, although some attempt had been made by Dixon and Marshall to prove this point, they themselves admitted the insufficiency of their evidence mainly on the grounds of inadequacy of material available for their investigation. It was decided that in the first stage, investigation would be confined to the fresh sterile serum of suitable subjects. It was realised that the quantity of hormone present would be small, but it was hoped that some indication of a difference between the sera of normal and parturient subjects might be detected. If a difference could be demonstrated, then it was proposed to extend the work by using dialysed extracts from the sera investigated, and, if necessary, by concentrating these extracts.

Blood was drawn under asceptic conditions and centrifuged when necessary after preliminary incubation and reduction to $0^{\circ}C.$ to favour clot separation. Blank determinations were made using serum from the blood of Professor Hicks and other males. It is evident that fresh serum has a stimulating effect upon uterine contractions, but it seems reasonable to assume, from a study of the cases presented, that there is a definite retraction of the uterine muscle when supposedly "active" sera are used.

As has been previously reiterated, the researches here recounted have been attempted contemporaneously because of the effort necessary to secure clinical material. Since the clinical difficulties are paramount, it seemed wise to follow as many paths as the ma-

terial collected would permit. This fact has restricted the distance
traversed in each instance, but as the present dissertation is pre-
sented it represents - so to speak - an interim report upon work which
is still in progress and which will be continued for some years to come.

The present section therefore deals with the preliminary at-
tempt to follow on the investigations cited earlier and to seek for
evidence of the presence of an oxytocic substance in the blood serum
of subjects in whom such might be anticipated at first.

As stated earlier in this section, the tests for the presence
of the well-known pressor action of "pituitary extract" were made by
injecting sterile sera into the blood stream of rabbits under complete
anaesthesia. The tracings attached are typical and indicate that there
was no pressor reaction.

In general, it is felt that the evidence warrants further
investigation using refined technique as regards the isolated uterus
method of testing for the oxytocic principle. As this is fraught with
considerable difficulty the preliminary investigation would appear to
be justified.

It will be necessary to secure larger amounts of blood from
the subject, and further, it seems advisable to dialyse the same to
secure protein-free extracts which can be concentrated in vacuo.
The following are the conclusions based upon the present stage of the
work:-

    1. The only certain difference between the serum of a subject
        in the first stage of labour and that of a non-pregnant sub-
        ject is the effect of the former upon "retraction".

    II. There also appears to be an increase in contraction, but the
   number of cases studied does not permit of a definite conclu-

sion being reached. Greater refinement of technique will be necessary in this connection, particularly in respect of the age and state of the uterus used.

III. Normal serum from non-pregnant female and also from male subjects causes a stimulation to contraction on the part of the surviving rat's uterus.

IV. No pressor effect could be demonstrated by injection of the suspected "active" sera into the circulatory system of an anaesthetised rabbit.

V. If the "retraction contraction" phenomenon cited under I and II be due to some hormone present in the blood of such subjects, that hormone has no pressor effect in similar doses.

VI. It is concluded that in order to follow up this work dialysis of the sera, in colloidion sacs, will ne necessary to remove the possibility of a protein effect upon isolated uterine contraction.

During these investigations a large number of tracings have been taken and some are here shown. It is desired to point out, however, that owing to the fact that we were breeding rats at the time of the investigation we were considerably handicapped in the number of available uteri, and also in the number of standard virgin rats. Some of the tracings therefore show a difference in the "normal" contractions. It must therefore be realised that the results here depicted are by no means conclusive and do not provide sufficient evidence until they are corroborated along the lines indicated in the text.

1.5 c.c. 6 mos.   1 c.c.ditto
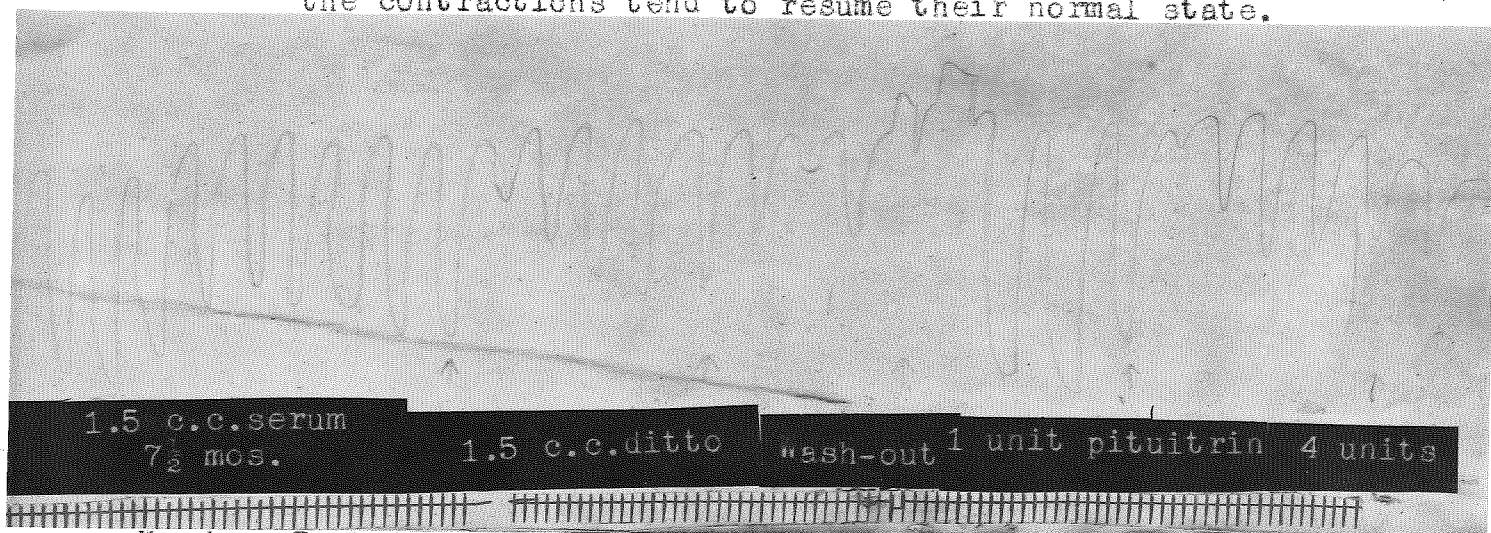pregnant
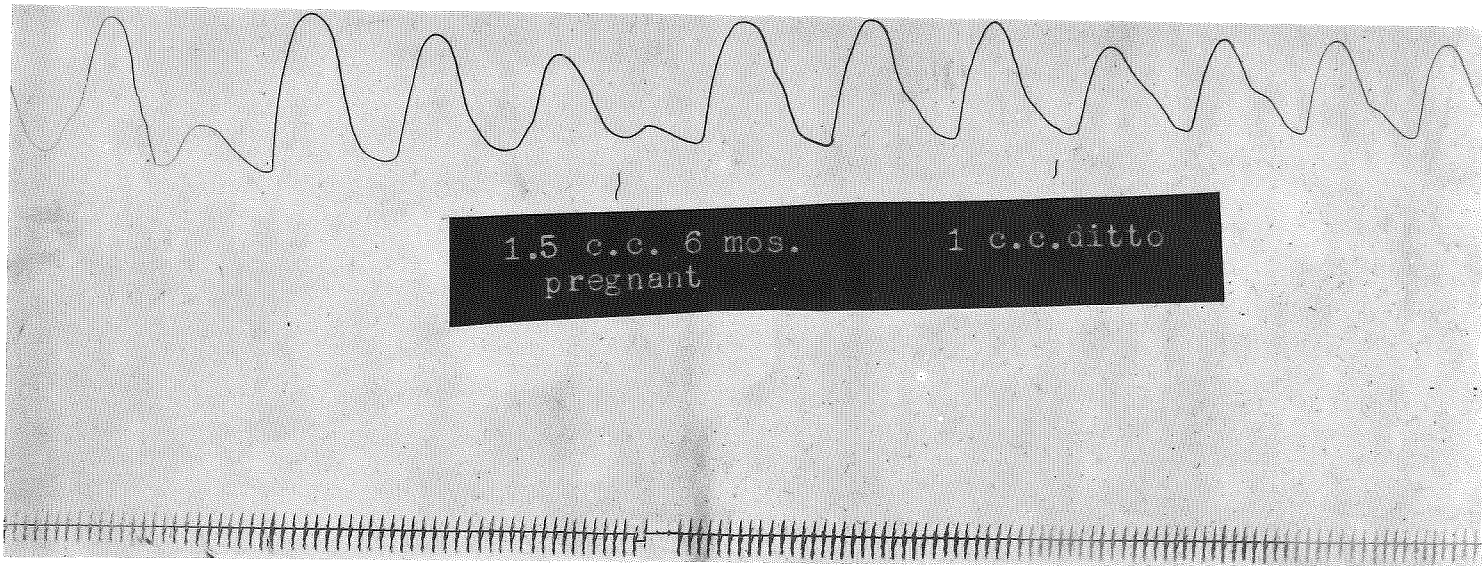
No.1.   Serum from patient 6 months pregnant added to Ringer Locke
        containing uterus.   No marked alteration occurred.   Each
   contraction took two minutes.

1.5 c.c.serum   Retractions
6 mos.pregnant
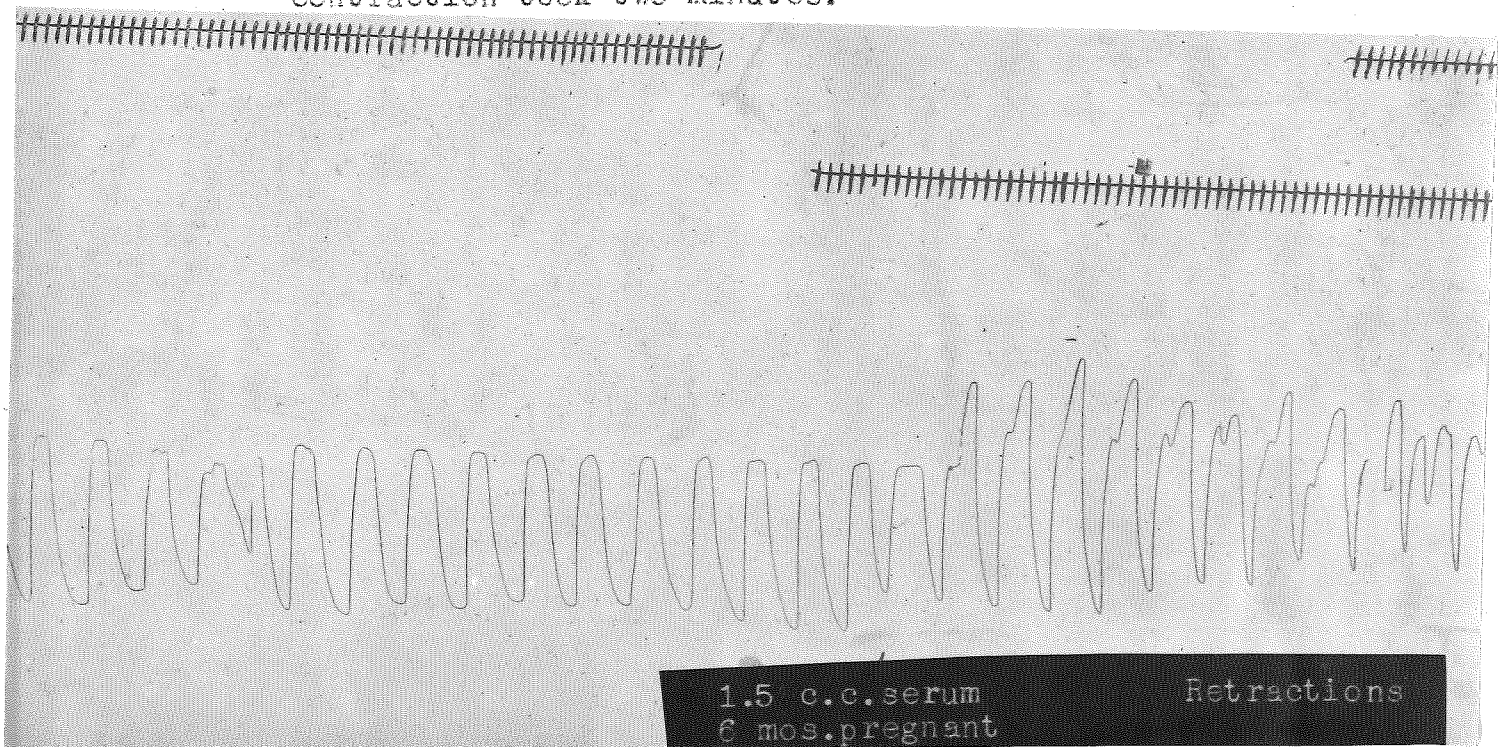
No.2.   Tracing of uterine contractions where contraction interval is
        46 seconds;after addition of serum from woman 4 months preg-
        nant the intervals lengthen to 55 seconds.   Contractions are
        accentuated and slight retraction occurs as shown by elevated
        base line.

1.5 c.c.serum
M.Roney 6½ mos.   1.5 c.c.ditto.
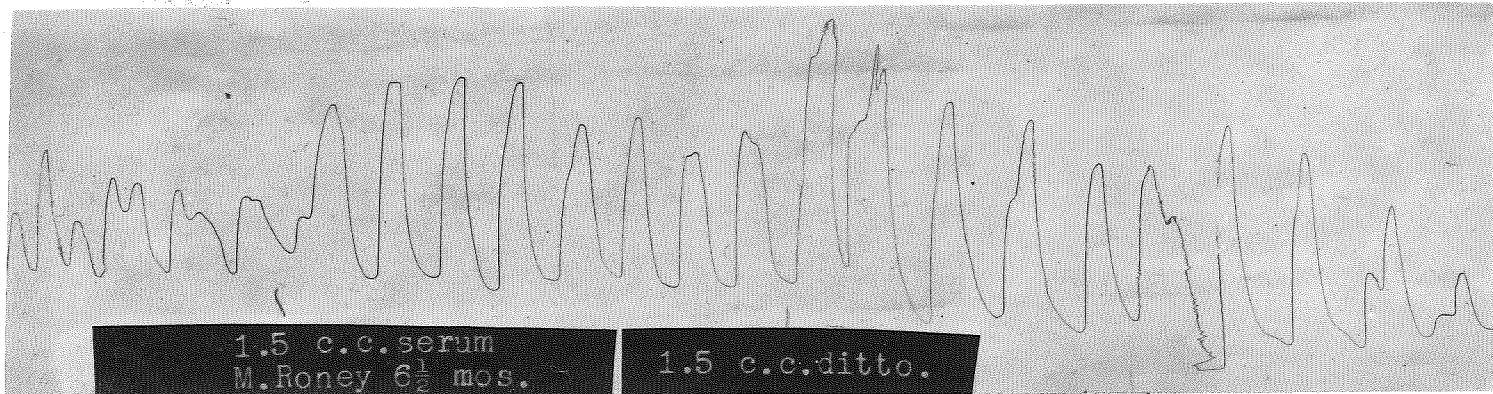
No.3.   Small normal contractions are here depicted and after addition
         of serum from a woman 6½ months pregnant the contractions are
         more marked but soon fall away until a second administration
         is made.   There is no retraction and it is again noted that
   the contractions tend to resume their normal state.

1.5 c.c.serum
7½ mos.   1.5 c.c.ditto   wash-out 1 unit pituitrin 4 units

No.4.   In this tracing normal large contractions are shown.   After
         the addition of 1.5 c.c. serum from 7½ months pregnant woman
         some contraction occurs, but soon goes off.   Subsequently
         another 1.5 c.c. are added and marked retraction occurs.
         After washing out the uterus further contractions occur of a
         more normal type.   1 I.N. unit of pituitrin produces similar
         results to the serum.   The large contraction and ready res-
         ponse may be due to the fact that the rat had been sensitised
         with the serum hormone.   This rat was used owing to depletion
         of virgin rats.   The contraction interval changes from 40 to
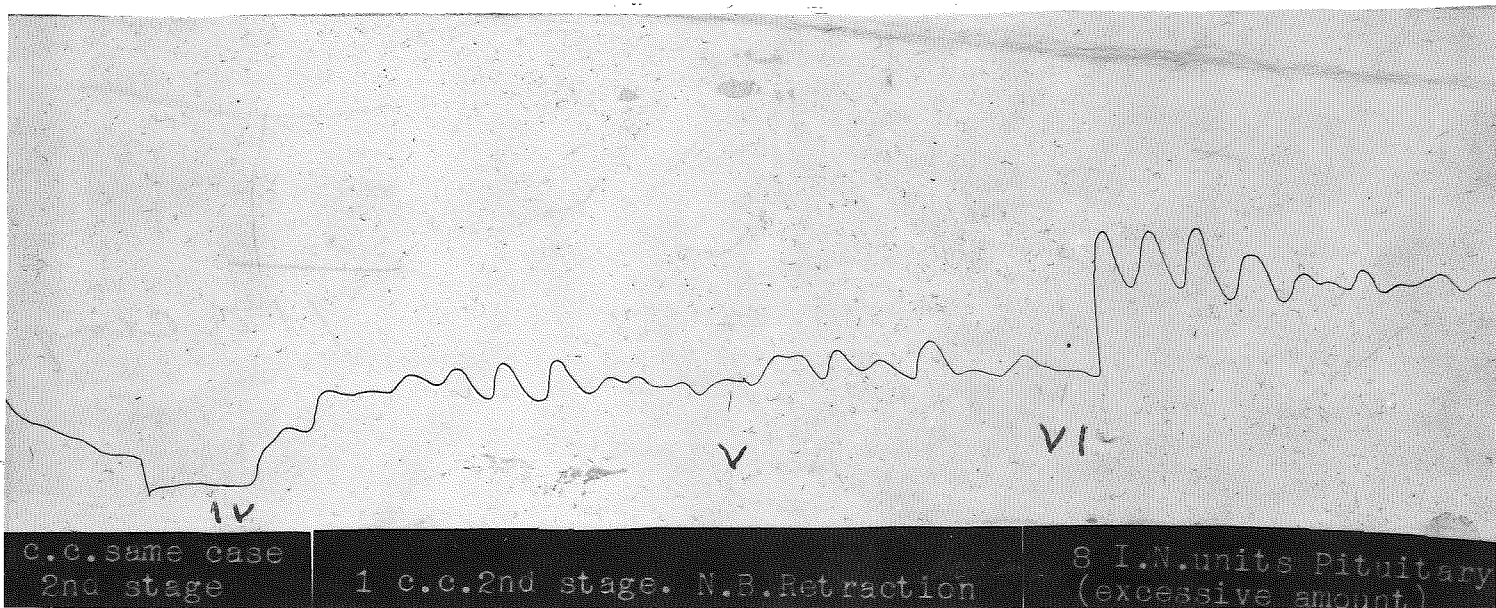   50 seconds.
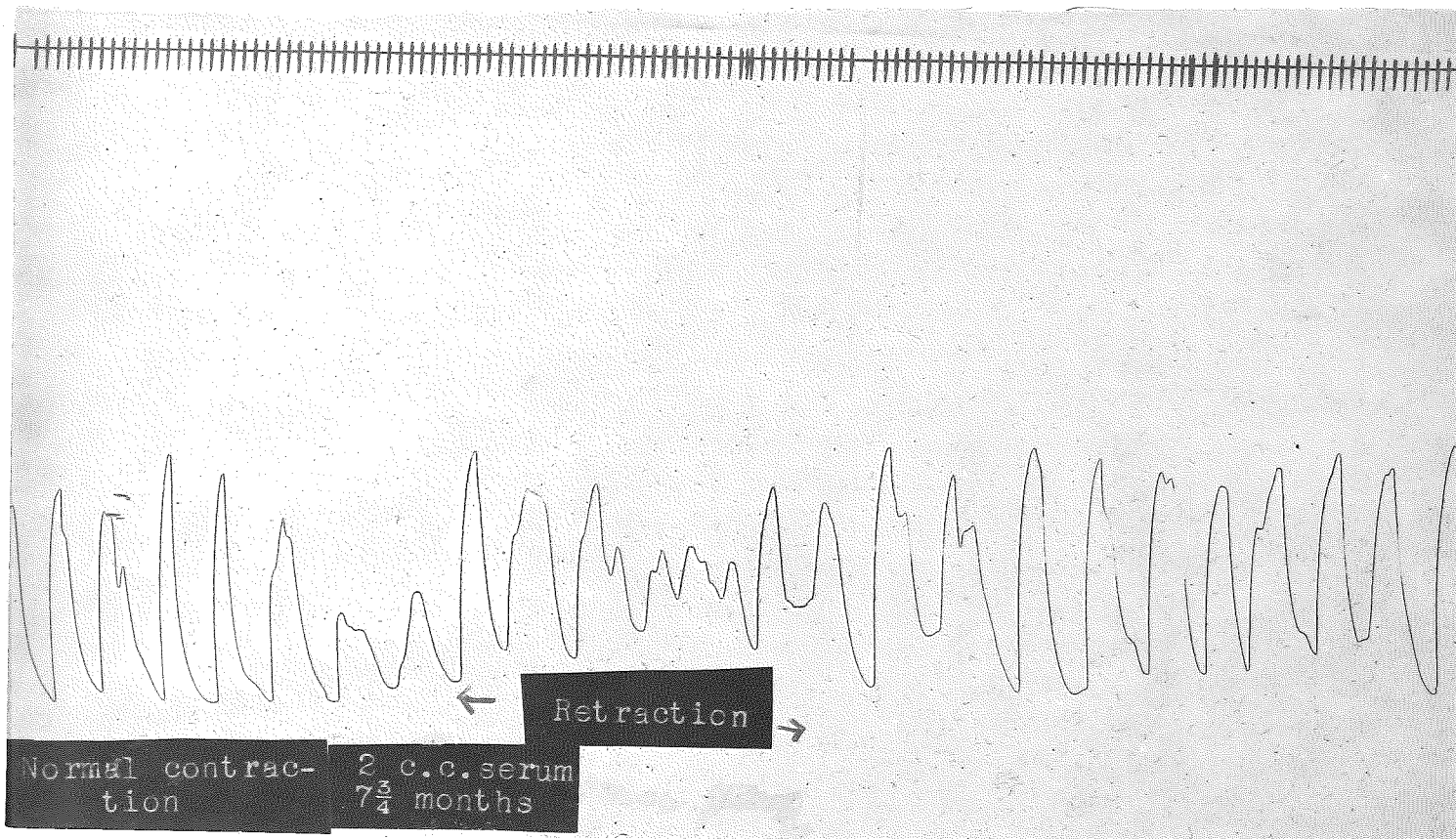
No.5.   Large contractions are here indicated and after adding 2 c.c.
of serum from a woman $7\frac{3}{4}$ months pregnant retraction is obser-
ved and the time interval changes from 50 to 66 seconds.
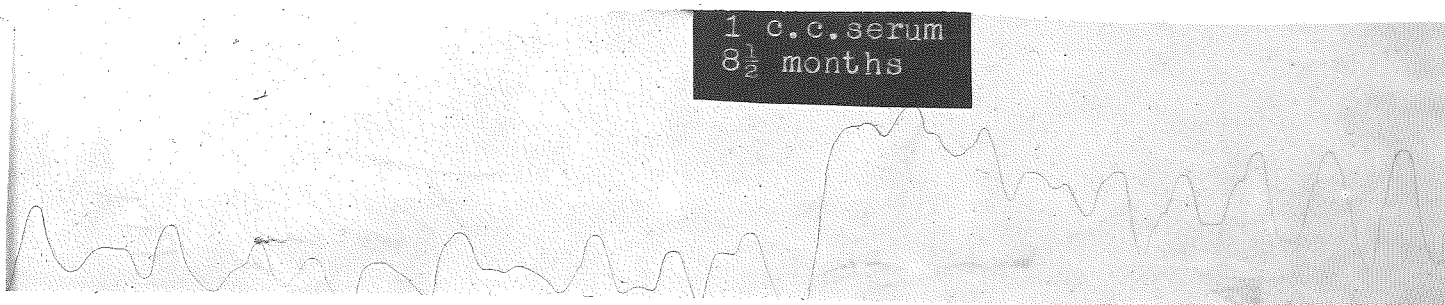
No.6.   This tracing indicates average contraction of the uterus until
1 c.c. of serum from a parturient woman advanced to $8\frac{1}{2}$ months
is administered, when marked retraction occurs.

1.c.c.serum 1st stage   1 c.c.serum 1st stage   Wash-out
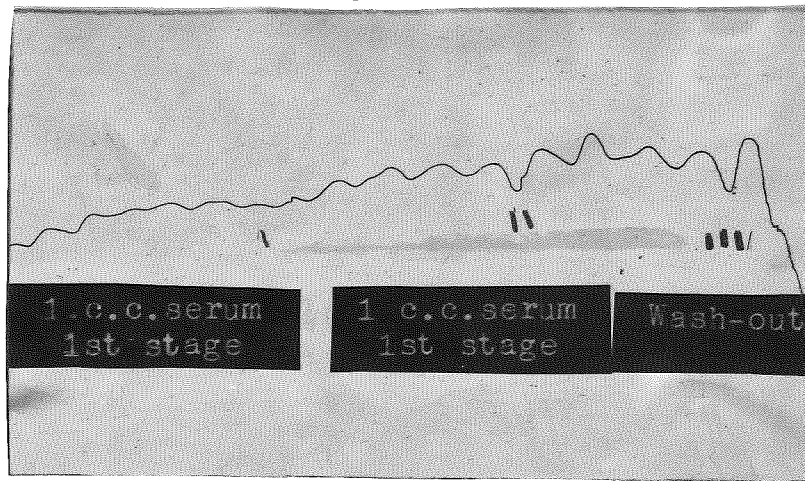
No.7.   Tracing depicts a poorly contracting uterus.   After addition
        of 1 c.c. of serum taken during first stage of labour there
        is some increase of contractility and retraction is marked.
        At II a second c.c. is given with further increase which re-
        mains until the Ringer Locke is washed out.   It is to be
   noticed that only 1 c.c. is used.

c.c.same case 2nd stage   1 c.c.2nd stage. N.B.Retraction   8 I.N.units Pituitary (excessive amount)

No.8.   Same uterus as No.7, and serum is from the same patient, but
        in the second stage of labour.   At IV 1 c.c. of second stage
        serum is used and definite retraction results.   At V. a
        further c.c. is added, with slight increase.   Retraction is
        well maintained.   At VI a very excessive amount of pituitrin
        was given, with still further retraction.   The comparison bet-
        ween the serum and pituitrin results are interesting.

1.5 c.c. serum 1st stage | 1.5 c.c. 1st stage | 5 I.N.units Pituitrin
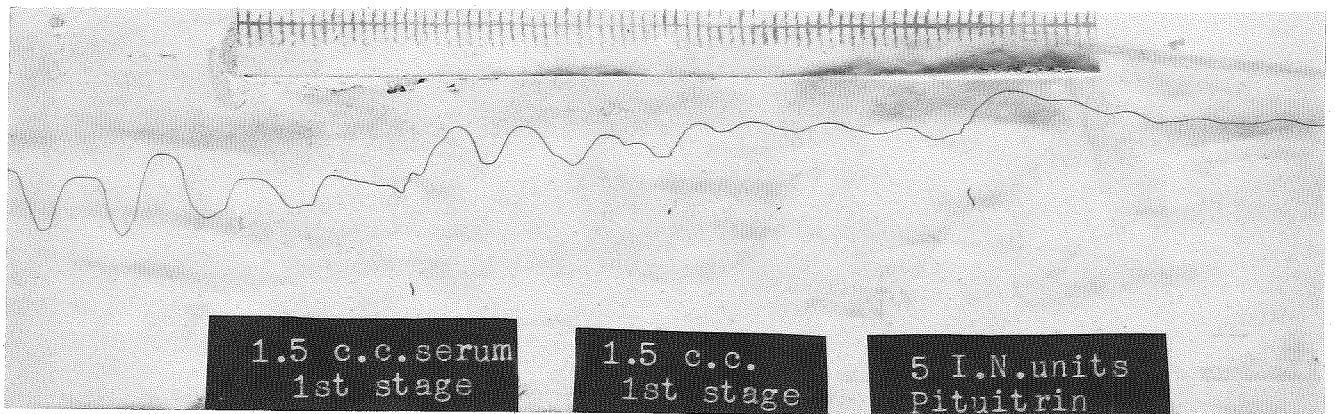
No.9.   This tracing shows the retraction which occurs into poorly
contracting uterus on addition of 1.5 c.c. serum, taken dur-
ing the first stage of labour.   Greater retraction occurs
on a further 1.5 c.c. being added.   Yet more pronounced re-
traction occurs from the addition of 5 international units
of post pituitary extract.


Poor contractions after Wash-out | 2 c.c.serum 1st stage
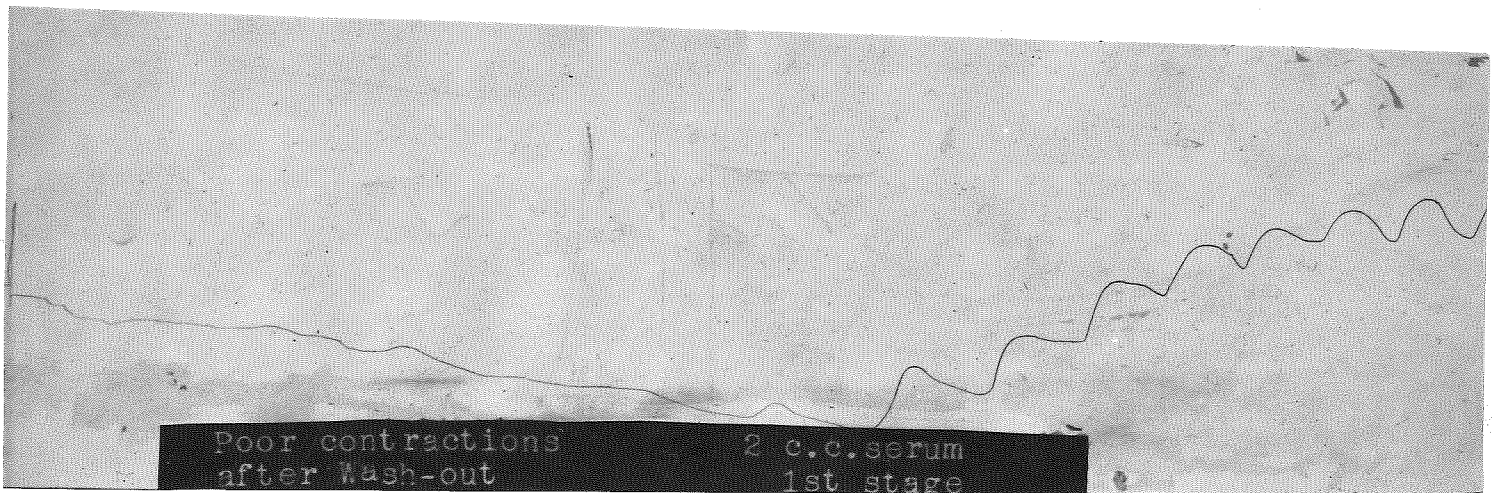
No.10.   Very few contractions are indicated on this tracing, but the
addition of 2 c.c. of serum from a subject in the first stage
of labour increases the contraction and produces retraction.

Normal contractions 1.5 c.c. serum 1.5 c.c. serum
of sensitised uterus   1st stage   1st stage
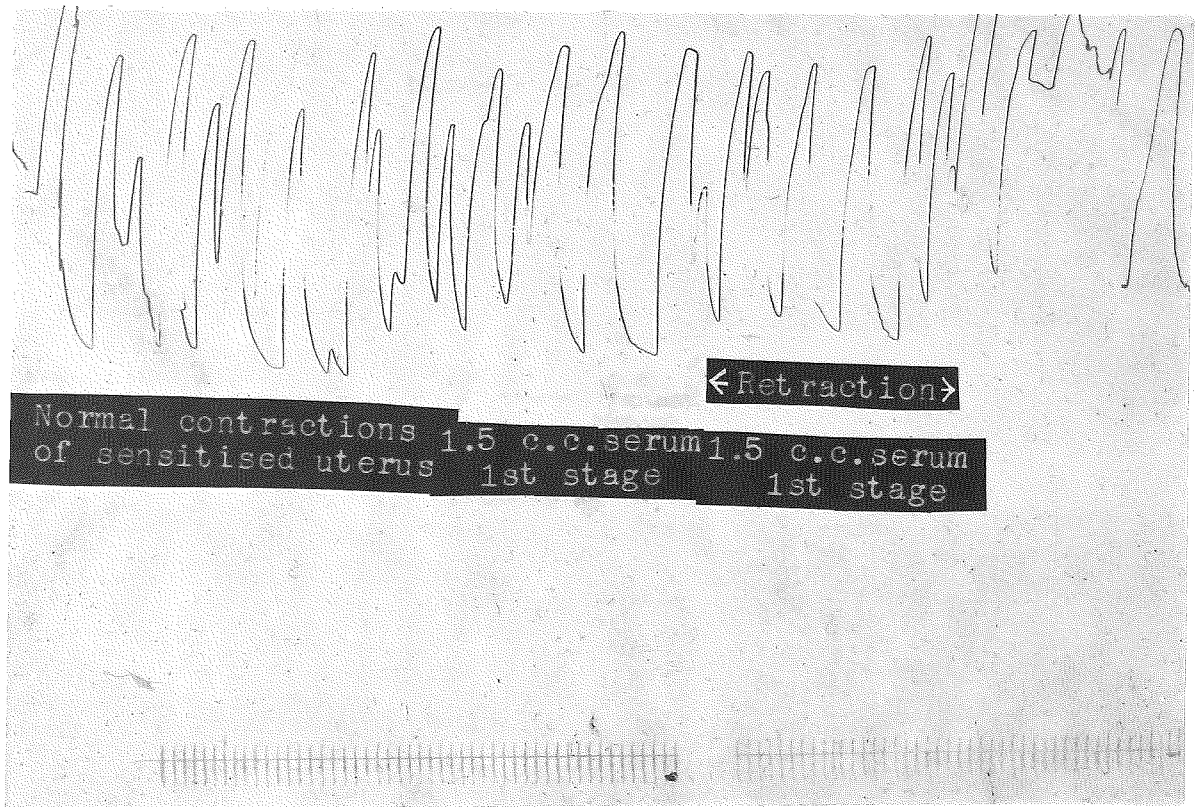
←Retraction→

No.12.   Exaggerated contractions are here represented and these are
due to injecting the rat with serum from pregnant women for
some days before it is destroyed.   The serum used in the
test is from a subject in the first stage of labour.   The
result of two 1.5 c.c. injections is slight retraction. The
same is observed in No.13 hereunder.

1.5 c.c.serum   1.5 c.c.serum
1st stage   1st stage
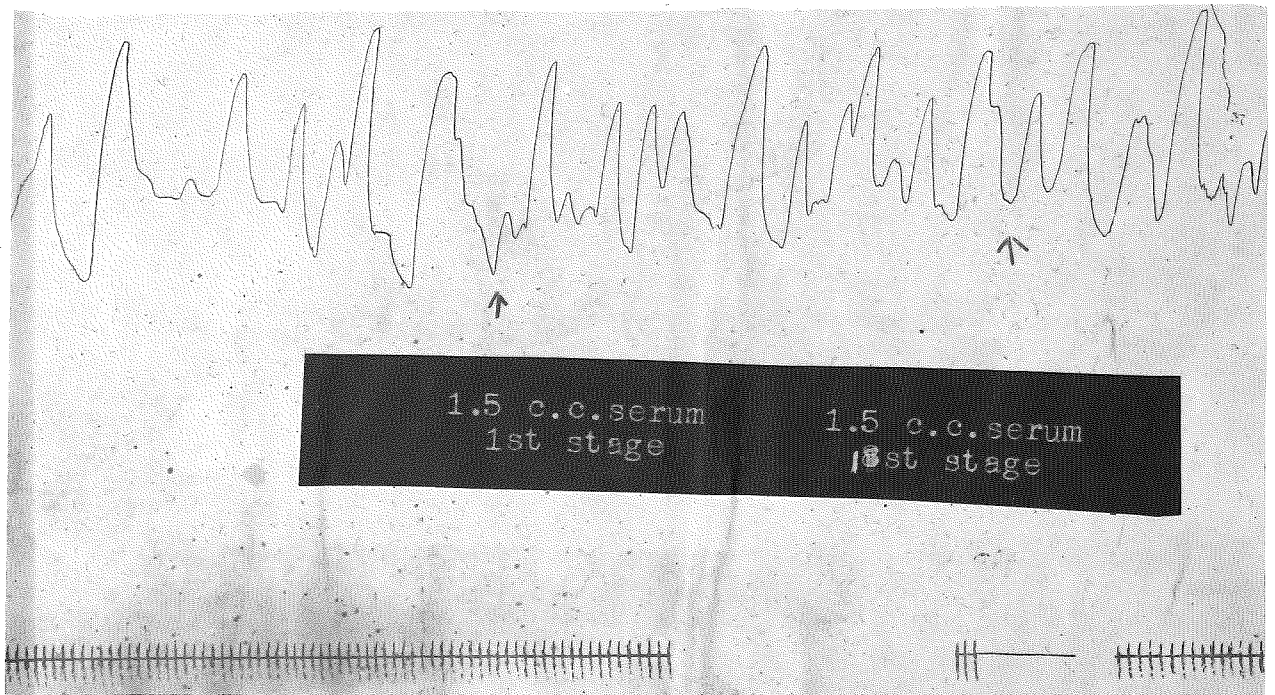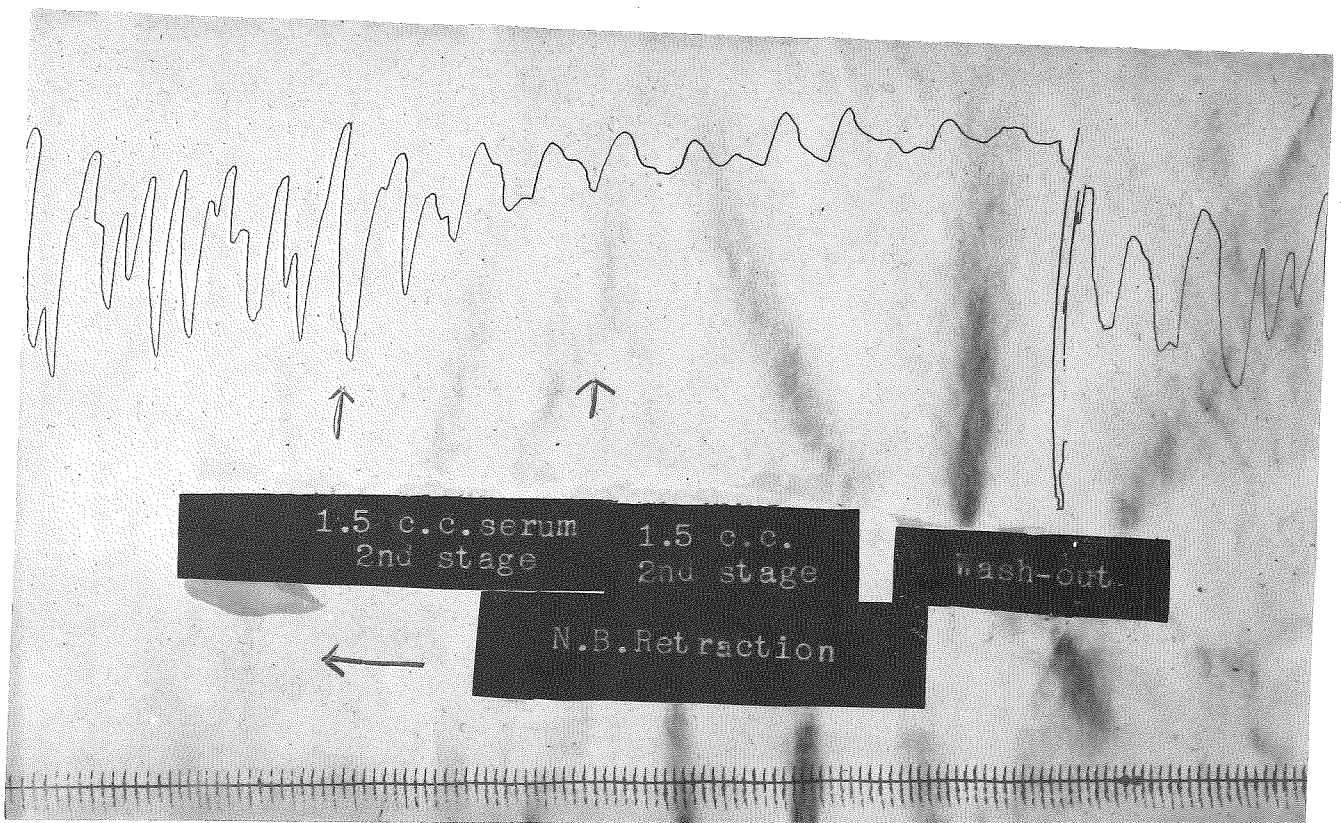
No.14.   This tracing determines very clearly the retraction produced by serum taken in the second stage of labour; 3 c.c. in all were given.
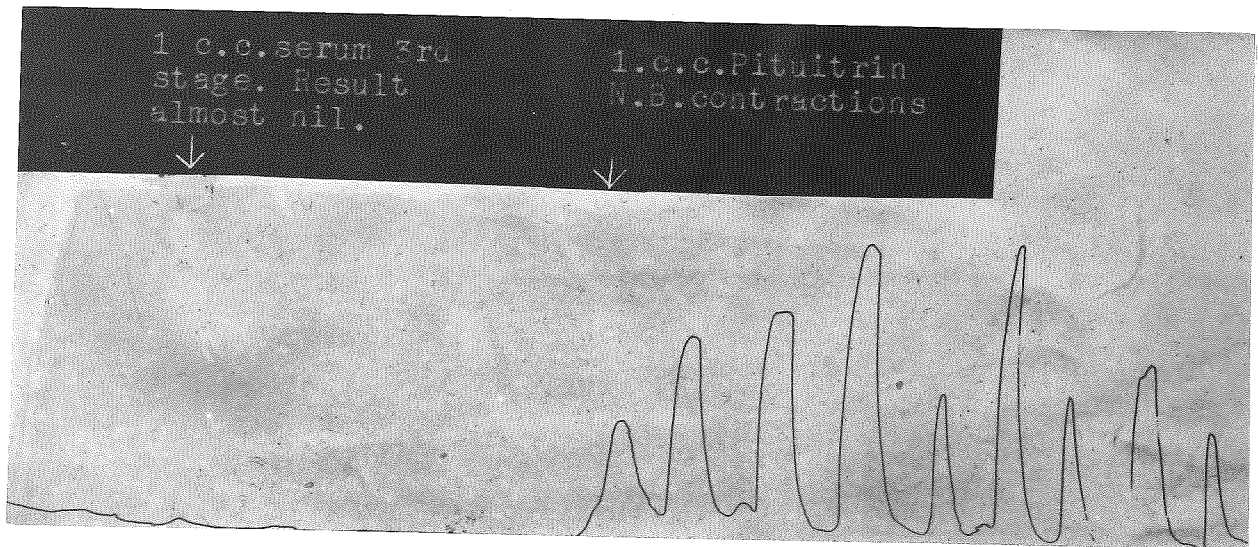
No.15.   Here is found the negative result of using serum taken from a patient after the third stage of labour.   There is only a perceptible result; 1 c.c. (large amount) of pituitrin provides merely contractions.   No retraction is noted.

.5 I.N.U.   .5 I.N.U.   4 I.N.Units
PITUITRIN.
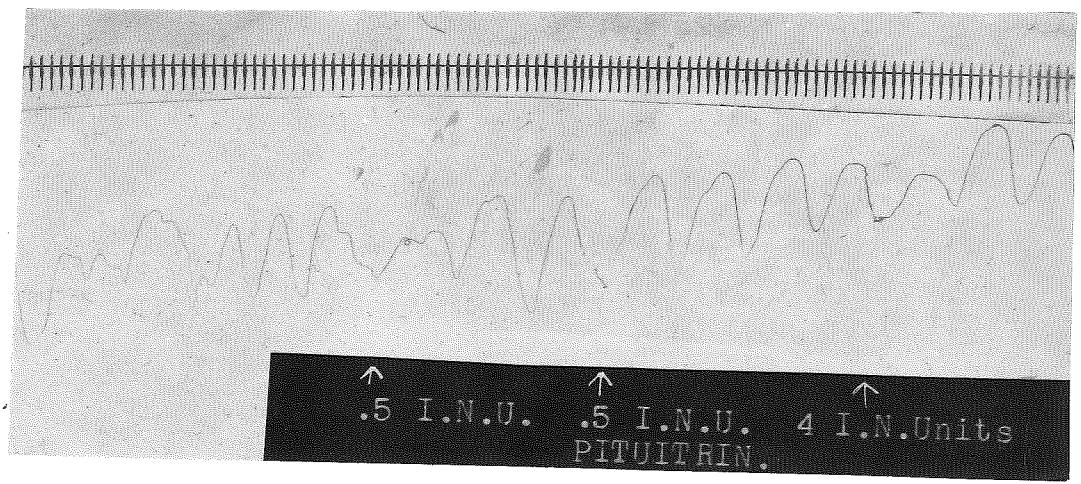
No.16.   Indicates the retraction effect of pituitrin in small doses.
No 17 (below) shows a very marked retraction following the
addition of .5 I.N. units of pituitrin.

Wash-out

.5 I.N.units Pituitrin
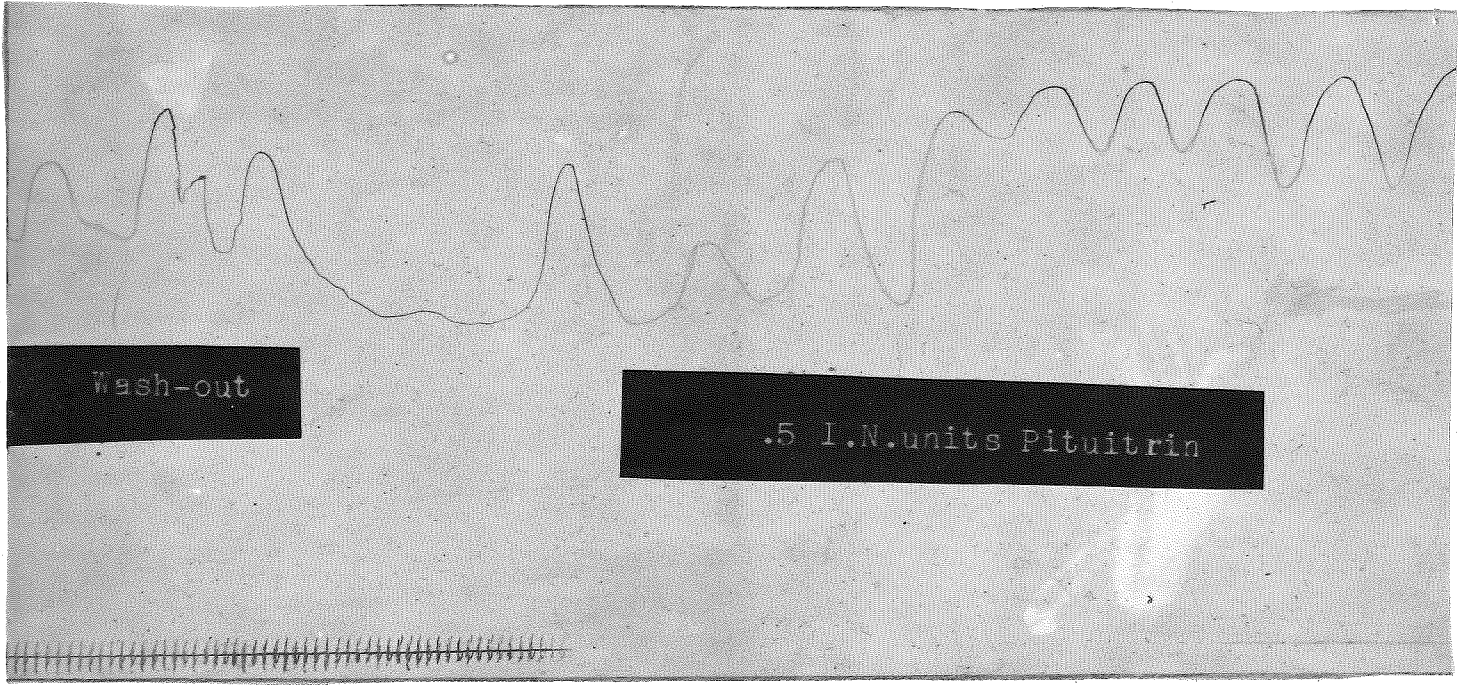
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INFLUENCE OF CORPUS LUTEUM IN MENSTRUATION AND PREGNANCY.

APPENDIX B.

Investigators of ovarian functions very early recognised in the Corpus Luteum a gland of internal secretion, but it remained for Prenant[1] in 1898 to postulate this theory.

During the last decade the main avenue of investigation has been towards oestrin, and in the course of these researches the follicular hormone has received most attention, while the corpus luteum has received the minor amount of consideration. Some work, however, in the main clinical, is here reported.

There have been many workers who have attempted to solve the activities of the corpus luteum and a fairly complete monograph was published by Matsumo[2] on this organ. The earlier workers Regaud and Policard[3] and Sanos[4], and later Cotte[5], Whitehouse[6] and Dawson[7] have given very clear statements regarding the relationship between the yellow body and the menstrual cycle.

Part of a translation of Matsumo's monograph is here given:- "The present day opinion is that the corpus luteum has monthly, by giving off specific substances, to transform the mucous membrane of the uterus into pregravid or premenstrual decidua. We know now, however, that the corpus luteum has to give only the final powerful impulse, for, according to the researches of Hitschmann and Adler[8] the utering mucosa even before follication rupture - and therefore long before the appearance of the corpus luteum - shows decidual reaction".

From the work of Halban and Kohler[9], since confirmed by Rensch[10], Seitz and Wintz[11], it is certain that the sorpus luteum exercises a definite influence upon the occurrence, intensity and

duration of the menstrual flow itself.

From his own investigations Matsumo says that:- "Removal of the corpus luteum during laparotomy is followed almost with certainty by a menstrual discharge after a lapse of two days".

Halban showed that the persistence of the corpus luteum - in the form of corpora lutea cysts - has a typical result of amenorrhoea, this is replaced by menstruation if the cyst be resected. When these researches were first instituted two cases of note had been seen. The first was at the Chelsea Hospital for Women, London, and the second at the Royal Infirmary, Edinburgh. In the first case the ovaries contained numerous luteal cysts and erratic luteal tissue was found throughout the ovaries. From this resulted an erratic decidual reaction which was found to be Chorio-Epithelioma. The ovaries of the second case were not unlike those of the first, and in this case an hydatiform mole was the result. The microscopical sections of the ovaries are not available for photographing.

Marshall and Jolly[12] extirpated the ovaries from pregnant dogs and rats. The pregnancy was discontinued in every case except one, in which a portion of the right ovary with the remains of two undoubted corpora lutea was found post mortem.

Beckwith Whitehouse[6] and Dawson[7] have shown that removal of the corpus luteum at operation has in each case been productive of menstruation very soon after the operation, and the same operation was shown by Cotte[5] of Lyons to produce menstruation. Cotte gives some twenty case reports of which two are given here:- (1) Woman aet.29. Regular menstruation every twenty-five days. L.M.P. May 24th to 27th. Operation June 11th, i.e. 16 days after last period began. Hysteropexy and excision of follicle with corpus luteum in

formation.   Result - Menstruated 13th June lasting three days.

(2) Woman aet.26.   Menstruation every 28 days.   L.M.P. 1st and

2nd April.   Operation April 21st (21 days after onset of last period).

Corpus luteum excised.   Result - Menstruated April 23rd.

In these present researches investigations have been mainly

on humans, but several rabbits were also used, and it was found that

the rabbits which were pregnant aborted/after excision of the corpus luteum,

although controls on whom operation was performed but the luteal tis-

sue left intact, did not abort.

One patient suffering from menorrhagia had her uterus cur-

etted in order to remove the thickened endometrium.   The ovaries

were temporally castrated by X-ray therapy and one menstrual period

allowed to pass to ascertain that menstruation did not occur.   After

this, extract of corpus luteum in oil solution was injected for three

weeks and then treatment was withdrawn.   This was followed by a slight

menstruation which did not recur at subsequent period times.   It is

considered that the corpus luteum extract built up the endometrium

into a pregravid state and when no more was given the luteal control

of the endometrium was lost and menstruation ensued.

Two cases of abortion were investigated.   Both patients

suffered from marked dyspareunia and subsequently at operation both

were found to have prolapsed ovaries.   One patient on whom operation

was performed soon after abortion, was found to have a prolapsed ovary

with a ruptured corpus luteum.   It is surmised that the rupture oc-

curred during coitus.

A patient who had been under observation off and on for

eight years, was a very fat nullipara who appeared to be of the pit-

uitary dystrophy type.   The patient first menstruated at 17 years

of age, but varied in her periods. Sometimes the interval would

as much as five months, when a haemorrhage would occur lasting perhaps

three weeks. The patient was given keratin-coated corpus luteum

extract tablets by mouth, on two occasions, and within two weeks in

each case marked menstrual flow ensued. It was considered that the

extra luteal influence produced sufficient congestion of the endome-

trium to permit menstruation to occur. On one occasion when the same

patient had been amenorrhoeic for over three months it was necessary

to operate for another condition. At the operation we removed a large

corpus luteum. The patient menstruated within two days.

Eight cases of amenorrhoea have been given corpus luteum by

mouth, and six cases subsequently menstruated. Two cases did not again

come under observation. The ages of these women were between twenty

and thirty years.

Administration of so-called "luteal extract" by mouth is

followed by some similar response, while oral exhibition of "ovarian"

extract produces no result. Possibly the activity of the former is

associated with its lipoid nature and corresponding resistance to di-

gestion. In debilitated cases, e.g. phthisis, etc., the corpora lutea

follicles have been shown to rupture normally, yet no menstruation oc-

curs. This is probably because of the failure of the corpus luteum

to build up the endometrium into a premenstrual state. The depletion

of fat generally affects also the lipoid tissue of the luteal secretion

and it is, therefore, possibly inert.

O'Donoghue[13] found that rupture of the follicles was not in-

variably followed by formation of the corpus luteum, and when corpora

lutea were not produced there was no growth of the mammary glands.

Two cases quoted by Matsumo are here given:- 1st case. "Wo-

man, about 44, has noticed for months a considerable increase in size

of the abdomen, combined with violent pains in the hypogastrium. The woman, who has had two confinements, had menstruated quite regularly till four months ago, since when complete amenorrhoea has existed. Objective finding, a myoma larger than a man's head and very sensitive to pressure; genitals otherwise normal. Operation is decided upon owing to pains, and by laparotomy supravaginal amputation of the uterus is performed. Both ovaries are macroscopically normal, no corpus luteum demonstrable. The adnexa are left. In the cavum recto-uterinum, on the posterior surface of the uterus corresponding roughly with the transition of the cervix into the corpus, is a body the size of a good bean, or irregular surface, greyish red colour, moderately firm, and fixed fairly firmly to the peritoneum of the uterus by several adhesions. This body, together with its uterine base, is excised and macroscopically, especially after section, gives the impression of a fairly old corpus luteum".

The histological examination (Professor Sternberg) gives the following finding:- "The greatest part of the preparation is taken up by a nodule composed of large, round or polygonal, light cells whose protoplasm is permeated by countless small interstices like a sieve and which possess a small readily stainable nucleus. These cells lie in a network of thick capillaries, on which for the most part they are situated in a perithelium-like fashion. On a small part of the circumferance of the nodule there runs near the surface and concentric with it a far broader, almost homogenous band, within which a few cell-nuclei are still visible, but the protoplasm of the cells is no longer distinguishable. At one pole of the nodule there is, in the latter, a spherical focus sharply marked off from its surroundings. This consists of homogenous, twisted bands staining, with eosin, regularly

red like hyaline.   In the immediate neighbourhood of this spheric
focus are extensive accumulations of a coarsely granular, dark yel-
lowish brown pigment, which gives intense iron reaction.

This nodule is imbedded in a loose cellular tissue trav-
ersed by very numerous and thick vessels.   In this tissue there are
also found near the surface a few fasiculi of smooth muscle.   The
surface is covered with a regular layer of cubical or flat cells.

If this nodule is followed up in an unbroken series of
sections, it appears that it has roughly a spherical form and shows
the above-described structure throughout its whole extent.   Both the
spherical focus composed of hyaline bands and the hyaline band running
near the surface can be followed up through a very large number of
sections.   In the surrounding tissue there also occur in the deeper
parts large, thick-walled, arterial vessels.

The histological examination then shows that the piece of
tissue examined contains a corpus luteum with well-developed lutein
cells.   In one of its lobes complete involution, the development of
a corpus fibrosum had incurred, and likewise in an extensive part near
the surface incipient involution was demonstrable.   In the tissue
bordering on to the corpus fibrosum were large layers of blood-pigment
as the remains of haemorrhage that had occurred here.

It is therefore certain that the <u>formation on the posterior
surface of the uterus was a prolapsed corpus luteum</u>.   The microscopi-
cal examination shows that it was in a regressive stage in which how-
ever numerous functional elements are still demonstrable.

The case is noteworthy in two respects: Firstly, because
it shows a complete prolapse of the corpus luteum, an occurrence which
in itself is not very frequent.   Still more rarely, however, in such

cases does an <u>implantation of the corpus luteum in the abdominal cavity</u> occur, for as a rule absorption would previously occur. We are of course not able to say with certainty how old the corpus luteum in our case was, still the fairly firm adhesions suggest with great probability that the healing must have already occurred some time before. Accordingly we shall probably not go far wrong if we connect the patient's amenorrhoea of several months' standing with this unusual finding, for she had previously always been quite regular in her menstruation. We may thus assume that the corpus luteum persistens acted like a permanent implantation and prevented the onset of the menstrual flow".

2nd <u>Case</u>. In January 1923 a 21-year old patient was admitted on account of severe haemorrhage. The <u>first</u> menses occurred at the age of 20 in January 1922, and lasted 3 days. Three weeks later the <u>second</u> menstruation. The next two months the patient was amenorrhoeic, until at the end of March the <u>third</u> not very profuse menstruation occurred, lasting 4 days. The <u>fourth</u> menses set in in the middle of April, painless as before, and lasted 4 days. On 28th June likewise normal menses of 4 day's duration. <u>Thenceforth the patient was amenorrhoeic till August</u>. From August there occurred at intervals of 4 - 14 days profuse haemorrhages which pulled down the patient and caused her to seek the hospital. She feels extremely limp and has violent headaches and giddiness.

<u>Gynaecological finding</u>: Uterus small, antev.flect.movable, hard, on the right side and in front of the uterus a movable, smooth firm tumour larger than a plum. On the left and behind the uterus a humpy, firm tumour scarcely as large as a plum and extremely sensitive to pressure. Diagnosis: Fibroma of the ovaries. <u>Operation</u>, 26th January 1923. Median laparotomy. After opening the abdominal

cavity the uterus appeared of normal size.   Right ovary transformed
into a firm body of humpy surface, about 8 cm. long, 5 cm. high, 2 cm.
thick.   To the right of this lies a cystic tumour about the size of
a mandarin, over which the normal right tube passes.   The left ovary
is transformed into a hook-shaped, lobed body, whose two shanks are
each about 8 cm. long and 2 cm. broad.   In the opinion that a malig-
nant neoplasm of the ovaries was present, both ovaries, in spite of
the youth of the patient, were extirpated, likewise the shrunken appen-
dix, which lay close to the right ovary.   Peritonealization of the
stumps, closure of the abdominal covering in three stages.

        Course, with the exception of an abscess of the abdominal
covering, normal.   Re-examination on 1/3/1923 gave nothing abnormal.
Since the operation the patient has had no more haemorrhage, but
suffers from headaches and occasionally from congestion.

        In this case then, in distinct contrast to the first, we
have irregular haemorrhages and the examination of both ovaries, which
were in a high degree fibrous, shows the complete absence of a corpus
luteum.   Here too we may well connect these two factors and must
assume that owing to the lack of corpus luteum function the menstrual
discharges have not experienced the usual inhibition.   Both cases,
therefore, would furnish an illustration of the correctness of the
view that the corpus luteum exercises an inhibitory influence upon
the onset of the menstrual flow, that with an excess of this function
as in corpus luteum persistens the result is atypical amenorrhoea,
with an absence of hypo-function atypical haemorrhages.

        These statements rather contradict Bland Sutton's[14] edict –
"concerning the cause significance and utility of menstruation we know
nothing".

CONCLUSIONS

1.   It is thought that:-

1. The luteal tissue in the ovary stimulates endometrial preparation for the embedding of the ovum.

2. The corpus luteum. which is controlled by the ovum, produces a hormone which retains the endometrium in a pregravid state.

3. Death of the ovum means atrophy of the corpus luteum, which in turn means atrophy and exfoliation of the endometrium which in the non-gravid means menstruation, and in the gravid abortion.

4. Luteal influence induces mammary development.

5. Ovulation occurs about the fourteenth day of the menstrual cycle and if the ovum be fertilised between this and the twenty-third day day the influence of the corpus luteum remains to retain the endometrium in a state of decidual production.

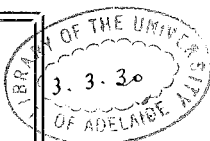6. Internal secretory function of the corpus luteum prevents ovulation during pregnancy.

Ovarian Malignancy with Special Reference to Krukenberg Tumours.

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