THE SIGNIFICANCE OF THE LUTEAL ACTION ON THE UTERINE MUSCLE IN THE MAINTENANCE OF GESTATION AND INITIATION OF PARTURITION.

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THE development of gestation is accompanied by certain definite alterations in the reactivity of the uterine muscle to oxytocin. In the early stages this posterior pituitary lobe hormone has no effect on the uterus, but later the sensitivity of uterine muscle to oxytocin develops, gradually increases, and reaches a maximum at parturition when very small doses of the hormone are capable of causing contraction. This is true both of the rabbit and of the human subject [Robson, 1933 *a, b*]. During the early stages of the puerperium, moreover, the reactivity of the muscle rapidly falls. That a hormone secreted by the corpus luteum plays a part in determining the inhibition of the reactivity of the uterus to oxytocin has been satisfactorily demonstrated both by Knaus [1930] and by Robson and Illingworth [1931], for the injection of certain preparations of the corpus luteum into non-pregnant animals will bring about an inhibitory effect similar to that observed in gestation. Later evidence, however, suggested that the mere withdrawal of the action of the luteal hormone is not sufficient to account for the high reactivity existing at parturition, and it appeared likely that the œstrous hormone might be an important factor in determining the alterations in the physiological state of the uterine muscle during gestation [Robson, 1933 *c*]. The experiments described in this paper were undertaken in order to study further the relation of the luteal secretion to the uterine changes and the factors responsible for controlling the development of uterine activity during the

later stages of pregnancy. They may conveniently be described under two headings, namely:

(1) Changes in uterine reactivity following the cessation of luteal secretion in pseudo-pregnancy, and

(2) Changes in uterine reactivity following the removal during pregnancy of the fœtuses with or without the placentæ.

METHODS.

All experiments were performed on mature female rabbits weighing about 2 kg.

The determinations of the *in vitro* reactivity of the uterus were performed as in previous investigations [Robson, 1933 a].

A purified preparation of the oxytocic hormone (pitocin) kindly supplied by Parke, Davis and Co. was used.

RESULTS.

Experiments on pseudo-pregnant animals.

Pseudo-pregnant animals were obtained by four methods, namely:

(1) By mating with vasectomized bucks.

(2) By implanting bovine anterior pituitary tissue together with an antiseptic, "quinanil."

(3) By the injection of an alkaline extract from bovine anterior pituitary.

(4) By the intravenous injection of a gonadotropic extract prepared from the urine of pregnant women.

As has been shown previously [Robson, 1932] the procedures described under (2), (3) and (4) result in alterations in the uterine musculature and endometrium similar to those observed in normal pseudo-pregnancy. In all experiments the existence of pseudo-pregnancy was confirmed by laparotomy and observation of the ovaries.

At various stages after the onset of pseudo-pregnancy the animals were operated on and strips of uterus were removed and their reactivity to oxytocin was determined in the usual way, the minimum dose of the hormone necessary to bring about a motor effect being accurately established. The results are collected in Table I. Since pseudo-pregnancy lasts for about 15 days, a number of observations was taken immediately afterwards over a period extending from the 16th to the 19th day. The results thus obtained show that although the cessation of pseudo-gestation is accompanied by a return of the uterine reactivity to oxytocin,

TABLE I. Showing the reactivity to oxytocin of uterine strips removed at various stages after the onset of pseudo-pregnancy.

For explanation of numbers in col. 2, see text.

Animal No. Ra	Methods of induction of pseudo-pregnancy	Reactivity units	Days after onset	Reactivity units	Days after onset	Reactivity units	Days after onset	Reactivity units	Days after onset	Reactivity units	Days after onset
388	(1)	0·5	7	—	—	—	—	—	—	—	—
337	(1)	—	—	0·2	18	—	—	—	—	—	—
366	(1)	<1·0	9	—	—	—	—	—	—	—	—
394	(3)	—	—	0·03	18	0·02	25	0·02	31	—	—
406	(2)	—	—	0·1	16	0·04	20	0·05	28	—	—
407	(2)	—	—	0·02	18	0·02	22	—	—	0·1	35
411	(2)	—	—	—	—	0·05	22	—	—	>0·3	34
412	(2)	—	—	>0·3	19	—	—	0·04	27	0·5	35
420	(4)	—	—	—	—	—	—	0·05	30	0·05	37
421	(4)	—	—	—	—	—	—	0·02	30	0·02	38

the doses of the hormone necessary to cause a motor effect are not particularly small, varying from 0·02 unit to a dose greater than 0·3 unit in a 100 c.c. of solution. Similar observations made at later periods also show that in no case did the muscle react to a dose of oxytocin smaller than 0·02 unit. Experiments were also performed at a later period between the 27th and 31st days after the beginning of pseudo-pregnancy, and thus corresponding to the time when parturition would have occurred had the animals become truly pregnant, but no further increase in the uterine reactivity was observed.

Incidentally it will be seen that the early stages of pseudo-pregnancy are not necessarily always accompanied by a complete loss of reactivity of the uterine muscle to oxytocin; though it must be emphasized that the two cases (Ra 366 and 388) which illustrate this point are exceptional.

The spontaneous rhythmic activity exhibited by the strips *in vitro* was also determined and the results are shown in Table II. In order to provide a basis of comparison with the findings during pseudo-pregnancy, data obtained during that period under exactly similar conditions are included in the table. (These latter experiments were described in a previous communication [Robson, 1932], in which, however, the spontaneous activity of the muscle was not given.) Further, the results obtained in rabbits during pregnancy [see Robson, 1933 a] are also given as they will be referred to in the discussion.

The spontaneous rhythmic activity of the uterine strips removed in the early stages of pseudo-pregnancy (3rd–7th days) is, on the whole, rather less than that observed later on (12th–14th day). But the cessation of pseudo-pregnancy (and hence of the specific luteal secretion) is not associated with any increase in the *in vitro* contractions of the uterine

TABLE II. Showing the spontaneous rhythmic activity of uterine strips removed at various stages after the onset of pregnancy and pseudo-pregnancy.

Animal No. Ra.	Condition	Spontaneous activity	Days after onset	Spontaneous activity	Days after onset	Spontaneous activity	Days after onset	Spontaneous activity	Days after onset	Spontaneous activity	Days after onset	Spontaneous activity	Days after onset
169	Pseudo-pregnant	+ +	5										
188	,,	+ − + +	5										
191	,,	+ − + +	3										
197	,,		3										
186	,,		6		12	+	16						
189	,,	−			14								
190	,,		6		12								
199	,,				12								
203	,,	+			11								
222	,,		3	+	14								
223	,,			+	12								
388	,,	+	7		13	+ + − · + + + +	18	+ +	25				
337	,,				12			+ +	20				
366	,,			· +	9	+ +		+ + − + +	22	+	31		
394	,,					+ +	18		22	+ +	28		
406	,,					+ + +	16	+					
407	,,					+	18	· +					
411	,,					+ + − · + + + +	19	· + + +		· + + + +	27	+	34
412	,,									+ + + +	30	+ − + +	35
420	,,							+		+ +	30	+ +	37
421	,,											+ +	38
334	Pregnant					+ + + + +	18	+ + +	25				
336	,,					+	18	+ +	21	+	27		
338	,,					+ + + +	18	+ ·	23				
335	,,					+	19						
346	,,						19	· +	25	+			
342	,,							· + +	25	· + + +	27	+	32
342	,,							+ +		+ + + +	30	+	
343	,,									+	31		32
340	,,									− − + + +	28	+ +	38
389	,,									− + + +	31	+ +	37
392	,,										32		
339	,,									− − + + +	33		
371	,,											+ +	35
352	,,											+ + + + +	39
368	,,												37
355	,,											+	37
364	,,												39

muscle which 16–19 days after the beginning of pseudo-gestation are not any more marked than at the earlier stage from the 12th to 14th day. Nor is there any increase at a still later period (22nd–25th days). Very high values for the spontaneous activity were, however, observed in three cases when the strips were removed 27 (one case) and 30 (two cases) days after the induction of pseudo-pregnancy.

Effects of removal of the fœtuses and placentæ.

In eight animals the fœtuses and placentæ were removed at various stages of pregnancy, from the 19th to the 27th day, and the condition of the uterine muscle determined at subsequent periods. The operations were performed with aseptic precautions; small incisions only were made in the uterus and the contents withdrawn; retraction of the muscle reduced the size of the aperture made, so that it could be closed by a single suture. An attempt was also made to remove the fœtuses alone, leaving the placentæ *in situ*, according to the technique first originated by Weymeersch [1912]. When this was performed fairly late in pregnancy, but before the 24th day of gestation, the animals occasionally survived (Ra 395 and 397); similar operations at later stages of pregnancy, however, invariably resulted in the death of the animals, usually within 24 hours. The impression was gained that death had resulted from the absorption of toxic substances from the placentæ which showed signs of degeneration. Mr Hammond very kindly suggested certain precautions which might be taken in the operation, but in spite of these (including strict asepsis) it was impossible to keep the animals alive when the fœtuses only were removed at the later stages of pregnancy.

The reactivity to oxytocin and spontaneous rhythmic contractions of animals in which the uterine contents were removed are given in Table III. An examination of the data shows that in no case was the reactivity to oxytocin particularly high, and that frequently it was very low. Such a result was obtained not only when the interval between the operation and the subsequent experiment was short (24 hours) but also when it was more prolonged. Thus, for example, in Ra 426 uterine strips were removed on the day after the operation and showed a reaction to 0·5 unit of oxytocin. In Ra 402, 2 days after the removal of the uterine contents the muscle was unaffected by 0·5 unit of the oxytocic hormone, and the same dose was just sufficient to cause a contraction of the isolated uterine muscle in animal Ra 349 several days after the removal of the uterine contents.

These results show that the increase in reactivity to oxytocin which occurs during normal pregnancy and culminates in parturition no longer

TABLE III. Showing the reactivity to oxytocin and the spontaneous rhythmic activity (S.A.) of uterine strips, following removal of uterine contents in gestation.
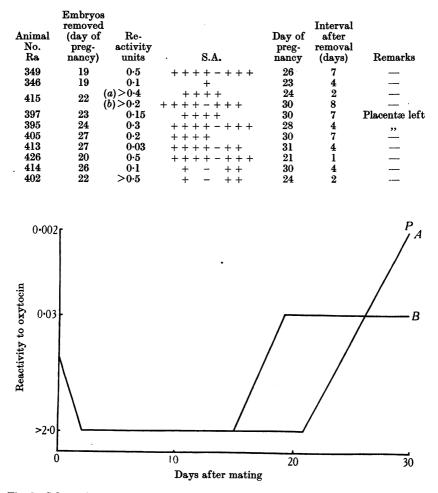
Animal No. Ra	Embryos removed (day of pregnancy)	Reactivity units	S.A.	Day of pregnancy	Interval after removal (days)	Remarks
349	19	0·5	+ + + + − + + +	26	7	—
346	19	0·1	+	23	4	—
415	22	(a) >0·4	+ + + +	24	2	—
		(b) >0·2	+ + + + − + + +	30	8	—
397	23	0·15	+ + + +	30	7	Placentæ left
395	24	0·3	+ + + + − + + +	28	4	,,
405	27	0·2	+ + + +	30	7	—
413	27	0·03	+ + + + − + +	31	4	—
426	20	0·5	+ + + + − + + +	21	1	—
414	26	0·1	+ − + +	30	4	—
402	22	>0·5	+ − + +	24	2	—

Fig. 1. Schematic representation of the uterine reactivity to oxytocin (expressed as the minimal number of units added to 100 c.c. of Ringer-Locke which causes a motor effect) at various stages of pregnancy and of pseudo-pregnancy. A, pregnancy; B, pseudo-pregnancy; P, parturition.

takes place after removal of the fœtuses and placentæ. This is brought out especially by an examination of the data obtained from strips removed at a time when parturition would have occurred if the uterine contents had not been disturbed. Thus in four cases in which uterine strips were

removed on the 30th day after mating (the uterine contents being removed on the 22nd–27th day of gestation), the dose of oxytocin necessary to cause contraction of the muscle was 0·1 unit or more. In one animal in which the fœtuses and placentæ were removed on the 27th day of gestation, the uterine muscle responded to a concentration of 0·03 unit of oxytocin on the 4th day after the operation.

An attempt was also made to determine whether the presence of the placentæ alone would be sufficient to allow of the development in reactivity observed in normal pregnancy. Two animals, in which the fœtuses alone were removed on the 23rd and 24th day respectively, survived the operation, and uterine strips were subsequently taken out (on the 30th and 28th days after mating), but though the placentæ had not been expelled, the minimum doses of oxytocin necessary to cause contraction of the muscle were still comparatively high. Thus in animal Ra 397 in which the fœtuses were removed on the 23rd day of gestation, the reactivity of the uterine muscle 7 days later was equal to 0·15 unit of oxytocin in 100 c.c. of solution, while in Ra 395, 0·3 unit of oxytocin was the minimal dose of the hormone which caused a motor effect on the uterine muscle 28 days after mating and 4 days after removal of the fœtuses.

The spontaneous rhythmic activity exhibited by the strips *in vitro* (Table III, col. 4) was, in seven out of the ten experiments, very high and equal to that observed during parturition (see Table II). In the other three animals (Ra 346, 402, 414), however, the rhythmic activity was low or medium. There appears to be no relation between the contractions exhibited by the muscle strips and the interval elapsing between the removal of the uterine contents and the subsequent determination of the state of the muscle.

DISCUSSION.

The experimental procedures used in this investigation have allowed of the determination of the state of the uterine muscle following a period of activity of the corpus luteum under two conditions, namely:

(1) Following a period of luteal activity not associated with pregnancy, and

(2) After the cessation of luteal activity occurring under completely normal conditions in a normal though incompleted gestation. In these experiments the pregnancy was interrupted by removal of the uterine contents and this, as Hammond [1917] has clearly shown, results in the degeneration of the corpus luteum of pregnancy and in cessation of its secretory activity.

If the state of the uterine muscle associated with parturition were dependent purely on a withdrawal of the luteal function, then it would be expected that a similar state would be observed when the luteal activity was interrupted under other conditions. Now the state of the uterine muscle at parturition, as far as the reactivity to oxytocin is concerned, can be expressed fairly accurately. In the rabbit the dose of oxytocin necessary to bring about a motor effect on the isolated strips at that stage varies from 0·001 to 0·01 unit in 100 c.c. of solution. It may be added that similar doses are effective, not only on the parturient human uterine muscle [Robson, 1933 b] but also on the uterine muscle of the mouse at parturition and at no other stage of gestation. (It is hoped to present details of these findings in the near future.)

An examination of the results obtained in this paper shows definitely that in no case was cessation of luteal activity under any conditions (except normal pregnancy) associated with a rise in the reactivity of the uterine muscle similar to that observed at parturition. Considering first the results obtained after a period of pseudo-pregnancy, it is found that the inhibition of the reaction to oxytocin observed during that period rapidly disappears, this process being dependent upon the cessation of secretion of the corpus luteum hormone. The height of the reactivity attained, is, however, never the same as that seen at parturition. For the purpose of comparison, the data of uterine activity following sterile and non-sterile mating are diagrammatically represented in Fig. 1 which shows that, though after a sterile mating the sensitivity of the uterus to oxy-tocin begins to increase at about the 15th day, and thus several days before a similar phenomenon is observed in true pregnancy, yet the degree of sensitivity developed by the muscle following pseudo-pregnancy is never as high as at parturition. The findings lead to the conclusion that, in pregnancy, the luteal activity, in as far as the action on the uterine muscle is concerned, is more prolonged than during pseudo-pregnancy, but that nevertheless it does not extend to the end of gestation. This conclusion is also supported by definite evidence that the inhibitory luteal activity during gestation begins to decrease even before the reactivity to oxytocin appears, for vaso-pressin is effective in causing contraction of the muscle in the rabbit shortly after the mid-period of pregnancy [Robson, 1933 a] and the inhibitory luteal hormone prevents the action of both the pressor and oxytocic substances on the uterine muscle of the rabbit.

The results following removal of the uterine contents during gestation have led to the same definite conclusion, for the reactivity to oxytocin

of the uterine muscle after such a procedure is in all cases low compared with the sensitivity at parturition. And this result was consistently obtained, even though the uterine contents were removed at different stages during the later part of gestation and the intervals between this removal and the subsequent determination of the state of the uterine muscle varied in the different experiments. These findings thus also lead to the inference that the cessation of the luteal secretion is not in itself sufficient to lead to an exaltation of the uterine reactivity similar to that associated with the physiological expulsion of the uterine contents.

In view of these findings it seems logical to conclude that during these later phases of pregnancy an active mechanism comes into play which brings about the condition of the uterine muscle observed at parturition. And as it has been found that œstrin, in large doses, is capable of causing a rise in the reactivity of the uterine muscle similar to that observed at parturition [Robson, 1933 c] it appears quite possible that the mechanism which comes into play during the later stages of pregnancy ultimately, to some extent at least, exerts its effects through the secretion of large amounts of œstrin. The exact nature of the mechanism is unknown, but the finding that it does not become effective when the uterine contents are removed suggests that it may originate in them; and the results obtained in the two cases where the fœtuses alone were removed, the placentæ being left *in situ*, further suggest that the developing embryo is the primary factor which activates this mechanism. It therefore appears justifiable to put forward, as a working hypothesis, the view that during the later stages of gestation the fœtus ultimately controls the development of the reactivity of the uterine muscle to oxytocin and that the secretion of œstrin plays an important part in this effect. The later stages of gestation must also be associated with a cessation of the inhibitory luteal activity, since even large doses of œstrin are unable to overcome the action of the inhibitory luteal hormone [Robson and Illingworth, 1931]; moreover, luteal activity is not essential for the maintenance of the condition of pregnancy in the uterus, as the maximum increase of uterine sensitivity does not result from the mere withdrawal of the luteal secretion. This is in harmony with the finding that, in the human subject, removal of the ovaries does not interfere with the normal course of gestation [Ask-Upmark, 1926].

As regards the spontaneous rhythmic activity of the uterine strips, the evidence derived from a study of pseudo-pregnant animals appears to demonstrate conclusively that cessation of the luteal secretion is not necessarily associated with any definite increase of the contractions

in vitro. And further, there seems to be no appreciable difference between the spontaneous behaviour *in vitro* of strips removed at various stages after a sterile and fertile mating respectively (Table II). On the other hand removal of the uterine contents often, though not always, resulted in the appearance of marked spontaneous contractions, although the reactivity to oxytocin was in these cases conspicuously low. These high spontaneous contractions are not necessarily the result of the cessation of luteal activity and, in fact, the results on the pseudo-pregnant animals suggest that they arise independently of it. In any event it appears unlikely that the rhythmic contractility of the uterine muscle, as exhibited *in vitro*, represents a mechanism involved in the onset of parturition, since, in the human subject at least, it can definitely be asserted that no convincing increase occurs during the course of gestation [Robson, 1933 *b*].

SUMMARY.

The reactivity to oxytocin and spontaneous rhythmic activity of isolated uterine strips were determined in rabbits:

(1) at various stages following the cessation of experimentally induced pseudo-pregnancy, and

(2) at various stages following the removal of the fœtuses with or without the placentæ during the later phases of gestation.

Under these conditions the cessation of luteal activity was not accompanied by an increase in the uterine reactivity similar to that observed at parturition.

The spontaneous rhythmic activity showed no definite increase following the cessation of pseudo-pregnancy, but was usually high in those animals in which the uterine contents had been removed.

REFERENCES.

Ask-Upmark, M. E. (1926). *Acta obst. gynec. scand.* 5, 211.
Hammond, J. (1917). *Proc. Roy. Soc.* B, 89, 534.
Knaus, H. (1930). *Arch. exp. Path. Pharmak.* 151, 371.
Robson, J. M. (1932). *Quart. J. exp. Physiol.* 22, 7.
Robson, J. M. (1933 *a*). *J. Physiol.* 78, 309.
Robson, J. M. (1933 *b*). *Ibid.* 79, 83.
Robson, J. M. (1933 *c*). *Ibid.* 79, 139.
Robson, J. M. and Illingworth, R. E. (1931). *Quart. J. exp. Physiol.* 21, 93.
Weymeersch, A. (1912). *Ann. Soc. Sci. méd. nat. Brux.* 70, 104.

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