THE REACTIVITY DURING ŒSTRUS AND PREGNANCY OF THE RAT UTERUS TO THE OXYTOCIC PRINCIPLE OF THE POSTERIOR PITUITARY GLAND

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STUDIES on the variation in sensitivity of uterine muscle to the oxytocic principle of the posterior pituitary with advancing pregnancy have been made by Robson (on the rabbit [1933*a*], human [1933*b*], and mouse [1934]), Knaus [1930] and others. A difference in the results obtained in the mouse and rabbit is noted by Robson. He finds that the mouse uterus is sensitive to the oxytocic principle throughout pregnancy, though the sensitivity increases markedly in the latter half, while in the rabbit the uterus is insensitive in the earlier stages. These differences in the rodents are emphasized by Hain [1935] who states that "œstrin and oxytocin do not appear to have the same synergism in rats as in mice". Consideration of these results justifies a systematic investigation into the sensitivity of the rat uterus to the oxytocic principle in its various reproductive phases.

METHOD

The rats were taken from the stock colony. Some were virgin stock and a few were parous. They were segregated for some time, vaginal smears being made daily. The rats used for the diœstrus group were taken 2 days after cornified cells had appeared in the vaginal smears. Those for the œstrus group were taken the day after nucleated cells had been found in the smears. The pregnant rats were killed for experiment 5, 10, 15 and 20 days after the finding of spermatozoa in the smear.

In all cases four strips of uterine muscle of equal length in the intact animal were taken. In the non-pregnant animal the strip comprised

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half the horn, divided longitudinally. In pregnant animals strips of approximately the same length and breadth were taken.

A technique which has been employed by some workers to obviate the difficulty of securing strips of approximately constant dimensions from pregnant and non-pregnant animals consists of the sterilization of one horn of the uterus before mating by unilateral ovariectomy, the sterile horn being used for the experiment.

This method was avoided in favour of that described, in view of the work of Markee & Hinsey [1935] and Markee et al. [1936] on the cat and rabbit, demonstrating a local factor in the control of the growth of the pregnant uterus in addition to the hormonal factors. It was thought that this local factor might also result in changes in sensitivity which would not be apparent if the sterile horn technique were used, and if the reactivity to the oxytocic principle is to be considered as a factor in parturition such changes might be of importance.

As soon as possible after removal from the animal the strips were suspended in 50 c.c. oxygenated Dale-Ringer solution in a muscle bath at a temperature of $38 \pm 1°$ C. Four tracings were taken simultaneously by light levers with frontal writing points on a large, slowly moving drum.

The pituitary preparation used was Parke Davis's "Pitocin" specially purified. Two series of doses were used: (a) 0·002, 0·02, 0·2 and 2·0 units, and (b) 0·001, 0·005, 0·010 and 0·015 units and the animals divided into two corresponding groups (a) and (b). The four doses of a series were administered as nearly as possible simultaneously to the four muscle strips from an animal, so that each dose was tested on a fresh strip. After the effect had been observed, the Dale-Ringer solution was changed, and time allowed for the muscle strips to settle down to the fresh solution. The four doses were then repeated on different strips so that a strip used, say, for testing the reaction to 0·002 units was used on the second occasion for one of the larger doses. Tests made on washed strips were regarded merely as confirmatory, and in no case was a strip used after more than one washing. Repeated washing of strips results in alteration in spontaneous activity and possibly in sensitivity, and it was this consideration which led to the adoption of this technique rather than that in which an effort is made to find the threshold value for each strip, the strip being washed between successive doses. The intervals between the values of the smaller doses were as small as possible. Closer approximation of these doses would have led to confusion of results owing to individual variation from animal to animal.

RESULTS

In the first group (a) of the experiments with doses of 0·002, 0·02, 0·2 and 2·0 units about half of the diœstrus group failed to react to the doses of 0·02 unit. In the case of the œstrus group, and in all four

pregnant groups, a reaction was obtained to 0·02 unit in all but two (which were 10th and 15th day pregnancies) out of 28 animals. With the dose of 0·002 unit no effect was obtained except in two experiments (which were on 10th and 20th day pregnancies). These discordant results were ascribed to individual variations in susceptibility, and it was concluded that the threshold in œstrus and pregnancy was between 0·002 and 0·02 units "Pitocin".

TABLE I. Sensitivity of the rat uterus to oxytocin

Group	Rat no.	Dose (units pitocin)							
		0·001	0·002	0·005	0·010	0·015	0·02	0·2	2·0
Diœstrus	0	.	−	.	.	.	?−	+	+
	2	.	−	.	.	.	+	+	+
	28	.	−	.	.	.	−	+	+
	11	.	−	.	.	.	+	+	+
	6	.	−	.	.	.	−	+	+
	41	−	.	−	−	−	?+	+	.
Œstrus	29	.	−	.	.	.	+	+	+
	10	.	−	.	.	.	+	+	+
	50	.	−	.	.	.	+	+	+
	42	−	.	+	+	+	.	.	.
	43	−	−	+	+	+	.	.	.
5th day pregnancy	12	.	−	.	.	.	+	+	+
	24	.	−	+	+	+	.	.	.
	40	−	.	−	?+	+	+	.	.
10th day pregnancy	4	.	−	.	.	.	+	+	+
	9	.	−	.	.	.	−	+	+
	7	.	−	.	.	.	+	+	+
	23	.	+	.	.	.	+	+	+
	22	.	−	.	.	.	+	+	+
	21	.	−	+	+	+	.	.	.
	35	.	−	+	+	+	.	.	.
	39	−	.	+	+	+	.	.	.
15th day pregnancy	1	.	−	.	.	.	+	+	+
	8	.	−	.	.	.	−	+	+
	13	.	−	.	.	.	+	+	+
	28	.	−	+	+	+	.	.	.
	36	−	.	+	+	+	.	.	.
20th day pregnancy	2	.	−	.	.	.	−	+	+
	3	.	−	.	.	.	+	+	+
	5	.	−	.	.	.	+	+	+
	25	−	.	+	+	+	.	.	.
	29	−	.	+	+	+	.	.	.
	26	−	+	+	+	+	.	.	.
	32	−	−	?	+	+	.	.	.

The second group of experiments (*b*) with doses of 0·001, 0·005, 0·010 and 0·015 units substantiated the conclusion that the diœstrus uterus was less sensitive since no reaction was obtained to these doses. This group also revealed a close agreement between the threshold at different stages of pregnancy and at œstrus. With one exception (in the case of

a 5th day pregnancy) 0·005 unit was a sufficient dose to excite activity, while 0·001 unit uniformly failed to give an effect. As in the first series 0·002 unit was also ineffective in almost all cases, the threshold of the uterine muscle in œstrus and at the four stages in pregnancy examined in these experiments would appear to be between 0·002 and 0·005 unit of "Pitocin". During diœstrus it appears to be higher, in the region of 0·02 unit.

DISCUSSION

The conception of the oxytocic principle of the posterior pituitary as the hormone responsible for the contraction of the uterine muscle at parturition has been built up on the results of several groups of experiments, all of which have a somewhat indirect bearing on the actual conditions at the time of parturition. Thus from the experiments of Robson [1933a, b; 1934] demonstrating the increase in sensitivity of the uterine muscle to the oxytocic principle with advancing pregnancy, in the rabbit, human and mouse, it is not unreasonable to suggest that such progressive lowering of the threshold will lead to the initiation of uterine contraction at parturition. Further support is given to this supposition by the observation of Robson [1933b] that the lower portions of the human uterus are less sensitive than the upper, and of Newton [1934, 1937] who finds that the cervix uteri is insensitive to oxytocin in the pregnant goat, rat and guinea-pig. The work of Parkes [1930] confirmed by Marrian & Newton [1935] on the synergism of œstrin and oxytocin, together with the observation that the concentration of œstrin in the urine increases during pregnancy in some animals, led to the œstrin-oxytocin theory of parturition.

There is, however, no evidence that the synergism between œstrin and oxytocin takes place physiologically. In the experiments of Marrian & Newton, when doses of œstrin of a supposedly physiological order were given, the dose of oxytocin used was far beyond that probably present at the end of pregnancy; while in those of Robson [1935] an excessive dose of œstrin was administered to obtain a degree of sensitivity similar to that of the parturient uterus. D'Amour & Dumont [1937] have administered œstrin and oxytocin in physiological doses to pregnant rats and conclude from the absence of abortions that the synergism is not effective in these animals. The same authors further report that they have been unable to hasten parturition (as distinct from terminating gestation by killing the fœtuses) by the use of hormonal factors of the hypophysis, follicular fluid, placenta and blood. A more satisfactory explanation of the complicated phenomena of labour than that based on

differences in threshold between parts of the uterine muscle with regard to the oxytocic principle appears to be required, though it is probable that these differences are of significance.

The present work demonstrates that there is no increase in sensitivity in the rat uterus as pregnancy advances and therefore offers no evidence for the œstrin-oxytocin theory of parturition. (A sudden rise in sensitivity between the 20th day of pregnancy and actual onset of parturition cannot, of course, be excluded.) The threshold found during œstrus and pregnancy is in close agreement with that found by Robson for uterine muscle just at parturition in the human, rabbit and mouse. That this threshold is higher than that at diœstrus may point to the synergism between œstrin and oxytocin being, after all, effective, but may on the other hand be due to the attainment of a constant reactivity resulting in any state in which there is myometrial proliferation. If the synergism is regarded as being effective the quantity of effective œstrin during pregnancy is not greater than that during œstrus, this in turn suggesting variation in the mechanism with the species. If, on the other hand, it is concluded from these experiments on the rat that the synergism is not operative as a physiological mechanism, and if the similarity between the sensitivity of the rat uterus throughout pregnancy and that of other species at parturition be taken into account, it is possible that the increasing reactivity observed in other species may be due to the withdrawal of an inhibitory influence acting in the earlier stages of pregnancy. The inhibitory action of the hormone of the corpus luteum on uterine reactivity to the oxytocic principle has been demonstrated by numerous workers in animals other than the rat, mouse and guinea-pig, the absence of the effect in these species having been noted by Siegmund [1930] and by Robson [1934]. The present work confirms the absence of the inhibitory effect in the rat.

Summary

The reactivity of the rat uterus to oxytocin during diœstrus, œstrus, and at four stages in pregnancy has been investigated. It is found that the reactivity during œstrus and pregnancy is constant, and is greater than that during diœstrus. The significance of these results with regard to hormonal factors and parturition is discussed.

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