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THE INFLUENCE OF THE OVARIAN HORMONES ON MYOMETRIAL BEHAVIOUR IN THE INTACT RABBIT

By BRENDA M. SCHOFIELD*

From the Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland, U.S.A.

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Investigations concerning the physiology of uterine muscle have accumulated a mass of valuable data in a variety of animals. Many of the findings are, however, contradictory and in the evaluation of these data one of the pressing questions is, can the differences be accounted for purely on the basis of species difference? The fact that wide variations are reported in the same species indicates that other factors are also involved.

It has been emphasized by Csapo (1954*a*) that for a more complete understanding of myometrial function it is necessary to break down the complexity of the myometrium and study it in steps at different levels of organization. This has been done in the last fifteen years on cross-striated muscle and much valuable information has been drawn from the observations (Szent-Györgyi, 1953). The study of uterine muscle is somewhat complicated by the regulating effect of the two ovarian steroids, oestrogen and progesterone, which determine its functional state. The influence of these hormones, therefore, has to be carefully controlled if any constancy in the results is to be obtained. Csapo (1950*a*, 1953*a*) has shown that uterine muscle can be closely compared with skeletal muscle at the molecular and cellular levels and that the same chain of events occurs during the contraction cycle in both types of muscle. Actomyosin is the intracellular contractile element deriving its energy from the high energy phosphate compounds adenosine triphosphate (ATP) and creatine phosphate. Moreover, the character of the isometric contraction which is developed by the myometrium on the application of electrical and pharmacological stimuli is closely related to the intracellular concentration of the monovalent cations. Csapo demonstrated by these observations that the uterus is built on a framework similar to that of skeletal muscle. Csapo & Corner (1952) have shown how this basic structure and function of the uterine muscle is

* Present address: Royal Veterinary College, London, N.W. 1.

modified by the superimposed influence of the ovarian hormones, and how much of the uterine behaviour is, in fact, a manifestation of the dominant hormone rather than a property of the muscle itself. From these observations one would infer that the basic principles of function of uterine muscle may be similar in all animals. The reported differences in behaviour may be partly due to differences in the techniques used, as was suggested by Reynolds (1949), or to the influence of superimposed regulations which only appear at or above the organ level.

Csapo (1954*b*) has shown how different the results can be when the same uterus is examined isotonically with different loads, or isometrically. It is clear from these observations that only isometric recording or isotonic recording with optimum load gives a quantitative measure of the working capacity of the uterus or the work done by the myometrium. When contractility is recorded isotonically with little or no load, the contraction of a few muscle cell bundles is recorded as maximum shortening, and such an 'all-or-none' system is unable to reveal the extent of activation of the myometrium. Conversely, isometric recording or isotonic recording with optimum load shows maximum shortening only when all the muscle cells are contracting, and hence the contractile capacity of the myometrium can be evaluated accurately only by using these methods.

Csapo & Goodall (1954) have shown in rabbit uterine segments *in vitro* that maximum active tension develops, as in skeletal muscle, only at the 'resting length'. The 'resting length' of a given uterine segment is such that the segment is not stretched to develop any resting tension, but is extended sufficiently for any tension developed to be recorded. The active (or extra) tension developed by a uterine segment is the difference between the tension recorded at the peak of isometric contraction and the resting tension. For a quantitative evaluation of contractility recordings should be made with the uterine segment at the resting length, since the same segment develops different tensions if its length is altered.

In a previous series of experiments it was shown that with carefully controlled experimental conditions and proper adjustments of techniques, comparable results can be obtained with the uterus *in vitro* (Csapo & Corner, 1952) and *in vivo* (Schofield, 1954). Experiments to be reported here are a continuation of the previous *in vivo* experiments using essentially the same methods as before.

Progesterone dominates the uterus at certain stages in the reproductive cycle and is closely concerned with the preparation for pregnancy and its maintenance. The literature on this subject is extensive but the conclusions drawn are contradictory and the preceding comments on experimental approach and methods apply as much to this section of uterine physiology as to any. The mechanism of the effect of progesterone on the uterus is not known, nor is the time required for its effect to be manifest. Some investigators claim

that progesterone effects develop in a period of about 1 hr, but others maintain that the latency period is more than 24 hr. It is important to have more accurate knowledge concerning progesterone action since it is an essential factor in the treatment of abortion and premature labour. In the experiments to be reported here the tension developed by fully oestrogen- and progesterone-dominated uteri are compared. The latent period for the manifestation of progesterone influence after a single progesterone injection is also studied and the hormone domination is established by observing the staircase effect.

The staircase phenomenon was first described by Bowditch (1871) on the perfused frog heart. After a period of rest the first stimulus elicits submaximal tension, and maximum tension is developed only after a series of subsequent stimuli at constant strength, giving rise to a staircase. Hajdu & Szent-Györgyi (1952) found that this phenomenon can be studied more quantitatively by using isometric recording and by varying the frequency of stimulation after a steady state has been established. Thus the more frequent the stimulus the greater the tension developed since each stimulus leaves behind a more favourable condition for subsequent tension development than it found. Csapo & Corner (1952) demonstrated the existence of the staircase effect in the isolated rabbit uterus. They found that the slope of the staircase when changing from one given frequency to another depends on the hormonal status of the animal from which the segment has been taken. Uterine strips from an animal in natural oestrus or one treated with oestrogen after ovariectomy exhibit a staircase similar to that demonstrated in cardiac muscle. They called this a positive staircase since there is a positive correlation between frequency and tension, that is, the higher the frequency of stimulation, the higher the tension developed. Strips from an ovariectomized rabbit treated with oestrogen + progesterone or from an animal in early pregnancy, show a staircase with negative correlation between frequency and tension—a negative staircase—and the higher the frequency of stimulation the lower the tension developed. Hence in these uteri each stimulus leaves behind a less favourable condition for subsequent contraction than it found. These authors also observed that uterine segments taken from ovariectomized rabbits without hormone treatment exhibit no staircase of either kind.

Thus the slope of the staircase was found to be an indication of the extent of hormone domination, the oestrogen-dominated uterus having a positive staircase, and the progesterone-dominated uterus having a negative staircase. A similar phenomenon occurs in the intact rabbit uterus (Schofield, 1954), and in over 100 rabbits studied, the slope of the staircase has always been an indication of the hormone domination. This finding is made use of in the experiments to be reported here and the latent period following a single injection of progesterone is investigated by observing the transition of the slope from positive to negative.

METHOD

Adult white New Zealand rabbits were ovariectomized and injected daily with 25 μg oestrogen for a week or more. The uteri of these animals were found to be similar to those in natural oestrus and were taken for all experiments as a standard upon which the further hormone modifications were superimposed. Thereafter they were divided into three groups and were injected daily with (*a*) 1 μg oestrogen for an oestrogen-dominated uterus, (*b*) 1 μg oestrogen + 4 mg progesterone for a progesterone-dominated uterus, and (*c*) 4 mg progesterone for a progesterone uterus. The first day of these secondary injections was taken as day 1 and the experiments were usually carried out on day 4. In addition, rabbits in natural oestrus and on the fourth day of pregnancy were used.

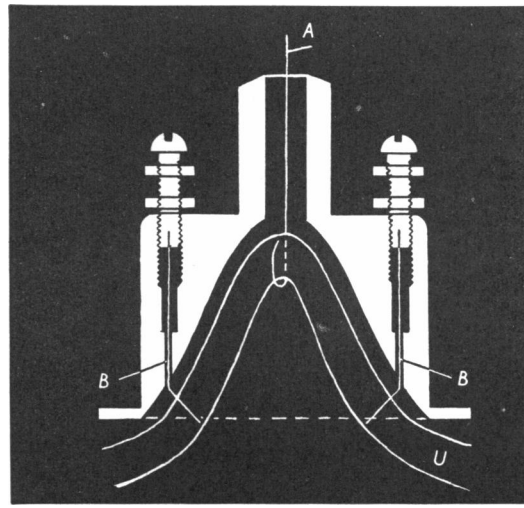

Fig. 1. Cross-section of the Plexiglas chamber which was inserted in the abdomen to fix the uterus and apply the electrodes. *U* = Uterus; *A* = platinum hook representing one pole of the stimulator and attached by thread to the isometric tension recorder; *B* = platinum electrodes representing the other pole of the stimulator.

The technique used for recording isometric tension in response to direct electrical stimulation was that already reported (Schofield, 1954) with certain modifications. The chamber (Fig. 1) made of methacrylate polymer (Plexiglas) bearing the electrodes was suspended on a rack and pinion support. The central platinum electrode (*A*) was temporarily clamped so that the central loop of the uterus (*U*) was held at the top of the chamber in the position shown. The thread to fix the ends of the segment on the other two electrodes (*B*) was then passed with a needle through the holes in the Plexiglas chamber on either side of the electrodes and around the uterus. The segment was pulled taut and pegged on to each electrode before the thread was tied. In this way approximately equivalent lengths of uterus were secured in the chamber in each experiment. Hook *A* was then released and the abdomen was sewn up above the flange. The rack and pinion support allowed the position of the chamber to be adjusted.

Determination of 'resting length' and length–tension curves

To show the relationship between the length of the segment and the tension developed by it, and to determine the resting length, the chamber was initially raised to maximum height in the abdomen. Hook *A* was fixed by a thread to the isometric tension recorder so that on electrical

stimulation of the uterine segment, minimum tension was recorded on the kymograph. Stimuli of 10 V (60 c/s a.c.) per segment of 2–2·5 cm and 5 sec duration were applied at a frequency of 1/min, and the chamber was lowered in 2 mm steps at every second stimulus. This was continued until the chamber could not be lowered further in the abdomen, or until the stretch on the uterus was such that little further tension could be developed. Length–tension curves were studied in this way also with lower voltages. The Plexiglas chamber was then raised again until the uterus was at the length which developed maximum tension when stimulated. This is the 'resting length' as shown in the paper of Csapo & Goodall (1954). At this length the resting muscle does not exert appreciable tension on the recording lever: when stretched beyond this length it is unable to relax to the original base line and an abrupt elevation of the base line is recorded as resting tension.

The staircase phenomenon. With the uterus thus at resting length, the staircase was observed by changing the frequency of stimulation.

Tension–stimulation strength curve. When the voltage applied to the uterus is significantly above the threshold, then the threshold is raised and only returns to its original value after a period of rest. Hence after observing the staircase the stimulator was switched off for about 1 hr and the uterus allowed to rest and develop spontaneous activity. In this way the uterus was again able to respond to the lower voltages. With a frequency of 1 stimulus/min a tension–stimulation strength curve was obtained by applying a rising series of voltages (with a 2–2·5 cm electrode distance) starting at 1, 2, 4 and in twos up to 20 V, and then in fives up to 70 V, applying one stimulus at each strength. The duration of stimulation was kept constant at 5 sec, this being the optimum as determined by Csapo & Goodall (1954). Such high voltages were applied only for theoretical reasons which will be discussed later.

Duration of contraction. The individual contraction–relaxation cycles were studied by increasing the kymograph speed and were subsequently compared with those observed in the isolated uterus (Csapo & Corner, 1952). The strength of stimulation was 10 V per 2–2·5 cm segment and 5 sec duration and the frequency was in the first place 1 stimulus every 2 min, then 1/1 min and 1/½ min, and studies were made after a constant tension had been attained for each frequency. The cycles were more uniform if observed at the beginning of an experiment and hence they were studied before the length–tension curve and after the resting length had been established with a few initial stimuli.

Measurements were made of the tension developed by the uterus when it was stimulated with 10 V at 2 min intervals. At the end of each experiment the uterine segment between the electrodes *B* was excised and weighed and measured so that the tension could be related to these quantities.

Administration of progesterone. To investigate an immediate effect of progesterone on uterine motility comparable with the effect observed *in vitro* (Csapo, 1954*b*), rabbits having oestrogen-dominated uteri were observed on day 3. The recordings were made with the uterus at resting length. Progesterone is soluble in water only to the extent of 5–10 μg/ml. and therefore the problem arose of how to make the progesterone immediately available to the uterus. Progesterone is soluble in propylene glycol and alcohol, and the alcohol can be diluted 50% without precipitation, but presumably the progesterone would subsequently precipitate with blood or body fluids. A number of methods of administration over a wide dosage range was tried in seventy-five rabbits. Progesterone was dissolved in propylene glycol or alcohol, sometimes diluted with saline (0·9% NaCl solution), and injected intravenously, subcutaneously, intramuscularly, or intraperitoneally. In microsuspension, and dissolved in serum albumin it was injected into the ear veins, and in oil intraperitoneally. Progesterone in solution in propylene glycol was injected into the lumen of the uterus with a long hypodermic needle. Krebs solution containing 10 μg/ml. was put inside the Plexiglas chamber to the extent of 100 ml. so that the abdominal cavity and chamber were filled. This reproduced *in vitro* conditions as nearly as possible. Finally the inferior mesenteric artery was cannulated and all arteries caudal to it except the uterine artery were ligated. Progesterone was then injected through the artery in amounts up to 2 mg when dissolved in Krebs solution and

up to 10 mg when dissolved in propylene glycol. Indian ink subsequently injected indicated that the solutions were in fact passing to the uterus. In some animals the uterus was stimulated with 10 V at 2 min intervals throughout the experiment, and in others it was stimulated at the beginning and end of the experimental run, spontaneous activity being recorded in between. The tension, both stimulated and spontaneous, was noted and stimuli of varying frequencies were applied to indicate the slope of the staircase at the beginning and end of the run. The experiments extended up to 12 hr.

Experiments were also carried out to elicit the period of time following a single progesterone injection when the myometrium developed 'progesterone-dominated' characteristics. Rabbits having oestrogen-dominated uteri were injected intramuscularly or subcutaneously with 4, 8 or 20 mg progesterone dissolved in oil or alcohol on day 3. (Those receiving 20 mg progesterone had 5 μg oestrogen instead of 1 μg.) Experiments were carried out on days 4 and 5 or 4 and 6 under sodium pentobarbitone (Nembutal) anaesthesia. Antiseptic precautions were taken during the operative procedure and the rabbits were injected with penicillin at the beginning of the experiment. They appeared to remain in a healthy condition. After the first experimental run the Plexiglas chamber was removed and the edges of the abdominal incision carefully sutured together. The animal was returned to its cage until the second experimental run when the uterus was set up as before in the Plexiglas chamber. Stimuli evoking a staircase effect were applied at the beginning and end of the 6 hr experiment which was carried out on the 2 days of observation. The sequence of stimulation frequencies was kept constant and was 1 stimulus/$\frac{1}{2}$ min, 1/1 min, 1/2 min and again 1/$\frac{1}{2}$ min, applying usually 6, 3, 2 and 6 stimuli respectively for each frequency. The criterion for classifying this intermediate staircase when the uterus was incompletely dominated by one hormone or the other was the direction of the slope at the transition from 2 min frequency to $\frac{1}{2}$ min frequency. When there was no slope this was taken as a transient staircase indicating that the uterus was influenced equally by oestrogen and progesterone.

RESULTS

Duration of contraction. Contraction–relaxation cycles observed in oestrogen- and progesterone-dominated uteri are shown in Fig. 2. They fall clearly into two groups as can be seen in Table 1. The cycle is longer in the oestrogen-dominated uterus than in the progesterone-dominated uterus and this occurs irrespective of the tension developed. The length of the cycle diminishes when stimulation is applied more frequently, the diminution being greater in the oestrogen-dominated than in the progesterone-dominated uterus.

Relation between length and tension. The length–tension curves of oestrogen- and progesterone-dominated uteri as obtained by changing the length of the segment with constant strength and frequency of stimulation are shown in Fig. 3. They indicate a clear difference between the two types of uterus. This is a characteristic difference and is best shown by drawing a straight line between the peaks of the first and the last tensions. The peak tensions do not approach this line in the progesterone-dominated uterus but over-step it in the oestrogen-dominated uterus. The curves are similar with lower voltages. The progesterone uterus and the 4-day pregnant uterus usually have a curve like that of the progesterone-dominated uterus, but with a lower tension. Uteri under the influence of both hormones so that neither is dominant develop tensions which lie on the straight line.

The staircase phenomenon. The staircase effect is shown at the resting length of the uterus by changing the frequency of stimulation at constant strength of stimulus. The character of the staircase thus observed divided the five groups of rabbits sharply into two. Those rabbits which had progesterone either injected or secreted by an active corpus luteum had uteri which always showed a marked negative staircase, whereas those uteri under oestrogen domination with no progesterone gave a positive staircase. Fig. 4 shows the staircases of uteri taken from rabbits in natural oestrus and early pregnancy respectively.

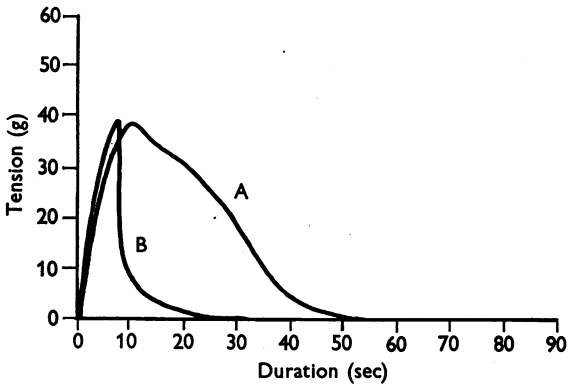

Fig. 2. Graph showing contraction-relaxation cycles of the uterus. A in a rabbit treated with oestrogen and having an oestrogen-dominated uterus; B in a rabbit treated with oestrogen and progesterone and having a progesterone-dominated uterus. Stimulus, 10 V; frequency, 1/2 min; duration, 5 sec.

TABLE 1. The average length in seconds of the contraction cycles of oestrogen- and progesterone-dominated uteri

Type of uterus (no. of rabbits in brackets)	Frequency of stimulation		
	1/2 min	1/1 min	1/½ min
Oestrogen-dominated (9)	42	29	19
Progesterone-dominated (10)	24	21	16

Relation between tension and strength of stimulation. When stimuli ranging from 1 V up to 70 V were applied to the uterus, two peaks of tension were observed (Fig. 5). The second rise in tension in these experiments is not, in fact, a peak since there was no subsequent decrease in tension, but for the sake of comparison with the *in vitro* work the second rise will be referred to as the second peak. With the oestrogen-dominated uterus the first peak and the inter-peak minimum tension occur later in the curve as compared with the progesterone-dominated uterus. The oestrogen-dominated uterus had the first peak at 12 V, and the following inter-peak minimum tension at 40 V (average from eleven rabbits). The tension subsequently developed was not as high as

the first peak tension. In the progesterone-dominated uterus, the first peak occurred at 7 V and the inter-peak minimum at 21 V (average from fourteen rabbits). Thereafter the tension increased and was higher than the first peak in five of the fourteen rabbits.

Measurements of tension in twenty-six oestrogen-dominated uteri, seventeen progesterone-dominated uteri, nine progesterone uteri and eight 4-day pregnant

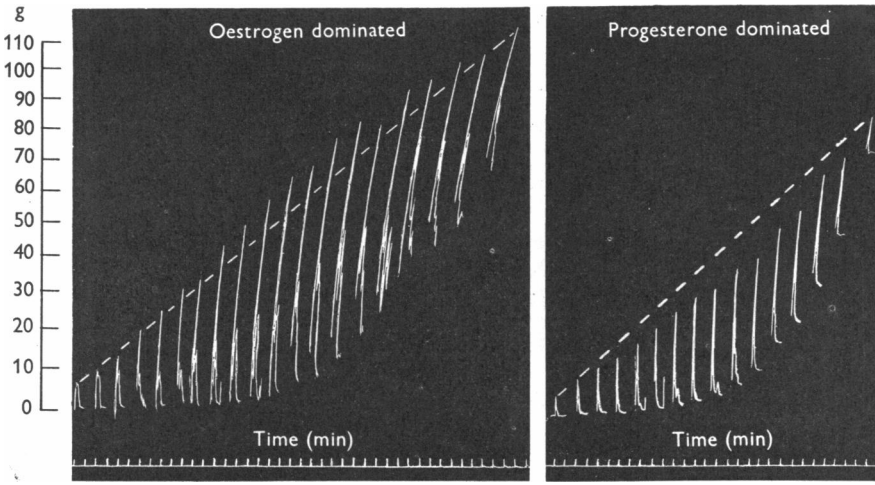

Fig. 3. Tracings of the ength-tension curve in an oestrogen-dominated uterus and in a progesterone-dominated uterus. The length of the uterine segment was increased by 2 mm at each step. Constant stimulus = 10 V; frequency, 1/min; duration, 5 sec. Every second response recorded. Tension calibrated in g.

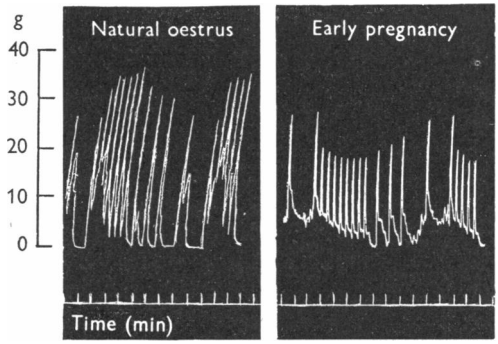

Fig. 4. Staircase effect shown by altering the frequency of stimulation with constant strength (10 V) and duration (5 sec) of stimulation. This figure shows the opposite relationship between tension and frequency of stimulation in uteri taken from a rabbit in natural oestrus and one in early pregnancy. The oestrogen-dominated uterus has a positive staircase and the progesterone-dominated uterus a negative staircase. Tension calibrated in g.

uteri were related to the weight and length of the segment from which they were recorded. Thus the tensions are in terms of g/g weight or/cm length. The means and statistical figures are shown in Table 2. The oestrogen-dominated

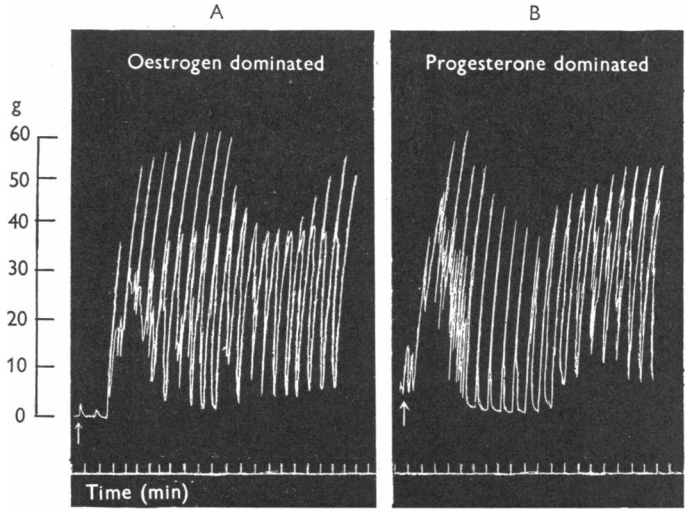

Fig. 5. The response of the uterus to consecutive stimuli of 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 45, 50, 55; 60, 65 V in B, and the same series +70, 75 V in A, showing the two peaks in an oestrogen-dominated and in a progesterone-dominated uterus. The first voltage was applied at the arrow. Frequency, 1/min; duration, 5 sec; tension calibrated in g.

Table 2. The means and statistical figures for tensions developed by the four groups: (1) oestrogen-dominated uteri, (2) progesterone-dominated uteri, (3) progesterone uteri, and (4) 4-day pregnant uteri

T = tension; W = weight; L = length

	Oestrogen-dominated uterus		Progesterone-dominated uterus	
	T/W	T/L	T/W	T/L
Mean tension (g)	26·8	11·9	20·1	8·1
Scatter	16·4–49·3	7·2–18·5	8·4–33·2	4–15·5
S.E. of the mean	1·47	0·59	1·85	0·71

S.E. of the difference $T/W = 2{\cdot}36$

$T/L = 0{\cdot}9$

For T/W, $t = 2{\cdot}89$ For T/L, $t = 4{\cdot}4$

	Progesterone uterus		4-Day pregnant uterus	
	T/W	T/L	T/W	T/L
Mean tension (g)	18·9	7·7	10·2	4·0

uterus apparently develops a higher tension than the progesterone-dominated uterus, and considering the t test, the difference between the means of tension per g weight is significant with P less than 0·01, whereas the difference in

tension per cm length is significant with $P=0{\cdot}01$. We can assume therefore that the tension developed is greater in the oestrogen- than in the progesterone-dominated uterus if the tensions are related to weight or length. The number of progesterone and early pregnant uteri studied is insufficient to justify any statistical analysis from which to draw conclusions. However, it appears that compared with the progesterone-dominated uterus, the progesterone uterus develops less tension and the 4-day pregnant uterus considerably less tension. More figures are needed to validate these results.

The effect of progesterone. Progesterone injected at the beginning of the experimental run had apparently no effect on the motility of the oestrogen-dominated uterus up to the end of a 6–12 hr record. The rabbit often died about 30 min after the injection of more than 0·5 ml. of propylene glycol, but in the interim period there was no manifestation of effect as judged by the character and amplitude of the spontaneous contractility or the tension developed in response to stimulation. Using these criteria and the slope of the staircase to indicate a positive effect, the other methods of administration were similarly ineffective over several hours, and there was either no change in activity, or small inconsistent variations which were likely to occur in a control uterus similarly recorded. That there was no reduction in stimulated tension comparable with the *in vitro* effect (Csapo, 1954*b*) was certain.

Observation of the staircase at different intervals of time after an injection of progesterone in oil or alcohol indicates that the latent period for the manifestation of the effect is approximately 21 hr, though there is wide variation. Of the twenty-seven experimental animals, nine showed negative staircases at 18–21 hr, four at 21–24 hr and five at a later stage (some of these had an additional progesterone injection). The other nine showed only transient staircases (i.e. no slope) at the times they were observed, but it is possible that these uteri passed through a progesterone-dominated stage during the night. Figs. 6 and 7 show the staircases of rabbits 9 and 26 to illustrate the criterion which was used for classifying the staircase. The slopes of the staircases are not marked, since after a single injection the uterus must be only slightly dominated by one hormone or the other. The true staircases indicating a complete domination are shown in Fig. 4 for a natural oestrous uterus and one in early pregnancy; the conditions of these uteri can be reproduced by injecting a castrate with oestrogen or progesterone respectively. Rabbits injected with oil instead of progesterone show good positive staircases throughout the experimental runs. The results are shown in Table 3. 8 mg is a more effective dose than 4 mg but as high a dose as 20 mg is apparently unnecessary. Those rabbits receiving one injection of progesterone showed a return to the positive staircase if observed on day 6 (nos. 12, 26, 27), but though 1 μg oestrogen is sufficient to maintain a uterus in oestrus initially, it is doubtful whether it is

sufficient to effect the transition from a partially progesterone-dominated uterus to a fully oestrogen-dominated uterus.

In all the stages of hormone domination described, spontaneous activity was observed to see whether it altered in character consistently with the hormonal changes. There was, however, no apparent change either in the pattern of contractility or in the tension developed. Under these conditions, therefore, observation of the spontaneous activity can tell us little about the hormonal control of the uterus and electrical stimulation of the muscle is necessary to elicit more standard behaviour.

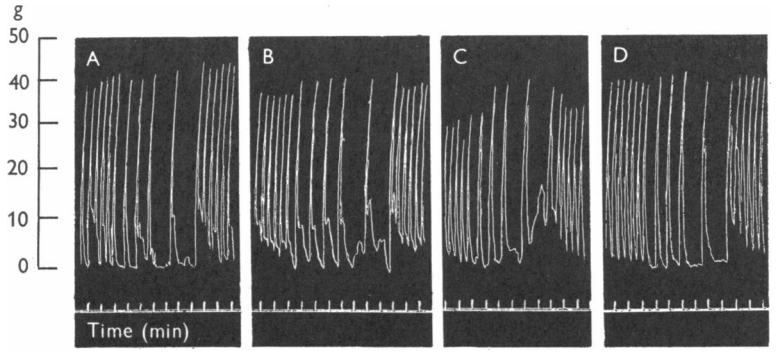

Fig. 6. Record of the staircases in rabbit 9 at 21(A), 27(B), 45(C), and 51(D) hr after one oil injection of 4 mg progesterone. Tension calibrated in g. The staircase is transient in A and D and negative in B and C.

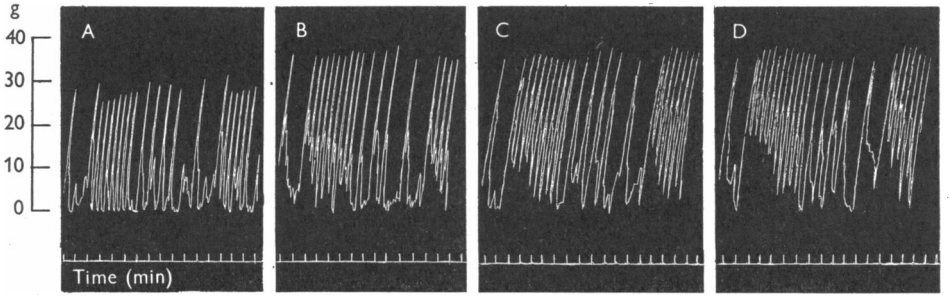

Fig. 7. Record of the staircases in rabbit 26 at 21(A), 27(B), 69(C) and 75(D) hr after one oil injection of 8 mg progesterone. Tension calibrated in g. The staircase is negative in A, transient in B and C, and positive in D.

DISCUSSION

The similarity between the results obtained with the uterine muscle *in vitro* and *in vivo* is reassuring since this indicates that *in vivo* results clearly reflect primary muscle properties not obscured by superimposed regulation in the more complex system of the intact rabbit. The *in vivo* behaviour of the uterus

TABLE 3. The slope of the staircase at the stated time after a single injection of oil or progesterone. 0 = transient. In nos. 8, 10, 15, 17 and 18 the initial progesterone injection was repeated the next day

No.	Time (hr)	Stair-case
	Oil	
1	12	+
	22	+
	28	+
2	24	+
	30	+
3	24	+
	30	+
4	19	+
	24	+
	42	+
	48	+
5	18	+
	24	+
	4 mg progesterone	
6	19	0
	24	0
	42	+
	48	+
7	18	–
8	21	0
	27	0
	45	–
	50	0
9	21	0
	27	–
	45	–
	51	0
10	21	0
	27	0
	45	–
	50	–
11	21	+
	27	0
12	24	+
	30	0
	72	+
	77	+
13	21	0
	8 mg progesterone	
14	18·5	0
	24	0
	42	+
15	21·5	–
	27	0
	45	–
	50	–
16	21	–
	27	–
	74	–
	117	0
17	21	0
	27	0
	45	–
	50	0
	69	0
18	21	–
	27	–
	44	–
	50	–
19	18	+
	24	+
	42	–
20	23	0
	29	+
	47	0
	53	+
21	18	–
	24	0
	42	–
	48	0
22	12	0
	18	0
23	18	–
	24	0
24	18	0
	24	–
	42	–
	45	–
25	18	+
	24	0
	42	–
	45	–
26	21	–
	27	0
	69	0
	75	+
27	24	–
	30	0
	72	0
	77	+
28	21	–
	27	–
	45	–
	51	0
29	21	0
	20 mg progesterone	
30	18	0
31	18·5	–
	24	0
	42	0
	48	0
32	18	0
	24	–
	42	0
	48	+

is not as regular as in the isolated muscle, but when differences are apparent, they can usually be explained on the basis of the experimental conditions.

The oestrogen-dominated uterus has a longer contraction cycle than the progesterone-dominated uterus, and this difference is similar to the one observed *in vitro* (Csapo & Corner, 1952). The time relationship between the two cycles with one stimulus/2 min is slightly less than 3:1 *in vitro* and slightly less than 2:1 *in vivo*. Each contraction of the myometrium results in a loss of intracellular potassium with subsequent return during rest (Csapo, 1953*b*) and, therefore, one would expect a net loss of potassium with higher frequencies since the next stimulation occurs before the potassium return has been completed. With diminished intracellular potassium the contraction cycle is shorter (Corner & Csapo, 1953) and thus the contraction cycles observed in these experiments and *in vitro* (Corner & Csapo, 1953) become shorter with higher frequency of stimulation. The change in length of the cycle of the progesterone-dominated uterus is not marked since the potassium content of the cells at rest is lower and because the muscle is probably nearer to the limit at which the duration of the cycle cannot be further shortened. The change in potassium content as the frequency of stimulation is increased is greater in the oestrogen-dominated uterus which has initially more intracellular potassium, and this probably accounts for the two types of uteri approaching each other in character with increased frequency of stimulation. At a frequency of 1/2 min the oestrogen-dominated cycle is 75% longer than the progesterone-dominated cycle, but at a frequency of 1/$\frac{1}{2}$ min only 19% longer.

The general shapes of the two length-tension curves are the same in these experiments as they are *in vitro* (Csapo & Goodall, 1954), and the oestrogen-dominated uterus has a convex curve while the progesterone-dominated has a concave curve relative to a straight line joining the first and last peak tensions. However, *in vitro* the oestrogen-dominated muscle stimulated at suboptimal voltage has a curve like that of the progesterone-dominated muscle stimulated at optimal voltage, namely a concave curve. This is not so *in vivo* where the shape of the curve is apparently not conditioned by the voltage. The dependence of the shape of the curve on the stimulating voltage can perhaps not be demonstrated in the intact animal because the uterus has a greater degree of spontaneous motility and, therefore, the margin between spontaneous tension and tension developed by a sufficiently low voltage is too narrow to permit demonstration of the curve at suboptimal voltage.

The high voltages applied across the uterus for the tension–stimulation strength curves were considered to be greatly above the physiological range. Csapo (1954*c*) has shown in the isolated uterus that with such a series of stimuli two peaks of tension development are seen. He suggested that at the first peak the normal mechanism of stimulation is put into effect by the depolarization of the membrane. The height of the first peak can be modified on altering

the potassium gradient, in the living animal by hormone treatment, or in the isolated uterus by changing the concentration of this cation in the bath. The second peak is not dependent on this gradient and tension remains maximal at this peak after the potassium gradient is abolished. High stimulation strengths applied to isolated segments could not be applied *in situ* owing to spread of current to the body of the animal. As far as spread of current was not apparent, however, the *in vitro* and *in vivo* experiments may be compared and it can be seen from the results that the curves obtained with the intact uterus simulate closely in principle those seen *in vitro*. Since progesterone modifies the activation process within the muscle (Csapo, 1954*c*) it modifies the first peak of tension. The second peak, however, is independent of the normal activation process and is not modified by progesterone. Thus the first peak is lower in the progesterone-dominated uterus as compared with the second peak. Since in these *in situ* experiments the voltage cannot be raised above 75 V, the apex of the second peak is not always reached and hence in only a few of the rabbits investigated is the second peak equal to the first in the oestrogen-dominated uterus and higher than the first peak in the progesterone-dominated uterus. These results, therefore, are in agreement with the *in vitro* observations as far as it is possible to test them.

If tension is a function of the weight or the length of the uterine segment, then it may be concluded from these results that when uteri are under full domination by one hormone or the other the myometrium develops a greater tension during direct stimulation in the oestrogen-dominated uterus than in the progesterone-dominated uterus. Oestrogen is necessary for the maintenance of the contractile proteins in the myometrial cell (Csapo, 1950*b*) and, therefore, the temporary absence of oestrogen in the progesterone uterus and the early pregnant uterus probably accounts for the decrease in tension observed. The tension is further diminished in the early pregnant uterus since the previous oestrogen domination is probably less in natural oestrus than in this experimental oestrus. However, since the oestrogen- and progesterone-dominated uteri receive equal amounts of oestrogen there must be equivalent amounts of contractile proteins present in the muscle (Csapo & Corner, 1951), and therefore some other factors must also influence the development of tension following direct electrical stimulation. The work of Horvath (1954) indicates that there are alterations in the intracellular potassium and sodium concentrations due to hormonal influences and this can influence both the development of tension and the behaviour of the uterus (Csapo, 1954*c*). Csapo (1954*d*) found by varying the electrode distances in the uterus that the conduction properties of the muscle depend on the hormone domination. It might be inferred, therefore, that the diminished tension observed in the progesterone-dominated muscle is due to one or both of these factors rather than to a reduction in the actual contractile capacity of the myometrial fibres.

An average of 21 hr was required for any demonstration of effect following a progesterone injection. This figure is in agreement with those of Csapo (1954*a*) who found that rabbits on the thirty-first day of pregnancy were protected from pituitary-induced premature labour by injection of progesterone 12–24 hr previously, and on the thirtieth day 6–12 hr previously. During pregnancy a high level of progesterone is assumed to be present, and therefore in late pregnancy when the level has begun to fall less time is required to raise the progesterone to its former level than is required in an oestrous rabbit having virtually no progesterone.

No immediate effect of progesterone could be demonstrated on the uterus *in situ*. This finding supports the early conclusion (Csapo, 1954*b*) that the *in vitro* effect of progesterone is non-specific since it results only in a decrease in tension without altering the staircase which is highly characteristic of the effect of progesterone.

SUMMARY

1. Observations were made of the isometric tension developed by the rabbit uterus *in situ* in response to direct electrical stimulation.

2. The behaviour of the muscle was studied when it was under the domination of injected or naturally secreted oestrogen and progesterone and the results were similar to those previously observed *in vitro*.

3. Characteristic differences, produced by the two hormones, were found: (*a*) in the duration of the contraction-relaxation cycle; (*b*) in the influence of muscle length on the tension developed; (*c*) in the relation of stimulus frequency to tension (staircase phenomenon); (*d*) in the relation of stimulus strength to tension.

4. A statistical analysis of the tensions developed in uteri fully dominated by oestrogen or progesterone indicates that the progesterone-dominated uterus develops less tension than the oestrogen-dominated uterus when this is related to the weight or the length of the segment.

5. Progesterone apparently produced no alteration in the character of spontaneous activity.

6. Injected progesterone has a latent period, and the period of time after the injection when the character of the muscle contractility begins to change from the oestrogen-dominated behaviour is about 21 hr.

7. The significance of these findings is discussed.

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