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The influence of the female sex hormones on the response of the pig uterus to electrical stimulation

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The response of the pig myometrium to electrical stimulation *in vitro* was used to assess the influence of the female sex hormones at different stages of the oestrus cycle. Optimal stimulation produced greater tension in the mature uterus than in the immature uterus and the tension was greater in the oestrogen-dominated uterus than in the progesterone-dominated uterus. The minimum (threshold) voltage necessary to elicit an isometric contraction was not influenced by the stage of the uterus in the oestrus cycle. Varying the frequency of stimulation caused changes in tension indicating the predominant sex hormone. The progesterone-dominated myometrium appears to bind calcium more strongly than the oestrogen-dominated myometrium.

IN many species spontaneous uterine motility is of different types according to the stage of the oestrus cycle. This was recognised by Corner (1923) who correlated the period of suppressed uterine motility with the presence of corpora lutea in the ovaries. The respective influence of oestrogens and progesterone on the myometrium has been studied using as a criterion the response of the uterus to electrical stimulation. Thus Csapo & Corner (1952) found in the rabbit uterus, that when the frequency of electrical stimulation was varied, the tension developed was proportional to the frequency of stimulation (positive staircase) in oestrogen-dominated uteri, and inversely proportional (negative staircase) in progesterone-dominated uteri.

The oestrus cycle in the pig is of three weeks' duration and has been described in detail by Burger (1952). Oestrus lasts for two or three days, ovulation usually occurring on the second day of oestrus. Corpora lutea reach maximum size in the second week of the cycle and after the 14th day decrease with the approach of oestrus. During this period there is a rapid growth of those follicles destined to rupture at the next oestrus period.

The work described in this paper is an *in vitro* study of the influence of the female sex hormones, revealed by electrical stimulation, on the myometrium of a polyoestrous animal, the pig. Non-pregnant uteri at different stages of the oestrus cycle were used to avoid the complications of hypertrophy and stretching of the uterus which occur during pregnancy.

Experimental

Collection of material. Uteri were collected from a nearby abattoir from freshly slaughtered Large White pigs; strips of uterus were set up in the tissue baths within 25 min of the animal being stunned. Immediately the uterus was removed from the animal it was examined to ascertain that it was non-gravid and that the ovaries appeared to be active. The anterior (ovarian) half of the right horn was removed and transferred to a thermos flask containing modified Krebs solution which had previously

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been cooled to 4° and gassed for 5 min with a mixture of 95% oxygen and 5% carbon dioxide. The modified Krebs solution was made up as follows (conc. g/100 ml): NaCl 0·69, KCl 0·035, $CaCl_2$ 0·028, $MgCl_2$ 0·011, $NaHCO_3$ 0·21, NaH_2PO_4 0·014, glucose 0·2. The weights refer to anhydrous salts.

Setting up the tissues. A longitudinal strip 3 cm long and 7 mm wide was cut from the antimesometrial border at the severed end of the uterine horn. The endometrium was removed and the strip of myometrium attached to a hook of platinum wire fixed to a Perspex frame in a 10 ml tissue bath; the hook also served as stimulating electrode. The upper end of the strip was connected by a thread to an isometric lever. A piece of silver wire inserted in the upper end of the strip acted as the second electrode. Krebs solution at 39° and gassed with 95% oxygen and 5% carbon dioxide was used in the bath.

The lever consisted of a watch spring, the tension on which could be adjusted to allow the uterine strip a maximum shortening of less than 5% of the resting length. A light lever arm was attached to the centre of the spring so as to give a 25 × magnification of the contractions which were recorded on a smoked drum.

For 10 min after being set up in the tissue bath, the strip elongated. At 2 min intervals during this period, the tissue was stretched slightly by lowering the Perspex strip in the tissue bath and the resting length was determined by the last stretch to which the uterus adjusted without developing resting tension (Csapo, 1954). The Perspex strip was marked at 2 mm intervals with the platinum hook as zero, and the resting length of the tissue was noted when stretching was completed; all the muscle strips used had a resting length of 32–38 mm.

Assessment of the stage in the oestrus cycle. The numbers of follicles or corpora lutea in each ovary were recorded. The stage of each uterus in the oestrus cycle was then determined as described by Corner (1921).

Threshold voltage and optimum voltage. After adjusting the strip to its resting length it was stimulated electrically at intervals of 1 min, each impulse of 5 sec duration, increasing the intensity of successive stimuli by increments of 1 V. When the muscle first contracted, the voltage which induced the contraction (threshold voltage) was noted, and also the voltage which induced maximal tension (optimum voltage). Voltage was then decreased with successive stimuli and the threshold voltage again determined. The strip was rested for 15 min.

The staircase effect was determined according to the method described by Schofield (1954). If the tension in the strip decreased when the frequency of stimulation was reduced, the effect was called a positive staircase, whereas if the tension increased with reduction in the frequency of stimulation, the staircase was negative. A transient staircase was designated as one in which there was no alteration in tension at different frequencies of stimulation.

Effect of calcium depletion on electrically induced tension. After recording the staircase effect, the tissue was rested for 15 min and then stimulated at the previously determined optimum voltage, each stimulus of 5 sec

duration at a frequency of 1/min until a steady state tension was established. Calcium-free Krebs solution was then substituted for the usual Krebs solution and this point of the experiment designated as zero time. Thereafter, at alternate 1 min intervals, the strips were stimulated at optimum voltage and the solution in the baths changed; recordings were continued for 1 hr from zero time.

Effects of progesterone. The effects of water-soluble progesterone (Intravenous Primolut, Schering) on spontaneous and electrically-induced uterine contractions were investigated using uteri in the oestrus stage of the cycle. After adjusting the strip to its resting length and determining the optimum voltage for stimulation, a period of $1\frac{1}{2}$ hr was allowed to elapse for the spontaneous contractions to become regular. Progesterone was diluted in Krebs solution to give final concentrations of 20 40, 60 and 80 μg/ml.

The bath was emptied and then filled with 20 μg/ml progesterone solution previously warmed to 39°; the solution remained in the bath until the maximum effect was observed before it was replaced by fresh Krebs solution. When spontaneous contractions became regular again, the experiment was repeated using 40 and 60 μg/ml progesterone.

After a resting period of 30 min, the strip was stimulated at a frequency of 1/min, each stimulus being of 5 sec duration at the previously determined optimum voltage; this was continued throughout the remainder of the experiment. When a steady state tension was established, the Krebs solution was replaced by successively greater concentrations of progesterone as described above.

Results

The staircase effect. The staircase effect, illustrated in Fig. 1, was studied in 64 non-pregnant sow uteri at different stages of the cycle and the results are shown in Table 1.

TABLE 1. THE TYPES OF STAIRCASE RECORDED FROM UTERI AT DIFFERENT STAGES OF THE OESTRUS CYCLE. The stage of the cycle was assessed on histological evidence and the figures in each group are the numbers of uteri from which the different staircases (positive, negative or transient) were recorded.

Type of staircase	Stage of the oestrus cycle			
	Oestrus	4–7 days	8–14 days	15–20 days
Positive	0	11	2	4
Negative	0	3	28	0
Transient	8	3	3	2

Negative staircases were recorded from those uteri in which the ovaries contained mature corpora lutea. The 5 specimens in the group in the 8–14 days stage of the cycle which did not show negative staircases were not typical of the group, since the ovaries contained large follicles in addition to corpora lutea. Blood levels of progesterone in sows during this stage of the oestrus cycle are comparable to the levels during pregnancy (Short,

1957, 1960; Rowlands & Short, 1959). It is therefore concluded that a negative staircase indicates a progesterone-dominated myometrium.

Histological examination of the corpora lutea of those uteri in the 4–7 days stage of the cycle revealed all stages of development from early post-oestrus, with a large blood clot in the centre, to practically fully formed corpora lutea. The 11 uteri in this group from which positive staircases were recorded were in the early post-oestrus stage of the cycle (3 or 4 days from the beginning of oestrus) in contrast to the remaining 6, the ovaries in which contained more mature corpora lutea. It has been shown that in the sow, oestrogen levels in the urine (Velle, 1960; Raeside, 1961), and in the ovaries (Allen & Doisy, 1927) are highest during oestrus. Thus it is concluded that a positive staircase indicates an oestrogen-dominated myometrium.

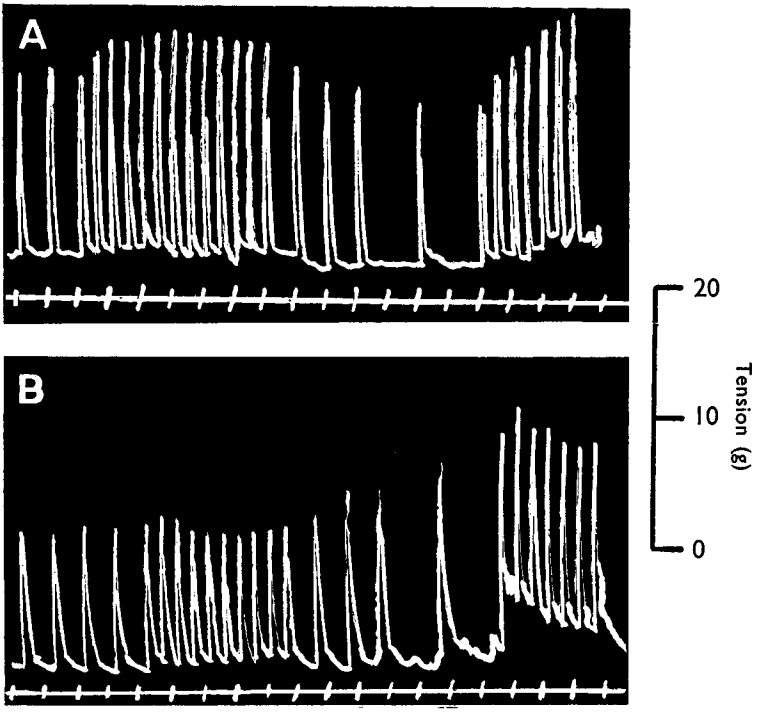

FIG. 1. A. A positive staircase recorded from a strip, the ovaries of which contained newly-ruptured follicles. The tension developed is proportional to the frequency of stimulation. B. A negative staircase recorded from a strip in the 8–14 days stage of the oestrus cycle; the ovaries contained mature corpora lutea. The tension developed is inversely proportional to the frequency of stimulation. Time marker 1 min.

The anomalous result that transient, rather than positive, staircases were recorded from uteri whose ovaries contained mature, unruptured follicles does not necessarily detract from the inferences already drawn. Spontaneous uterine motility at this stage of the cycle is characterised by

contractions of very regular amplitude and frequency (Keye, 1923; Wislocki & Guttmacher, 1924; King, 1927; Adams, 1940). Furthermore, in such strips oxytocin does not cause a significant increase in amplitude of contractions, but produces either an increase in the frequency of contractions, or a more sustained contraction (Adams, 1940). It is not unexpected, therefore, that strips from uteri in the oestrus stage of the cycle respond with maximum tension to optimal electrical stimulation.

Some of the uteri whose ovaries contain either developing corpora lutea (4–7 days stage), or degenerating corpora lutea with developing follicles (15–20 days stage), would be expected, from the evidence cited above, to be neither oestrogen-dominated nor progesterone-dominated; that transient staircases were recorded from such uteri is in accord with this argument.

The staircase effect was also studied on 16 immature gilt uteri. In all instances, electrical stimulation caused only small responses with no change in tension when the frequency of stimulation was altered.

Threshold voltage. The minimum (threshold) voltage causing an isometric contraction was measured in each strip after adjusting the resting length; it was expressed as V/cm of the resting length. The uteri were subsequently divided into 2 groups on the basis of histological examination and the type of staircase. One group consisted of 24 uteri in the oestrus (transient staircases) and the early post-oestrus stage of the cycle (positive staircases); that is, the myometrium was oestrogen-dominated. The second group consisted of 25 uteri in the 8–14 days stage of the cycle (negative staircases) with a progesterone-dominated myometrium.

A statistical analysis of the mean values for threshold voltage shows no significant difference between the 2 groups.

Attempts were also made to measure threshold voltages in strips from immature gilt uteri. However, by the time the strips were adjusted to the resting length there was marked spontaneous motility and it was impossible to determine with accuracy whether the smaller voltages induced contractions or whether the contractions were spontaneous.

Steady state tensions. Before studying the response to varying the frequency of electrical stimulation (staircase effect), the strips were stimulated at optimum voltage at a frequency of 1/min until a steady state tension was established and this value was measured for each strip. The sow uteri were subsequently divided into the same 2 groups as previously described; strips from immature gilt uteri were also examined and the results are summarised in Table 2.

TABLE 2. THE MEAN VALUES FOR STEADY STATE TENSION IN A GROUP OF IMMATURE GILT UTERI AND 2 GROUPS OF SOW UTERI AT DIFFERENT STAGES OF THE OESTRUS CYCLE

	Sows	
Immature gilts	Oestrogen-dominated	Progesterone-dominated
Tension (g) ± s.e. 7·6 ± 0·55 (16)	Tension (g) ± s.e. 16·7 ± 1·14 (24)	Tension (g) ± s.e. 11·1 ± 0·62 (25)

No. of uteri in parentheses

The mean tension developed in the gilt uteri is significantly less ($P < 0{\cdot}01$) than that in the sow uteri. Furthermore, a comparison of the 2 groups of sow uteri shows that the mean tension in the progesterone-dominated group is significantly less ($P < 0{\cdot}01$) than that in the oestrogen-dominated group.

Effect of calcium depletion on tension. The effect of repeatedly rinsing strips in calcium-free Krebs solution was studied in 29 uteri, 8 of which were from immature gilts and 21 from sows. On the basis of histological evidence and the staircase effect, the sow uteri were classified into 10 which were oestrogen-dominated and 11 which were progesterone-dominated.

The results are summarized in Fig. 2, which shows that tension was reduced most rapidly in the oestrogen-dominated strips. A statistical comparison of the 2 groups of sow uteri was made by determining graphically the time at which the electrically induced tension in each strip was reduced to 50% of the steady state tension. The difference between the 2 groups is highly significant ($P < 0{\cdot}01$).

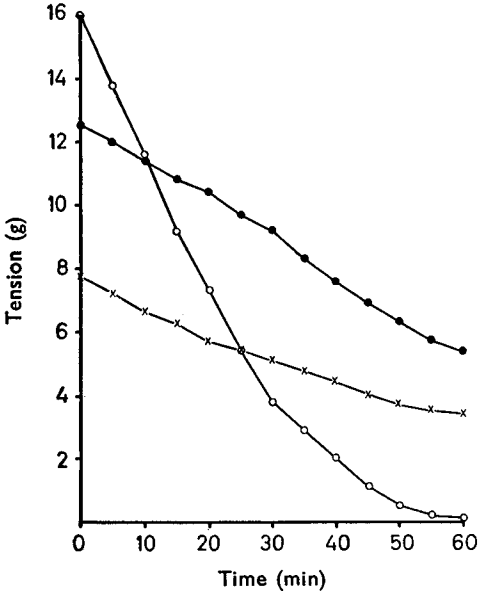

FIG. 2. The rate of reduction of electrically-induced tension in calcium free Krebs solution in strips at different stages of the oestrus cycle. The open circles represent the mean tensions of 10 oestrogen-dominated strips. The filled circles and the crosses represent respectively the mean results from 11 progesterone-dominated strips and 8 strips from immature gilt uteri. At zero time, calcium-free Krebs solution was substituted for normal Krebs solution.

Effects of progesterone. In these experiments, 6 uteri in the oestrus stage of the cycle were used; during this stage spontaneous motility is pronounced and regular, thus any effects of progesterone could be unequivocably demonstrated, and furthermore could be accurately

expressed as a percentage reduction in tension. In all cases spontaneous motility was completely abolished by 60 μg progesterone/ml. Progesterone also reduced the tension of electrically induced contractions, and Fig. 3 shows that there is a satisfactory correlation between dose and response.

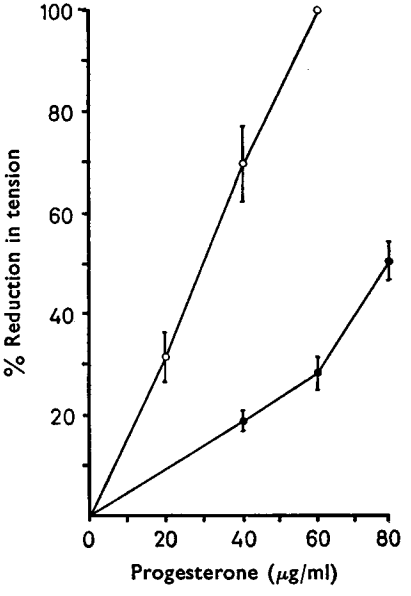

FIG. 3. The reduction in tension produced by progesterone on spontaneous contractions (open circles) and electrically-induced contractions (filled circles). Each point represents the mean of 6 experiments, and the bars indicate $\pm$s.e. of the mean.

Discussion

This investigation has shown that the type of staircase recorded from the pig myometrium is correlated with the stage of the oestrus cycle. The evidence for the staircase effect in the pig uterus is circumstantial, for final proof would necessitate ovariectomising animals and treating some with oestrogen and others with progesterone before recording staircases. Nevertheless, it is concluded that the staircase effect (with the exception of the uteri in oestrus) is similar to that described in the rabbit (Csapo & Corner, 1952; Schofield, 1954) and sheep (Bengtsson & Schofield, 1960).

The threshold voltage required to produce an isometric contraction in the oestrogen-dominated pig uterus is not significantly different from that in the progesterone-dominated uterus. This is contrary to the finding of Csapo & Goodall (1954) that in the rabbit, the progesterone-dominated uterus has a significantly higher threshold for electrical stimulation than the oestrogen-dominated uterus. Their results for the rabbit are in accord with the observations that progesterone influences the myometrium by raising the membrane potential to a higher value than that in the oestrogen-dominated myometrium (Goto & Csapo, 1959; Kuriyama & Csapo, 1961;

Marshall & Csapo, 1961). The evidence that the membrane potential in the progesterone-dominated myometrium is greater than that in the oestrogen-dominated myometrium is not however, unequivocal, for in the rat, Jung (1964) found that there was no difference in membrane potential between oestrogen-dominated and progesterone-dominated uteri. The results of the present study are more readily explicable in the light of Jung's (1964) observations.

Csapo (1950) demonstrated that the synthesis of actomyosin in the myometrium is directly related to the influence of oestrogen and is not affected by progesterone. Further, it has also been shown (Csapo & Corner, 1953) that, other factors being equal, the tension developed by the myometrium to optimal electrical stimulation is proportional to the actomyosin concentration. It is concluded, therefore, that the greater tension produced by electrical stimulation of the sow myometrium when compared with the gilt, is due to a higher concentration of contractile protein in the former, produced by the influence of oestrogen. Unpublished observations by Csapo (cited by Reynolds, 1951), in which actomyosin concentration in the sow myometrium was measured directly, revealed no differences in concentration at different stages of the oestrus cycle. It is therefore concluded that the tension induced by electrical stimulation in the progesterone-dominated myometrium is less than that in the oestrogen-dominated myometrium, not because of any difference in actomyosin concentration, but because of the effect of progesterone. This conclusion is further substantiated by the experiments in which progesterone was added to tissue baths containing strips from uteri in oestrus. There was a reduction both in the tension developed during spontaneous contraction and in the tension developed to optimal electrical stimulation, with a correlation between dose and response. It has also been demonstrated that progesterone depresses uterine motility *in vitro* in the rabbit (Kuriyama & Csapo, 1959), rat, human (Jung, 1964) and the guinea-pig (Sullivan, 1963). The finding that the mean steady state tension in the progesterone-dominated group of sow uteri is less than the mean steady state tension in the oestrogen dominated group, despite similar actomyosin concentrations in each, is in agreement with conclusions drawn by Schofield (1955) from *in vivo* studies in rabbit uteri.

The role of calcium in the response of smooth muscle to drugs has been discussed by Daniel (1964). The present work has shown that the progesterone-dominated sow myometrium binds calcium more strongly than the oestrogen-dominated myometrium; similar findings have been reported in rabbits (Csapo, 1956; 1961), but preliminary studies in the guinea-pig (Schofield, 1964) have not shown this effect. Although the evidence as yet is insufficient to draw firm conclusions, the different effects of the female sex hormones on calcium binding in the myometrium may help to explain the relative refractoriness of the progesterone-dominated uterus to stimulation.

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