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CHANGES IN ELECTRICAL PROPERTIES OF RAT MYOMETRIUM DURING GESTATION AND FOLLOWING HORMONAL TREATMENTS

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SUMMARY

1. The membrane properties of the rat myometrium, during gestation and following ovarian hormone treatment, have been investigated with the micro-electrode technique.

2. Spontaneously generated bursts of electrical activity alternating with silent periods were recorded from non-pregnant, pregnant and post-partum myometria. The membrane potential was highest during the middle stage of gestation, but the spike amplitude within a burst was not uniform. In the final stage of gestation and during parturition, the membrane potential was low and the spikes within a burst were of low frequency and uniform amplitude.

3. During parturition and post-partum, a gradual depolarization of the membrane, accompanied by an increase in membrane resistance, occurred before the generation of a burst.

4. Excitability of the membrane fluctuated from a peak just before the generation of a burst to a low after the cessation of a burst.

5. Displacement of the membrane potential by electrical current or by lowering the temperature modified the slope of the slow spontaneous depolarization, but the fluctuations of excitability persisted. The Q_{10} value for the frequency of spontaneous bursts, measured between 36 and 30° C, was 3·8.

6. Hyperpolarization of the membrane increased the maximum rate of rise of the spike, but beyond -70 mV, the rate of rise was reduced. Half-inactivation of spike generation occurred at a membrane potential less negative than the interburst potential, indicating that the current carrying system was not fully activated during parturition.

7. In both normal and spayed rats, oestradiol hyperpolarized the membrane and the burst of spikes was generated on a sustained depolarization. Progesterone slightly hyperpolarized the membrane and

burst discharges occurred without a sustained depolarization. Simultaneous treatment with progesterone and oestradiol produced a plateau potential of long duration during burst discharges.

8. The thickness of the muscle layer, length constant of the tissue and time constant of the membrane were measured during gestation and from spayed rats under various hormonal conditions. The length constant of the tissue was increased by oestradiol and was further increased by simultaneous treatment with oestradiol and progesterone. The increase in tissue thickness appeared to have the most marked influence on the length constant.

9. The resting and active membrane properties of the progesterone treated myometrium were similar to those observed during the middle stages of gestation. The oestradiol-treated myometrium did not resemble that during the last stages of gestation and parturition, which was simulated by combination of the two hormones, oestradiol preceding progesterone.

INTRODUCTION

Bozler (1941) recorded, simultaneously, the electrical and mechanical activity of the uterus, and reported that during anoestrus the muscle is electrically inexcitable and exhibits very weak and uncoordinated contractions. Treatment with oestrogen, however, was shown to increase myometrial excitability and to initiate strong spontaneous contractions. Periodic changes of myometrial activity are known to occur during the oestrous cycle (e.g. Reynolds, 1949, 1965).

Membranes of the pregnant and post-partum rat myometrium produce spontaneous bursts of electrical discharges between periods of quiescence. A slow depolarization of the membrane preceding each burst (prepotential or pacesetter potential) can be recorded from most muscle fibres during parturition and post-partum but not always during the pregnancy. A similar slow depolarization accompanied by a gradual increase in membrane resistance has also been observed for oestradiol and progesterone treated guinea-pig myometrium (Bülbring & Kuriyama, 1973). It has been shown in rat myometrium that the membrane excitability during gestation differs from that during post-partum and is not related to the membrane potential. Moreover, periodic excitability changes are not a consequence of the specific membrane properties of the pacesetter cells but occur also in the propagating cells (Casteels & Kuriyama, 1965).

Suppression of uterine motility during pregnancy is known as 'inactivation' (Reynolds, 1965) or 'progesterone block' (Csapo, 1956), and has also been referred to as 'the defence mechanism of pregnancy' (Csapo, 1956, 1961). During the early and middle stages of gestation the uterus is called

'the progesterone dominated uterus' (Csapo, 1961; Marshall, 1962). This term is also applied to the uteri of spayed animals which have been primed with oestrogen and then treated with progesterone. During the last stage of gestation, parturition, and following delivery, when uterine motility is augmented, the condition is known as 'the oestrogen dominated uterus' (Csapo, 1961; Marshall, 1959, 1962, 1970; Abe, 1971), and this refers also to the uterus of spayed animals injected with oestrogen.

Though the specific effects of ovarian hormones on the electrophysiological properties of the myometrium have been studied by many investigators (see reviews by Abe, 1970; Marshall, 1970) the factors which determine the pattern of the spontaneous activity and the changes in the passive electrical membrane properties are not yet understood.

The present experiments investigate further the effects of ovarial hormones, administered *in vivo*, on the electrical properties of the rat myometrial membrane in comparison with the physiological changes during gestation.

METHODS

White rats of the Wistar–King variety were used for all experiments (fifty-two non-pregnant, pregnant and post-partum rats and 84 spayed and hormone treated rats). Normal virgin females were placed in separate cages with a male rat and allowed to copulate. The period of gestation was calculated from the day after the male and female were placed together. The time after delivery was calculated from the birth of the first rat. For comparison, virgin female rats (200–250 g and 2–3 months old) were anaesthetized with Nembutal (30 mg/kg; sodium pentobarbitone, Abbott Lab.) and picrotoxin (1 mg/kg; Wako Pure Chem. Ind. Ltd) by intraperitoneal injection and ovariectomized. Seven to ten days after ovariectomy, either progesterone (10 mg/day; synthetic progesterone, Mochida Pharm. Co. Ltd), oestradiol (10 μg/day; oestradiol-17 benzoate, Teikokuzoki, Pharm. Co. Ltd) or both simultaneously was injected subcutaneously. For one series of the experiments twenty rats were used. The following symbols define the hormone treatment: P_n = n days treatment with progesterone, E_n = n days treatment with oestradiol, E_nP_n = n days treatment with progesterone after n days treatment with oestradiol successively, EP_n = n days treatment with oestradiol and progesterone simultaneously.

For electrophysiological experiments, the rats were stunned and bled. Longitudinal muscle strips (1 mm × 15 mm) were excised from the uterus near the mesometrial attachment. A muscle strip was weakly stretched across a small chamber (2·5 ml. capacity), which was divided into two compartments for electrical stimulation and recording, using the method described in detail by Abe & Tomita (1968). Glass capillary micro-electrodes filled with 3 M-KCl were used throughout. The amplitude and shape of the electrotonic potential were measured from photographic oscilloscope records and the electrical activity over longer periods was recorded using an inkwriting oscillograph.

The preparation was superfused with saline at 35° C at a rate of 3 ml./min. The saline was a modified Locke solution containing 154 mM-NaCl, 8 mM-$NaHCO_3$ 5·6 mM-KCl, 2·2 mM-$CaCl_2$ and 5·5 mM glucose, equilibrated with a mixture of 97 % O_2 and 3 % CO_2.

The drug concentrations are given in g/ml.

For structural comparisons, excised myometrial tissue, which was the same tissue as that used for electrophysiological recording, was rapidly frozen to $-20°$ C with liquid nitrogen and cut in 10 μm sections. The thickness of the longitudinal muscle layer was measured with a calibrated micrometer.

RESULTS

Changes in electrical activity recorded from the myometrium before, during and following pregnancy

The resting membrane potential of the myometrium before pregnancy was -56 mV ($\pm 3{\cdot}5$ mV S.D., $n = 50$). During gestation the resting potential gradually increased, reaching a maximum (-78 mV) on the 11th–15th day (mean value -68 mV ($\pm 3{\cdot}1$ mV S.D., $n = 50$)). In the late stages of gestation and during parturition, the membrane potential returned nearly to the pre-pregnancy level (-54 mV ($\pm 2{\cdot}8$ mV S.D., $n = 50$)). These observations, in general, confirm the observations of Casteels & Kuriyama (1965), though our values for the non-pregnant myometrium are somewhat higher (cf. -42 mV *vs.* -56 mV).

Electrical activity of the myometrial membrane at different stages is shown in Fig. 1. In all cases the electrical activity consists of burst dis-

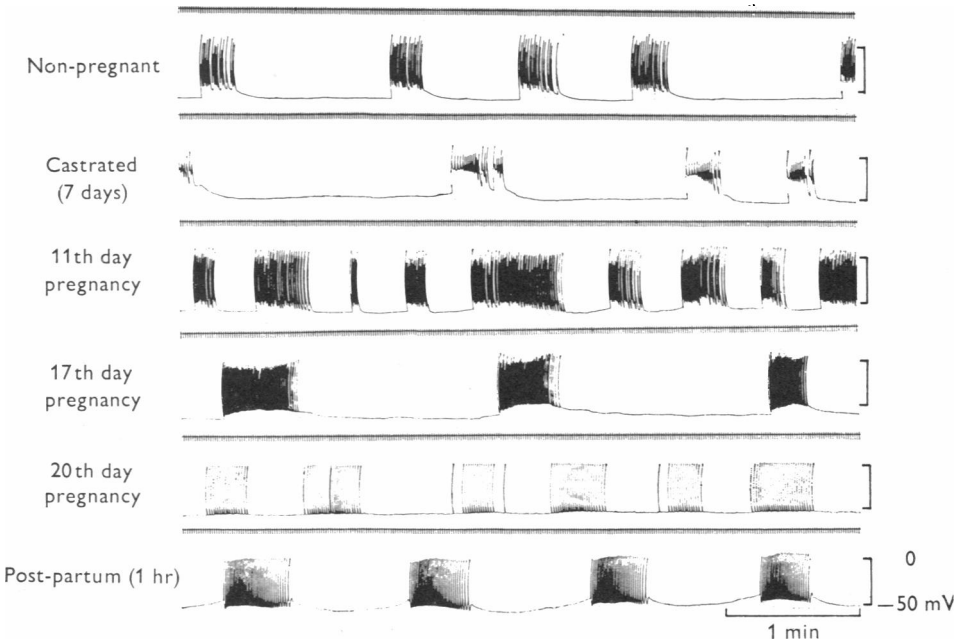

Fig. 1. Electrical activity of rat myometria recorded under various conditions as indicated.

charges alternating with silent periods. Overshooting spike potentials were recorded. At least three different patterns of electrical activity have been observed. (i) In non-pregnant and ovariectomized myometrium, the amplitude and frequency of the spikes within a burst were irregular. Especially in the ovariectomized myometrium, spikes were generated on a sustained depolarization. (ii) During mid-gestation, spikes of irregular amplitude, but with the highest frequency were generated. The maximum rate of rise and fall of the spikes was 24 ± 4 and 28 ± 3 V/sec ($n = 20$), respectively. (iii) During the last stage of gestation the amplitude of the spike in a train was regular but the maximum rate of rise and fall was lowered to 12 ± 3 and 13 ± 3 V/sec ($n = 20$) respectively.

The appearance of burst discharges depended on the degree of stretch of the muscle strip. Severe stretching resulted in continuous spike generation without a silent period. This suggests that the occurrence of bursts and silent periods may be related to physiological stretching of the muscle, though this problem has not been investigated.

Periodic changes of membrane resistance associated with the spontaneous electrical activity

Spontaneous bursts recorded from the rat myometrium during parturition are shown in Fig. 2. When inward and outward current pulses were applied to the membrane during the slow depolarization (*C* and *D*), the amplitude of the electrotonic potential gradually increased from just after the cessation of the burst until just before the generation of the next burst, i.e. the gradual depolarization of the membrane was accompanied by an increase in membrane resistance (*C*). Outward current pulses evoked an electrotonic potential of increasing amplitude until, during the progress of the depolarization, the local potential and the spike were triggered before initiation of the spontaneous burst (*D*). Tetrodotoxin (10^{-7} g/ml.) did not affect any feature of the membrane activity.

The intervals between bursts were prolonged by application of outward current pulses and were shortened by inward current pulses, i.e. the slope of the depolarization was increased by successively applied inward current pulses and decreased by outward current pulses. The results indicate that the increased amplitude of the electrotonic potential during the slow depolarization may not be simply due to the passive properties of the membrane.

The influence of the membrane potential on electrical activity

The effect of membrane potential displacement on the slope of the slow depolarization and the appearance of burst discharges are shown in Fig. 3. When the membrane potential was displaced in the depolarized direction

(+8 mV), a burst was generated but they were no longer periodic. Displacement of the membrane potential did not increase the frequency of the spikes in a burst but the over-all number of spikes was increased (*B* and *C*). When short inward current pulses were successively applied to the

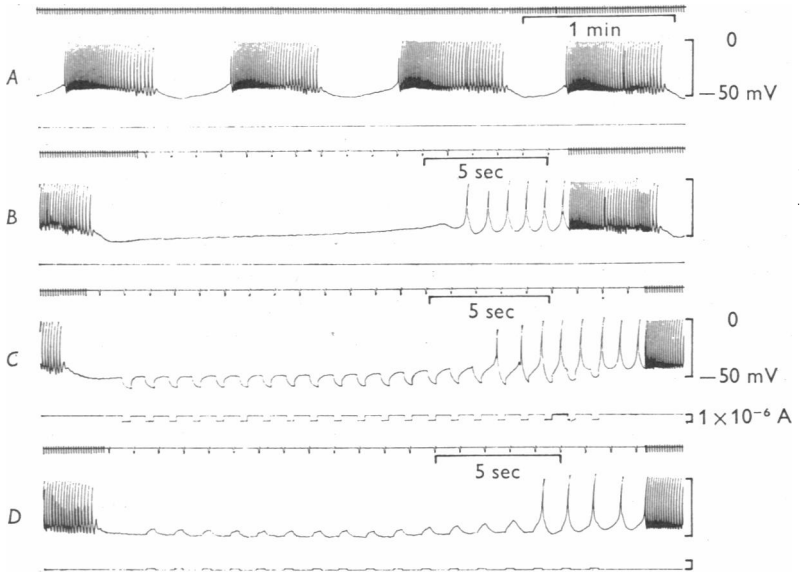

Fig. 2. Shape of the slow depolarization before generation of burst discharges and changes of the electrotonic potential during the slow depolarization (8 hr after delivery). *A* and *B*, controls at two different speeds. *C*, application of inward current pulses (300 msec). *D*, application of outward current pulses (300 msec).

tissue during conditioning depolarization, membrane responses were evoked by the cessation of the current pulses (anodal break responses); they appeared periodically and were similar to spontaneous periodic burst discharges (*C*).

When the membrane potential was displaced in the hyperpolarized direction (−9 mV), the generation of the bursts was suppressed (*D*). Successively applied inward current pulses, however, evoked anodal break responses periodically (*E*). Thus, periodical changes in membrane excitability persisted even when spontaneous spike activity was suppressed by either depolarization or hyperpolarization of the membrane. During conditioning depolarization, the slope of the slow depolarization preceding a burst becomes low, but the slope of the repolarization on breaking an anodal current pulse is high and is able to initiate spikes. On the other hand, conditioning hyperpolarization may prevent the slow depolarization from reaching firing level, while anodal break excitation is still produced.

The critical membrane potential for generation of bursts was low for a hyperpolarized membrane and high for a depolarized membrane as well as quite variable; therefore we were unable to determine quantitatively the slope of the pacesetter potential for various membrane potentials.

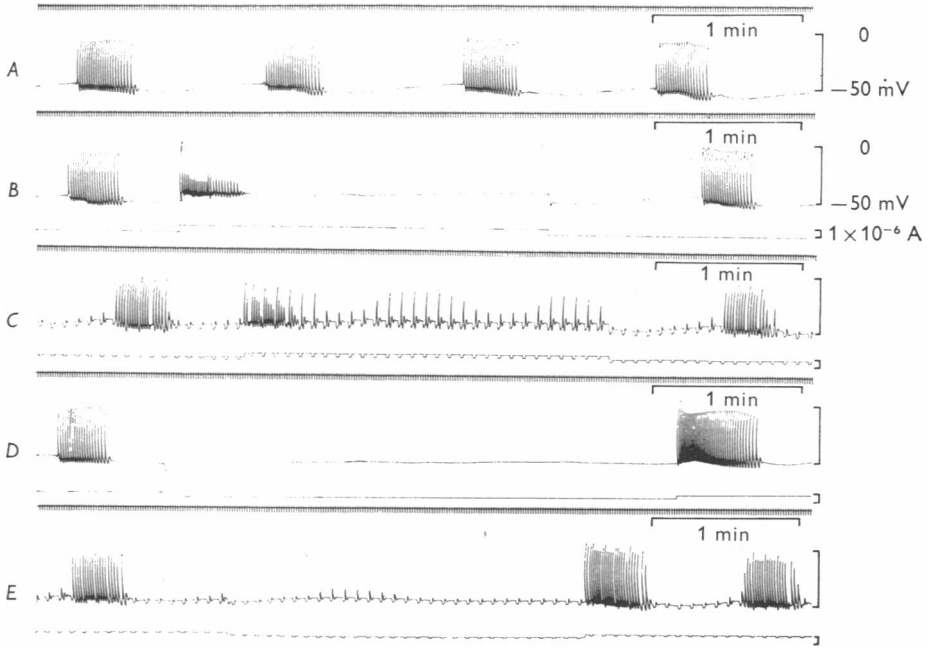

Fig. 3. Effects of membrane potential displacement on membrane activity (4 hr after delivery). *A*, control; *B*, application of outward current; *C*, effect of anodal current pulses during the application of outward current; *D*, application of inward current; *E*, effect of anodal current pulses during the application of inward current.

The influence of temperature on electrical activity

The effects of temperature on the generation of membrane activity in the post-partum rat myometrium are shown in Fig. 4. When the temperature of the organ bath was lowered from 36 to 26° C, the membrane was depolarized from $-54 \pm 2{\cdot}1$ ($n = 20$) to $-38 \pm 2{\cdot}6$ mV ($n = 20$), and the frequency of spikes within a burst decreased. The silent periods between bursts were prolonged due to reduction of the slope of the slow depolarization, which decreased in proportion to the decrease in temperature (from 36 to 29° C). The amplitude of spikes within a burst became irregular below 33° C. When the temperature was lowered to 26° C, spontaneous activity ceased (*D*). However, when a single inward current pulse was applied to the tissue, a burst was evoked as a break excitation.

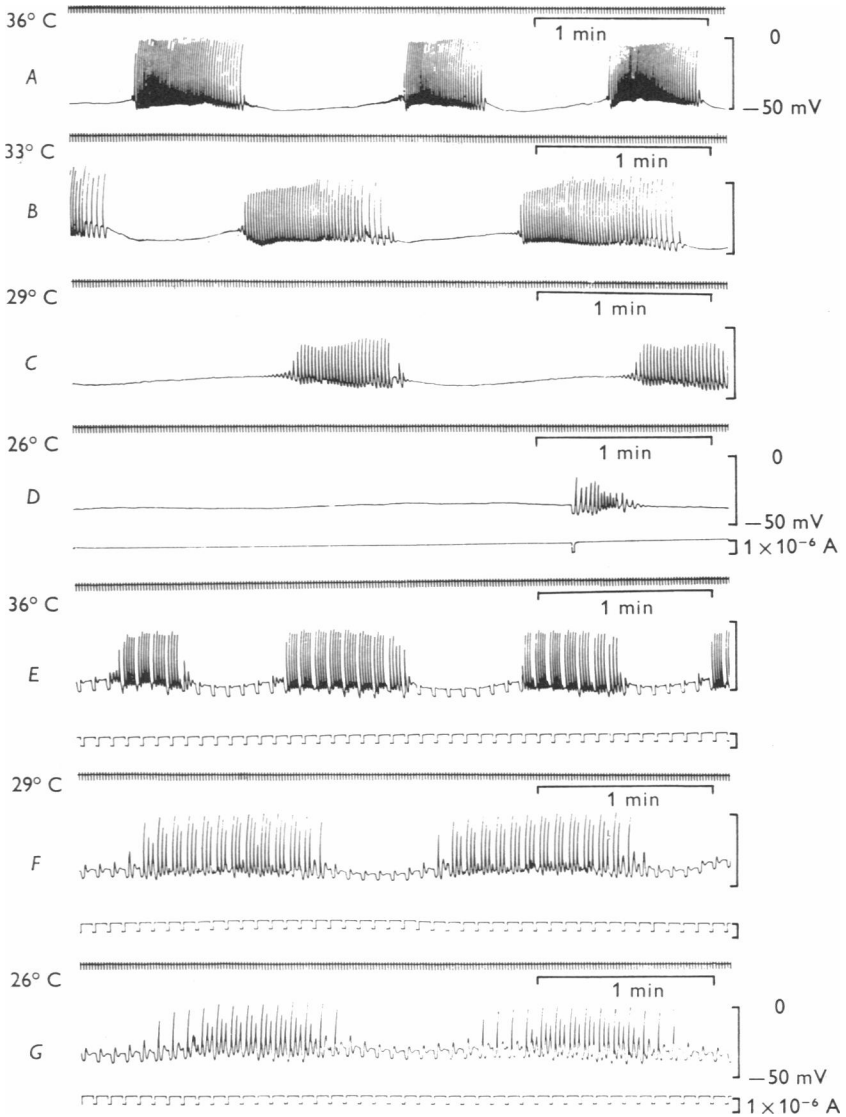

Fig. 4. Effects of lowering the temperature on membrane activity (8 hr after delivery). *A*, control, 36° C; *B*, 33° C; *C*, 29° C; *D*, 26° C; single inward current pulse (300 msec) was applied; *E*, inward current pulses (300 msec) were applied successively at 36° C; *F*, inward current pulses (300 msec) were applied successively at 29° C; *G*, inward current pulses (300 msec) were applied successively at 26° C.

The displacement of membrane potential induced both by electric current and by lowering the temperature modified the firing level of the burst discharges even though the periodic changes of excitability were preserved. As shown in Fig. 4*F* and *G*, the periodical changes of excitability were still observed at 29 and at 26° C, i.e. successively applied inward current pulses periodically evoked spikes by anodal break excitation. The amplitude of the break excitation gradually increased and then, periodically, gradually decreased again.

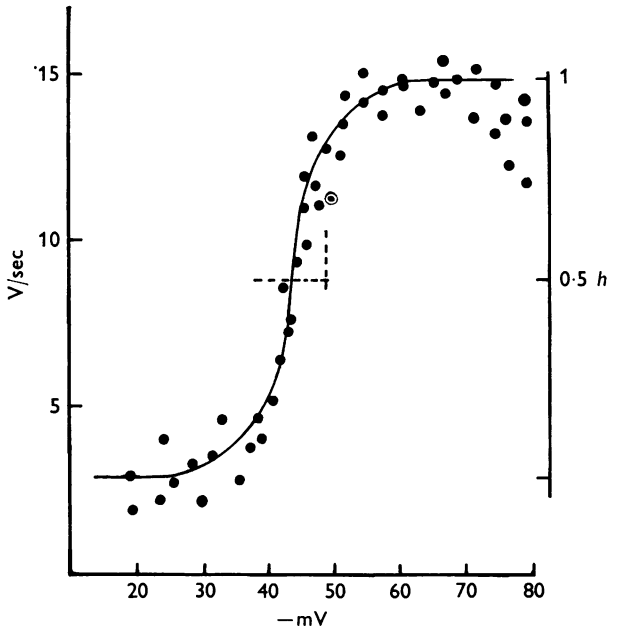

Fig. 5. Relationship between the maximum rate of rise of the spike and the membrane potential (8 hr after delivery). V/sec: the maximum rate of rise of the spike. *h*: the peak value of V/sec was recorded as unity. Interrupted vertical line indicates the resting membrane potential during the interburst period; horizontal line indicates half-activation of the maximum rate of rise of the spike (0·5 *h*).

The Q_{10} for the frequency of bursts, measured over a temperature range of 36–30° C, was 3·8 (n = 3). This value is influenced by two main factors, namely the slope of the pacesetter potential and the inactivation of the spike generating mechanism due to membrane depolarization at the lowered temperature.

Fig. 5 shows the relationship between membrane potential and the maximum rate of rise of the spike during post-partum (8 hr). Hyperpolarization of the membrane increased the maximum rate of rise. Beyond

−58 mV the maximum rate of rise of the spike did not further increase (maximum value was 14·5 V/sec). When the membrane was hyperpolarized more than −70 mV the maximum rate of rise of the spike declined. As seen in Fig. 5, the observed values lie along a sigmoidal curve, the half inactivation of spike generation ($h/2$) being −44 mV. This value was slightly more positive than the membrane potential measured during the interburst period.

Electrical activity of hormone treated myometria

The patterns of electrical activity following hormonal treatment of castrated rats are shown in Fig. 6. The myometrium of spayed rats exhibited spikes of irregular amplitude which were generated on a more or less sustained depolarization (*A–C*). Depolarization of the membrane during the generation of a burst discharge was more frequently recorded and better maintained after treatment with oestradiol (E_6 in *D* and E_5 in *G*). The sustained depolarization (plateau formation) was triggered by the initial spike in a train. On the other hand, in the progesterone treated myometrium (P_8 in *E*), the burst discharge of higher frequency appeared without a plateau. The sustained depolarization was gradually reduced when oestradiol treatment was followed by progesterone (*G–I*), and gradually enlarged in amplitude when progesterone treatment was followed by oestradiol (*F–L*). Clear differences were observed after treatment with either hormone alone (*D*, *E*, *G*, *J*). However, when oestradiol and progesterone were administered simultaneously, the influence of oestradiol was dominant and caused a sustained depolarization of more than 30 sec duration (EP_6 in *F*). Although the dose of progesterone administered (10 mg/day) was 1000 times greater than that of oestradiol (10 μg/day), oestradiol had a much greater effect on the membrane activity.

The membrane potential of myometrium from spayed rats increased significantly following oestradiol treatment (E_6) from $-52 \pm 3{\cdot}5$ to $-65 \pm 2{\cdot}8$ mV ($n = 50$). Following progesterone treatment (P_5), the membrane potential was only slightly increased ($-56 \pm 3{\cdot}1$ mV, $n = 50$) except after pretreatment with oestradiol (E_5P_6) ($-64 \pm 2{\cdot}5$ mV, $n = 50$).

The effects of various hormones on the myometrium of non-castrated virgin rats are shown in Fig. 7. At least three different patterns of burst discharges (*A–C*) were observed in the non-pregnant myometrium, probably associated with the oestrous cycle, but this was not examined in detail.

In the presence, as in the absence, of the ovary, the oestradiol-treated myometrium generated bursts with a sustained plateau formation, whereas the progesterone-treated myometrium generated bursts without plateau formation (*D–G*). Following the administration of oestradiol (3 days),

simultaneous treatment with oestradiol and progesterone up to 3 days still produced a marked plateau potential (*i*). However, continued treatment with progesterone decreased the plateau, until, after 5 days, it was no longer recorded.

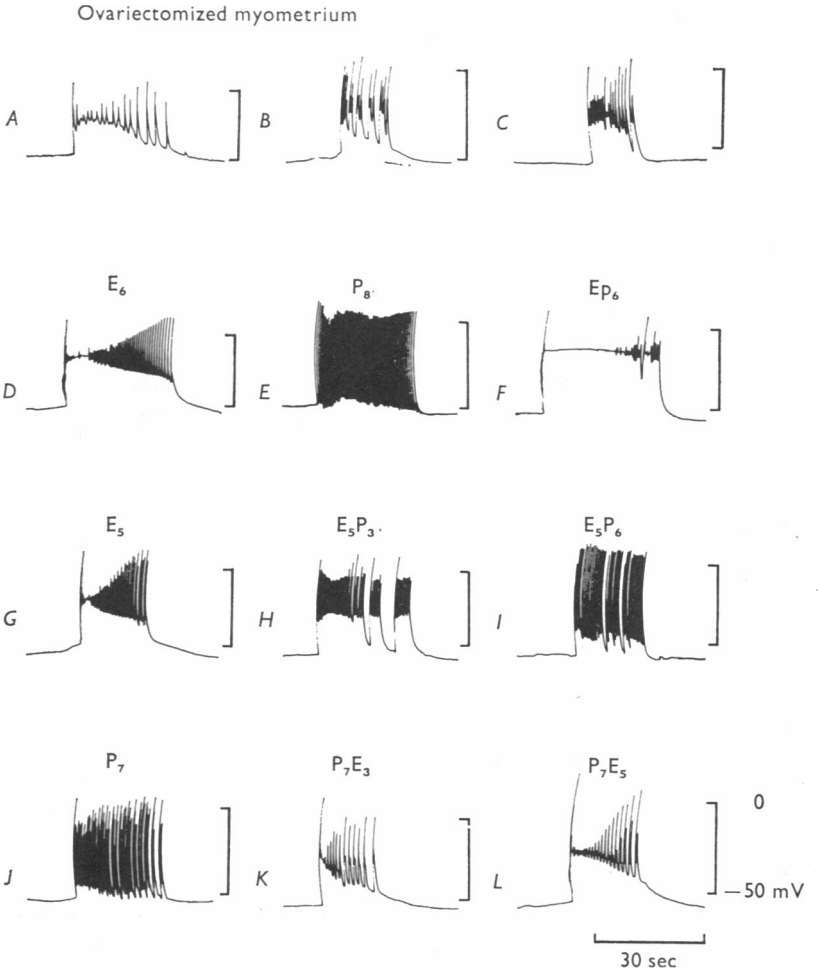

Fig. 6. Electrical activity recorded from hormone-treated myometria of castrated rats. *A–C*, non-treated myometria. *D–L*, hormone-treated myometria. See text for explanation of symbols.

To summarize. (i) Ovarian hormones produce the same patterns of electrical activity in the presence or absence of the ovary; (ii) the oestradiol-treated myometrium generates a sustained depolarization on which the burst of spikes is superimposed, but the progesterone-treated myometrium does not generate a sustained depolarization; (iii) if the two hormones are

administered one after the other, the pattern of electrical activity is determined by the second hormone; (iv) if the two hormones are given simultaneously, the activity pattern resembles more closely that recorded after oestradiol treatment than that after progesterone treatment; and (v) the so called 'oestrogen-dominated myometrium' and 'oestradiol-treated myometrium' produce bursts of different shape.

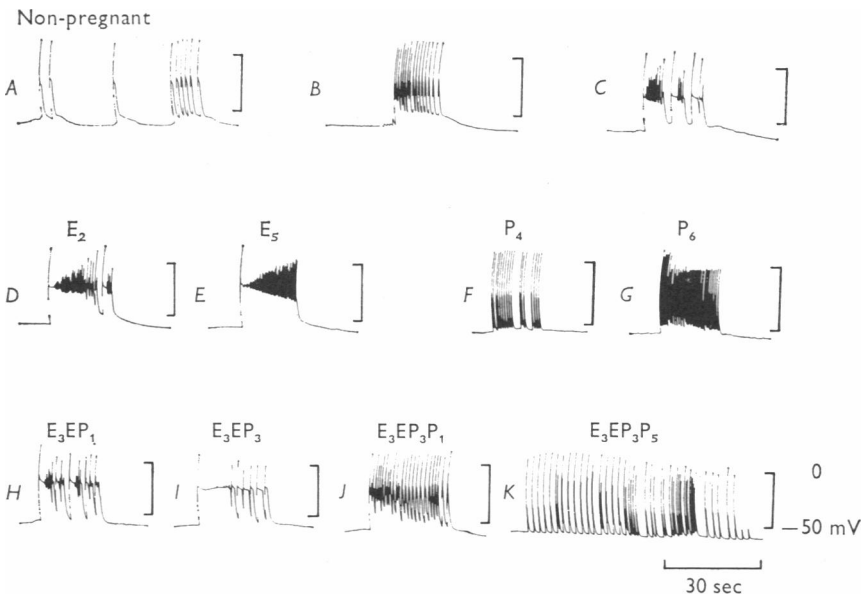

Fig. 7. Train discharges recorded from hormone-treated myometria. *A–C*, patterns of electrical activity recorded from non-pregnant, virgin rat myometria. *D–K*, hormone-treated myometria. See text for explanation of symbols.

Passive electrical properties of rat myometria

The length constant (λ) of the longitudinal muscle of the myometrium was determined by applying square pulses of 1 sec duration to the tissue and recording the current–voltage relationship at various distances from the stimulating electrode. This was roughly linear between the resting membrane potential level and the amplitude of the electrotonic potential up to about 20 mV hyperpolarization. Fig. 8 shows three examples recorded from the myometrium of spayed rats. Records of electrotonic potentials produced by inward current pulses and the spike evoked by outward current pulses at three different distances from the stimulating electrode are shown. The length constant of the myometrium, 7 days after ovariectomy, was 1·3 mm (S.D. = $\pm$0·2 mm, n = 11). This value is nearly the

same as that measured from the non-pregnant myometrium ($1{\cdot}5 \pm 0{\cdot}2$ mm, $n = 7$).

The time constant of the membrane (τ_m) was determined from a plot of the half-time of the final steady amplitude of the electrotonic potential against distance from the stimulating electrode (Hodgkin & Rushton, 1946). The time constants of the longitudinal muscle membrane of the

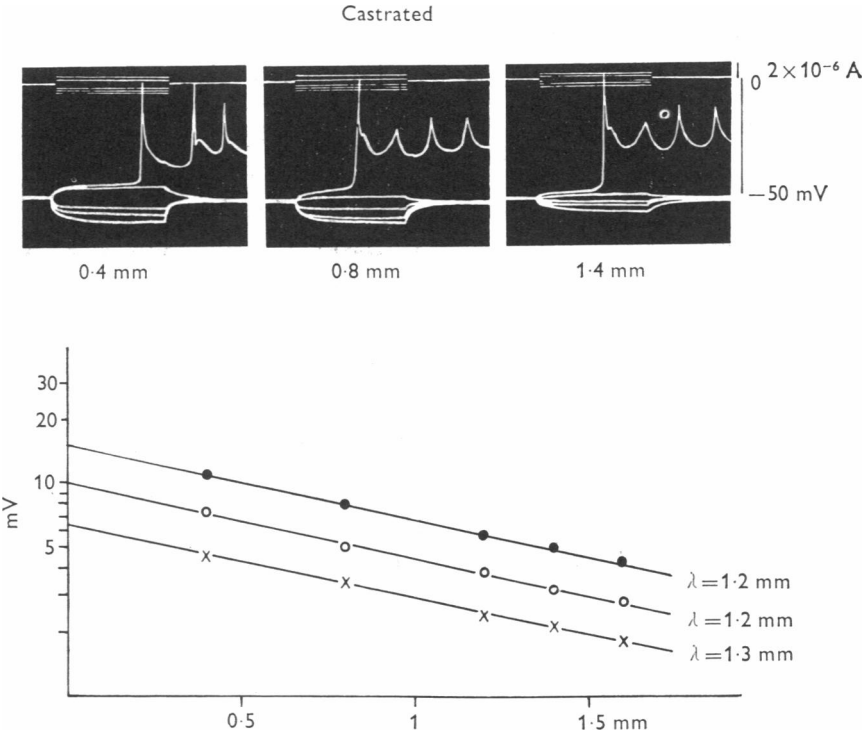

Fig. 8. Changes in amplitude of the electrotonic potential recorded at various distances from the stimulating electrode. Upper records: the electrotonic potentials and the spikes were recorded following inward and outward current pulses at distances of 0·4, 0·8 and 1·4 mm. Lower graph: relationship between the logarithm of the amplitude of the electrotonic potential and distance from the stimulating electrode. Amplitudes of the electrotonic potential were measured at three different intensities. The length constant (λ) was calculated from the decay of the electrotonic potential at e^{-1}.

spayed rat and of the non-pregnant myometrium were found to be 114 and 128 msec, respectively.

The effects of inward and outward current pulses (1 sec) on the myometrium in two different conditions (19th day of gestation and parturition) are shown in Fig. 9. Membrane responses were recorded at four different

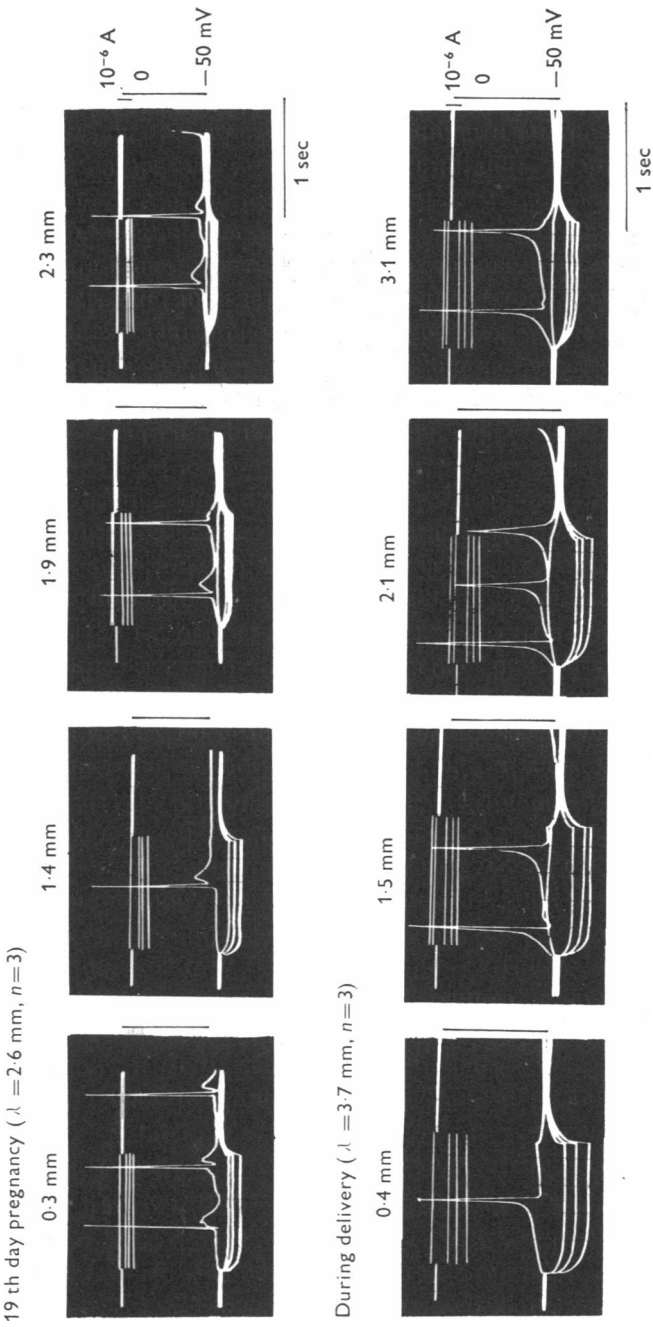

Fig. 9. Changes of electrotonic potential and spike evoked by inward and outward current pulses at different intensities. The records were obtained from the myometrium after 19 days pregnancy (upper records) and during parturition (lower records). Distances were measured between the recording and stimulating electrode. Mean length constants are indicated for both experimental conditions.

distances from the stimulating electrode. The shape of the spike evoked by outward current pulses differed at these two stages. An after-depolarization is apparent in records taken on the 19th day of gestation but not during parturition. The spatial decay of the electrotonic potential was faster at 19 days pregnancy than during parturition. The mean values of the length constant measured at the middle stage (13–15th day), last stage of gestation (19th–20th day) and just after delivery were $2{\cdot}5 \pm 0{\cdot}5$ mm ($n = 8$), $2{\cdot}9 \pm 0{\cdot}6$ mm ($n = 7$) and $3{\cdot}2 \pm 0{\cdot}3$ mm ($n = 8$), respectively. These values are significantly greater than the values obtained from myometria of non-pregnant and spayed rats. Table 1 summarizes the various

TABLE 1. Passive properties of the membrane during physiological changes

	Membrane potential (−mV, $n = 50$)	Maximum rate of rise of the spike (V/sec, $n = 20$–30)	Length constant (mm, $n = 6$–8)	Time constant (msec)
Non-pregnant	$56 \pm 3{\cdot}5$	16 ± 6	$1{\cdot}5 \pm 0{\cdot}2$	128
Middle stage of gestation (11–15 day)	$68 \pm 3{\cdot}1$	24 ± 4	$2{\cdot}5 \pm 0{\cdot}5$	180
Late stage of gestation (18–19 day)	$64 \pm 2{\cdot}6$	21 ± 3	$2{\cdot}6 \pm 0{\cdot}4$	198
Last day and during parturition	$54 \pm 2{\cdot}8$	12 ± 2	$2{\cdot}9 \pm 0{\cdot}6$	228
Post-partum (6–10 hr)	$51 \pm 2{\cdot}1$	10 ± 2	$3{\cdot}2 \pm 0{\cdot}3$	241

membrane properties of the myometria before, during and just after the delivery.

After treatment with oestradiol (E_6), the length constant increased to $2{\cdot}5 \pm 0{\cdot}2$ mm ($n = 7$). Treatment with progesterone (P_4) did not increase the length constant: $1{\cdot}0 \pm 0{\cdot}4$ mm ($n = 5$). When progesterone was administered after pretreatment with oestradiol (E_5P_6), the length constant increased from $1{\cdot}3 \pm 0{\cdot}2$ to $1{\cdot}8 \pm 0{\cdot}1$ mm ($n = 8$). This value is larger than that produced by progesterone alone (P_4) but smaller than that produced by oestradiol (E_6). The results suggest that the length constant is increased by oestradiol, but slightly decreased by progesterone. When progesterone and oestradiol were given simultaneously (EP_6), the length constant was $2{\cdot}6 \pm 0{\cdot}6$ mm ($n = 8$). Moreover, when progesterone and oestradiol were given simultaneously following pretreatment with oestradiol (E_3EP_3), the length constant increased to $2{\cdot}8 \pm 0{\cdot}4$ mm ($n = 3$). This value is nearly the same as that observed during the last stage of gestation.

The thickness of the longitudinal muscle layer in the myometrium of castrated rats was 52 ± 10 μm S.D. ($n = 100$) and this value increased to

100 ± 12 μm S.D. ($n = 104$) after treatment with oestradiol alone (E_6), and to 194 ± 18 μm S.D. ($n = 100$) after simultaneous treatment with oestradiol and progesterone (EP_6). After treatment with progesterone alone (P_7), the tissue diameter was 56 ± 13 μm S.D. ($n = 104$). Table 2 summarizes the various passive properties of the membrane following hormonal treatment calculated from cable equations.

TABLE 2. Passive membrane properties following hormonal treatments

	Membrane potential ($-$mV $\pm$ S.D. n = 50–100)	Thickness of the longitudinal muscle layer (μm $\pm$ S.D. n = 60–120)	Length constant (mm $\pm$ S.D. n = 3–11)	Time constant (msec)
Castrated	$52 \pm 3{\cdot}5$	52 ± 10	$1{\cdot}3 \pm 0{\cdot}2$	114
P_6	$56 \pm 3{\cdot}1$	56 ± 13	$1{\cdot}0 \pm 0{\cdot}4$	82
E_6	$65 \pm 2{\cdot}8$	100 ± 12	$2{\cdot}5 \pm 0{\cdot}2$	135
E_5P_6	$64 \pm 2{\cdot}5$	89 ± 15	$1{\cdot}8 \pm 0{\cdot}1$	112
EP_6	$68 \pm 2{\cdot}1$	194 ± 18	$2{\cdot}6 \pm 0{\cdot}6$	197
E_3EP_3	$66 \pm 2{\cdot}4$	185 ± 16	$2{\cdot}8 \pm 0{\cdot}4$	181

DISCUSSION

Spontaneous electrical activity of the rat myometrium

The observations described indicate that periodical changes of excitability and of the activation mechanism for spike generation are the basis for the rhythmic co-ordinated membrane activity in the rat myometrium. Spontaneous electrical discharges appear as bursts of spikes alternating with quiescent periods of 10–60 sec duration. At short intervals there are few spikes within a burst; at long intervals there are many. During the fluctuations from the active to the inactive state, a change of excitability occurs such that, during the silent period, a stimulus fails to evoke a spike until shortly before a spontaneous burst. These excitability changes occur also during gestation but without any marked change of the membrane potential (Casteels & Kuriyama, 1965). The periodical changes of excitability are also observed during the suppression of the spontaneous slow depolarization and spike generation by either cooling or current injection. Therefore the excitability changes may not be directly related to the slow depolarization of the membrane. Moreover, when the stimulus intensity is increased, spikes are regularly triggered in the pregnant rat myometrium, but the generation of bursts is suppressed (Casteels & Kuriyama, 1965; Abe, 1970; E. Bülbring, personal communication).

The pacesetter potential, i.e. the gradual depolarization leading to a

burst of spikes, was recorded from all parts of the longitudinal muscle layer during parturition. The membrane depolarization was associated with a gradual increase of membrane resistance, presumably due to a reduction of K conductance. Tomita & Watanabe (1973) postulated for the guinea-pig taenia coli that the reduction of K conductance may be caused by acceleration of a Ca-pump activity which reduces the amount of Ca bound at the inner surface of the cell membrane. Bülbring & Kuriyama (1973) assumed a similar mechanism for the guinea-pig myometrium. However, we have no direct evidence yet for a causal relationship between the pacesetter potential and a change of K conductance by Ca pumping at the membrane. The spike in the rat myometrium may be partly due to a Ca and partly to a Na current, and, in the non-pregnant uterus, under normal physiological conditions, inactivation of the inward current carrying system is produced at a low membrane potential. During parturition, the membrane potential, the maximum rate of rise of the spike and the spike frequency in the bursts were much lower than those recorded during the middle stage of gestation. Under these circumstances, a slight depolarization of the membrane rapidly reduced the maximum rate of rise of the spike and blocked spike generation, while hyperpolarization beyond -70 mV also reduced the rate of rise.

So far, then, three factors seem to contribute to the manifestation of rhythmic activity: fluctuations of the membrane excitability, the membrane resistance and the nature of activation and inactivation of the inward current carrier. The integration of these factors, however, has to be clarified further.

Ovarian hormones and rat myometrium

The resting membrane potential of the myometrium differs in different species in different states of hormonal influence (Abe, 1970). Casteels & Kuriyama (1965) showed that the K equilibrium potential remained constant at about -70 to -80 mV under different hormonal conditions, and that the gradual increase in membrane potential during the progress of gestation was probably due to an increase in K permeability, while the depolarization of the membrane during the last stage of gestation and parturition was due to an increase in Na permeability.

In the present investigation an attempt was made to correlate the physiological changes of membrane potential with the effect of individual ovarian hormones. It was found that the term 'oestrogen dominated myometrium', generally used to describe the behaviour of the uterus at the end of gestation, during parturition and following delivery, is incorrect since its pattern of activity does not resemble that of the 'oestradiol treated myometrium'. The oestradiol treated myometrium showed a

higher membrane potential than that at the end of pregnancy and also higher than the progesterone-treated myometrium. Moreover the shapes of the train discharges recorded from the oestradiol-treated myometrium were markedly different from those recorded from the myometrium during the last stage of gestation and parturition. The former developed a plateau potential during a burst but not the latter. On the other hand the progesterone-treated myometrium, after pre-treatment with oestradiol, had nearly the same membrane activity as was observed at the end of pregnancy. This result agrees with observations on guinea-pig myometrium (Bülbring, Casteels & Kuriyama, 1968) in which the increase of the membrane potential on the last day of pregnancy was similar to that produced by 8 daily injections of oestradiol and additional progesterone on the last 4 days (corresponding to E_4EP_4).

During the middle stage of gestation, the behaviour of the myometrium is nearly the same as that induced by progesterone treatment, but by the last stage of gestation and during parturition it is strongly influenced by oestradiol.

The length constant of the myometrium increased with the progress of gestation and oestradiol injection, but not with progesterone treatment. Since the myometrium possesses cable properties, the length constant of the tissue can be expressed by the following equation (Hodgkin & Rushton, 1946): $\lambda = \sqrt{(\frac{1}{2}a)} . \sqrt{(R_m/R_i)}$, where a is the radius of the functional syncytium, R_m is the membrane resistance and R_i is the longitudinal resistance of the myoplasm, including intercellular junctional resistances. In the myometrium, hormonal treatment markedly increased the thickness of the muscle layer. For instance, treatment with oestradiol (E_6) increased the thickness of the longitudinal muscle layer from 52 μm to 100 μm, and simultaneous treatment with progesterone and oestradiol (EP_6) increased the thickness to 194 μm. After treatment with progesterone, however, the thickness was not increased (56 μm). The ratio of the increase in thickness to the thickness of non-pregnant myometrium, by EP_6, E_6 and P_6 is 2, 4, and 1 respectively. The increase of the above values following hormone treatments are due to increase in numbers and diameter of the cells. The radius of the functional syncytium, expressed as a, was not obtained from the present experiments. However, it is conceivable that the change in length constant of the tissue might be related to the changes in the morphological structures rather than a relative change of R_m/R_i ratio. These calculated values suggest that oestradiol treatment modifies the membrane properties more markedly than does progesterone treatment.

The change in membrane properties during pregnancy cannot be explained by the action of one hormone alone; more specifically, the metabolic alterations of the myometrium *in situ* are probably due not only to

progesterone and oestradiol but also to other factors, such as relaxin, adrenocorticosteroids, testosterone, and their metabolites, all acting in concert.

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REFERENCES

ABE, Y. (1970). The hormonal control and the effects of drugs and ions on the electrical and mechanical activity of the uterus. In *Smooth Muscle*, ed. BÜLBRING, E., BRADING, A., JONES, A. & TOMITA, T., pp. 396–417. London: E. Arnold.

ABE, Y. (1971). Effects of changing the ionic environment on passive and active membrane properties of pregnant rat uterus. *J. Physiol.* **214**, 173–190.

ABE, Y. & TOMITA, T. (1968). Cable properties of smooth muscle. *J. Physiol.* **196**, 87–100.

BOZLER, E. (1941). Influence of estrone on the electric characteristics and the motility of uterine muscle. *Endocrinology* **29**, 225–227.

BÜLBRING, E., CASTEELS, R. & KURIYAMA, H. (1968). Membrane potential and ion content in cat and guinea-pig myometrium and the response to adrenaline and noradrenaline. *Br. J. Pharmac.* **34**, 388–407.

BÜLBRING, E. & KURIYAMA, H. (1973). The action of catecholamines on guinea-pig taenia coli. *Phil. Trans. R. Soc.* B **265**, 115–121.

CASTEELS, R. & KURIYAMA, H. (1965). Membrane potential and ionic content in pregnant and non-pregnant rat myometrium. *J. Physiol.* **177**, 263–287.

CSAPO, A. (1956). Progesterone 'block'. *Am. J. Anat.* **98**, 273–291.

CSAPO, A. (1961). Defence mechanism of pregnancy. *Progesterone and the Defence Mechanism of Pregnancy. Ciba Fdn Study Grps* **9**, 3–27.

HODGKIN, A. L. & RUSHTON, W. A. H. (1946). The electrical constants of a crustacean nerve fibre. *Proc. R. Soc.* B **133**, 444–479.

MARSHALL, J. M. (1959). Effects of estrogen and progesterone on single uterine muscle fibers in the rat. *Am. J. Physiol.* **197**, 935–942.

MARSHALL, J. M. (1962). Regulation of activity in uterine smooth muscle. *Physiol. Rev.* **42**, 213–227.

MARSHALL, J. M. (1970). Adrenergic innervation of the female reproductive tract: anatomy, physiology and pharmacology. *Ergebn. Physiol.* **62**, 6–67.

REYNOLDS, S. R. M. (1949). *Physiology of the Uterus*, 2nd edn. New York: Hoeber.

REYNOLDS, S. R. M. (1965). *Physiology of the Uterus*, 2nd edn. New York: Hafner.

TOMITA, T. & WATANABE, H. (1973). Factors controlling myogenic activity in smooth muscle. *Phil. Trans. R. Soc.* B **265**, 73–85.