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MEMBRANE POTENTIAL AND IONIC CONTENT IN PREGNANT AND NON-PREGNANT RAT MYOMETRIUM

By R. CASTEELS* and H. KURIYAMA
From the Department of Pharmacology, University of Oxford

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It is well known that the membrane potential and excitability of uterine muscle as well as the production of tension are under hormonal influence. Thiersch, Landa & West (1959) observed that, during the progress of gestation, the membrane potential of the rat myometrium gradually increased and that the placental site had a higher membrane potential than non-placental sites. Goto & Csapo (1959) extended the observations to the post-partum uterus, using rabbits, and they found that the membrane potential rapidly fell within 2 days after delivery to the same level as that before pregnancy. Similar observations have been made by other workers on different animal species (for references see Kuriyama, 1961).

Membrane-potential changes in non-pregnant uterus resembling those of pregnant and post-partum uterus can also be produced by treatment with oestradiol and progesterone (rat: Marshall, 1959, 1962; Jung, 1960, 1964; rabbit: Goto & Csapo, 1959; Kuriyama & Csapo, 1961; guinea-pig: Bülbring & Kuriyama, unpublished observations). These changes in membrane potential may be due either to a change in internal ionic contents or to a change in ionic permeability of the cell membrane.

Ionic contents of myometrium have been measured by many authors in different animal species after different hormonal treatment (Rat: Cole, 1950; Kalman, 1957; rabbit: Horvath, 1954; Daniel & Daniel, 1957; Daniel, 1958; Kao, 1961; Bitman, Cecil, Hawk & Sykes, 1959; Daniel & Robinson, 1960*a*, *b*; cat: Daniel & Daniel, 1957; Daniel, 1958; human: Daniel & Boyes, 1957; Daniel, 1958). On the other hand, data about the ionic content of the myometrium during the oestrus cycle and pregnancy are not available. Bitman *et al.* (1959) found that endometrium of the progesterone-treated rabbit uterus had a high potassium and low sodium concentration compared with the oestrogen-treated rabbit endometrium. However, the myometrium showed only slight changes throughout. Kao (1961) also found only a slight change of the ionic contents in the rabbit myometrium after treatment with oestradiol and progesterone.

In the present experiments a systematic study was undertaken in order

* Riker Fellow.

to find out, first, whether there was a change in the internal ionic content during pregnancy and, if so, how far the change in membrane potential was correlated. Secondly, we studied the relation between the external potassium concentration and the membrane potential in the different conditions. Thirdly, membrane activity, spontaneous as well as that evoked by electrical stimulation, was observed in different stages of pregnancy. The results showed that the changes of membrane potential and membrane activity during pregnancy were not due to changes in ionic content but probably were due to changes in membrane permeability.

Some of the observations have been reported before (Kuriyama, 1964; Casteels & Kuriyama, 1964).

METHODS

Female albino rats weighing 110–130 g were stunned and bled. The uterus was removed from the abdomen and, in pregnant animals, the foetuses and placentae very gently squeezed out. The procedure was easy after the 20th day of gestation, but more difficult earlier.

Solutions. The strips of the uteri were dissected at room temperature in a solution containing (mM): Na^+ 144, K^+ 5·9, Ca^{2+} 3·7 and Cl^- 157·3.

The standard solution used in all experiments was modified Krebs's solution prepared from isotonic stock solutions (Krebs & Henseleit, 1932). It contained (mM): Na^+ 137·5, K^+ 5·9, Ca^{2+} 2·5, Mg^{2+} 1·2, HCO_3^- 15·5, $H_2PO_4^-$ 1·2, Cl^- 134·1 and glucose 11·5, and was equilibrated with a gas mixture of 3 % CO_2 and 97 % O_2. The pH at 36° C was 7·4.

TABLE 1. Composition of solutions

		Normal K	2·5	5×	10×	20×
Solution I	K	5·9	14·7	29·5	59	118
	Cl	134·1	134·1	134·1	134·1	134·1
	Na	137·4	137·4	137·4	137·4	137·4
II	K	5·9	14·7	29·5	59	118
	Cl	134·1	134·1	134·1	134·1	134·1
	Na	137·4	128·6	113·8	84·3	25·3
III	K	5·9	14·7	29·5	59	118
	Cl	134·1	54	27	13·4	6·7
	Na	137·4	128·6	113·8	84·3	25·3

Three different types of solution were used in which the external potassium concentration was increased from 5·9 to 118 mM (Table 1). For a reduction of the external chloride concentration, this ion was replaced by ethanesulphonate (Goodford & Ing, 1959).

In solution I the sodium and chloride concentrations were kept constant. The potassium concentration was increased by adding potassium ethanesulphonate; this produced a progressive increase of the tonicity of the solution.

In solution II, the increase of the potassium concentration was achieved by replacing part of the sodium chloride by potassium chloride. The solutions prepared in this way were isotonic and had a constant chloride concentration. Their sodium concentration decreased with increasing potassium concentration.

Solution III was prepared to keep the product of $[K]_o \times [Cl]_o$ constant. The solution is of the Boyle-Conway (1941) type and was used to investigate if the membrane potential was a Donnan-equilibrium potential. As in solution II, the increase in potassium concentration was balanced by a decrease in sodium concentration. The chloride concentration was

reduced by replacement with ethanesulphonate. This solution was isotonic and had variable potassium, sodium and chloride concentrations.

In some experiments the calcium concentration was increased by adding an appropriate amount of a concentrated calcium chloride solution. For concentrations over 10 mM a reduction of the $H_2PO_4^-$ concentration was necessary to avoid precipitation.

Recording of membrane activity. Uterine strips of 10–15 mm length and 3–5 mm width were used. The experimental procedures were those described by Bülbring (1955), and Bülbring & Kuriyama (1963*a*, *b*), for taenia coli. Electrical stimulation of the myometrium was done by field stimulation as described by Kuriyama (1963*a*).

Measurements of ion content. The remaining strips of the same uterus from which electrical activity was recorded were dissected free of endometrium. The number of preparations which could be cut out varied between 8 and 35 according to the size of the uterus. The weight of the strips of myometrium varied between 3 and 9 mg. The preparations were suspended isometrically on Perspex rods approximately at the *in vivo* length in modified Krebs's solution at 36° C. A minimal recovery period of 90 min was allowed, as it was found that at least 60 min were necessary to bring the ionic content of the tissues into a steady state. The immersion period in solutions of different potassium concentration lasted 25–40 min since it was found that 20 min was sufficient to reach a steady state. At the end of the immersion period the preparations were cut between the ligatures and dried by blotting them with filter paper (Whatman No. 54). The weight was determined to an accuracy of 0·1 mg and the tissues were transferred to a Pyrex test-tube or a small Pyrex pot.

The water content of the tissues was determined by comparing the wet weight and the dry weight. The dry weight was obtained by leaving the tissues in an oven at 95° C for 20 hr and then cooling them in a calcium chloride desiccator for 1 hr. Hydrogen peroxide (1 ml. Analar) was added to each test-tube and the contents were evaporated to dryness in an oven at 95° C. To determine, on the same sample, the potassium, sodium, chloride and calcium contents, a modification of the chloride estimation method of Menis, House & Raines (1957) was used. Concentrated $AgNO_3$ solution was added to the H_2O_2 to obtain a concentration of 1·4 μM. All chloride in the tissue was precipitated as AgCl during the ashing procedure. The dry ash was dissolved in the test-tube with 2 ml. of a solution containing 1 N-HNO_3 and 0·02 M-H_3PO_4 (Analar). The tubes were left for at least 36 hr in the dark. This procedure dissolved all salts apart from the AgCl. The Na, K, Ca and excess Ag concentrations of the supernatant were determined by flame photometry at wave-lengths of 589, 769, 422 and 328 mμ (McIntyre, 1961). The flame photometer was a Zeiss spectrophotometer PMQII with flame attachment.

Inulin space. After equilibration of the tissues for 90 min in Krebs's solution at 36° C, some pieces were transferred to an identical Krebs's solution containing 0·5 % inulin for another 90 min; it was found that the inulin space did not show significant differences for immersion times between 20 and 180 min. To determine the inulin spaces in the different potassium concentrations, the tissues were soaked in inulin-Krebs's solution for 60 min, and for another 30 min in excess potassium solution with the same concentration of inulin. The inulin was extracted at room temperature for 90 min in 1 ml. of the dissection fluid. The inulin concentration was determined by the method of Kulka (1956).

Ethanesulphonate space. Intercellular structures, mainly mucopolysaccharides, may limit the uniform distribution of the large inulin molecules over the whole extracellular space (Ogston & Phelps, 1960; Goodford & Leach, 1964). Therefore the uptake of a smaller molecule such as ethanesulphonate (Goodford & Ing, 1959) was investigated as done in other tissues by Goodford & Lüllmann (1962).

^{35}S-labelled ethanesulphonate was supplied by the Radiochemical Centre, Amersham. The tissues were suspended for different periods in a Krebs's solution containing ^{35}S-ethanesulphonate and varying amounts of inactive ethanesulphonate. The ^{35}S-labelled ethanesulphonate was extracted for 12 hr by putting the tissues in 1 ml. of isotonic Na-ethane-

sulphonate at room temperature, and the activity of this extraction fluid was determined by liquid scintillation counting using the scintillation mixture described by Bray (1960).

A steady value of the ethanesulphonate space was observed for an immersion period ranging between 5 and 40 min. Thereafter a slow continuous increase was observed. Up to 40 min immersion period the ethanesulphonate space exceeded the inulin space by 150 ml./kg wet weight. In the present experiments a standard immersion time of 9 min has been used throughout.

RESULTS

The membrane potential and membrane activity during the oestrus cycle, pregnancy and postpartum

Membrane potential. The rat uterus was spontaneously active in all conditions. Since activity occurred in bursts, the membrane potential could be measured during the silent periods. Figure 1 shows the changes during the oestrus cycle, during pregnancy and after delivery. In non-pregnant uterus the membrane potential was low, ranging between 34–46 mV (mean 42 mV ± 0·7 S.E., n = 78), and no significant differences were observed between oestrus and anoestrus. During the progress of gestation,

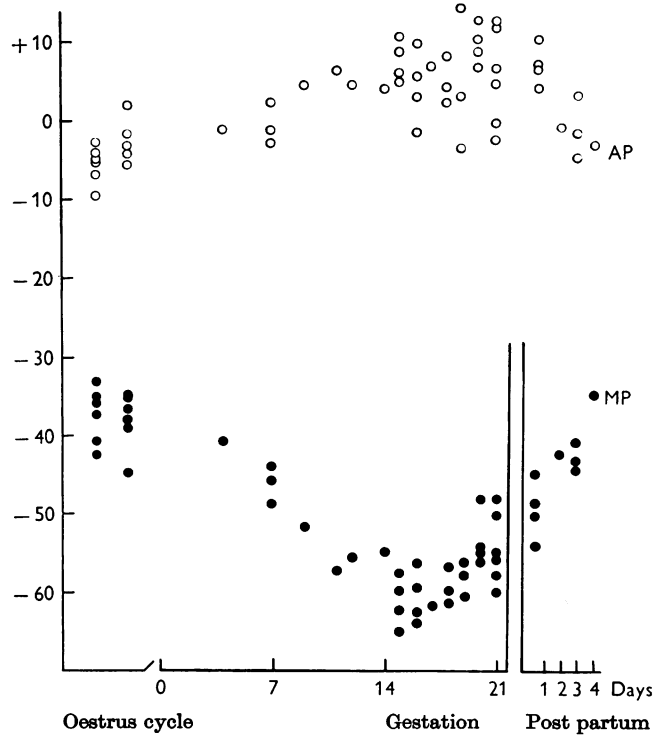

Fig. 1. Changes of the membrane potential (●) and spike amplitude (○) during the oestrus cycle, pregnancy and post-partum in rat myometrium. Each dot represents the mean value of 10–15 impalements.

the membrane potential increased and reached its maximum on the 15th or 16th day (mean 60·5 ± 0·5 S.E., n = 95) the highest mean value in one tissue being 65 mV. During the last stage of gestation (20th–21st day) the membrane potential was again slightly lower (mean 54·5 mV ± 0·5 S.E., n = 105) and the mean values from individual tissues showed a wide scatter from 49 to 62 mV. In the course of 2–3 days after delivery the membrane potential rapidly decreased to the same level as that before pregnancy. After the 7th day of gestation, the membrane potential of the placental site was consistently 5–8 mV higher than that of the non-placental regions. One day after delivery, no potential difference between the placental and non-placental site was observed.

Action potential. Spikes appeared as trains of discharge. Two types of cell activity could be seen. In some cells the membrane gradually depolarized from the resting state (2–11 mV over 80–1200 msec). When the depolarization reached the threshold it triggered an action potential of the type characteristic for pace-maker potentials in cardiac muscle. In other cells, the spikes were triggered without preceding depolarization of the cell membrane; these spikes were presumably propagated.

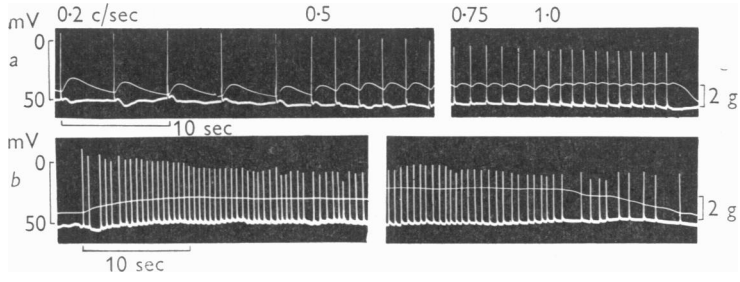

Fig. 2. Action potentials and tension developments of rat uterus on the 20th day of pregnancy (intracellular records). (*a*) Spikes triggered by field stimulation (10 V 1 msec). The frequency was varied from 0·2 c/s to 1·0 c/s. (*b*) Spontaneous discharges in a train.

The amplitude of the spikes whether discharged spontaneously or evoked by electrical stimulation, increased during the progress of pregnancy as shown in Fig. 1. In non-pregnant uterus an overshoot potential was rarely seen, but from the 14th day onwards it was seen consistently. The maximum rate of rise of the spike also changed during gestation; it was 3–8 V/sec in the non-pregnant uterus, 7–16 V/sec on the 15th day, 5–12 V/sec on the 19th–20th day, and 2–6 V/sec on the first day after delivery.

During the silent periods the response of the uterine muscle cell membrane to electrical stimulation followed the 'all or none' law. However, with repetitive stimulation at a frequency of more than 0·5 c/s, the spike amplitude became smaller (Fig. 2*a*). This may be because the spikes were

evoked during the relative refractory period, since the same phenomenon was seen during spontaneous bursts of fast repetitive discharge (Fig. 2*b*). When, during a spontaneous burst, the spike frequency became very high a depolarization of the membrane was also observed and the reduced spike amplitude may be partly due to this.

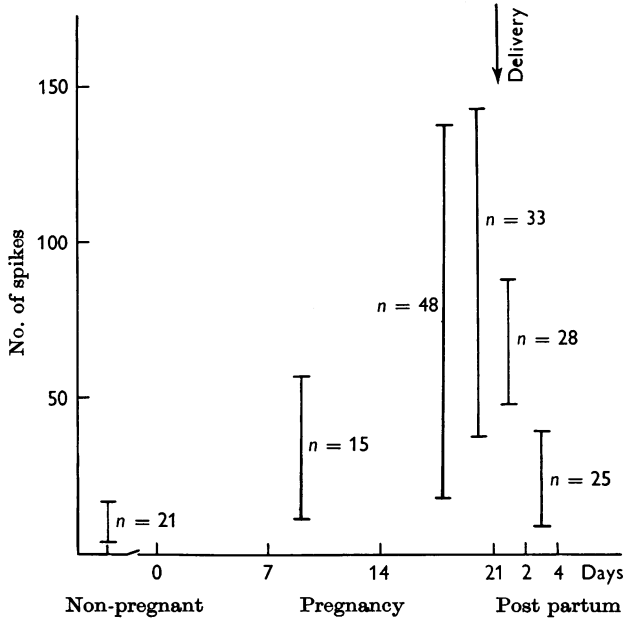

Fig. 3. The range of the variation in numbers of spikes in a train at different stages of pregnancy (limited vertical lines). Ordinate = the number of spikes. Abscissa = stage of pregnancy in days. n = number of trains which were investigated at the respective stages.

Figure 3 shows the variation of the number of spikes in a train under different conditions of the uterus. Non-pregnant uterus had long silent periods between bursts consisting of 3–18 spikes. During the progress of gestation, the number of spikes in a train increased and the range of variation became greater (on the 18th day 17–38 spikes). The irregular amplitude of the tension developed at this stage of pregnancy may be due partly to the variation in the number of spikes in a train and partly to asynchronous activity. After delivery (6–24 hr post-partum) the number of spikes in a train decreased, the range of variation became smaller (48–87 spikes in a train) and the tension development became more uniform.

Changes of excitability to electrical stimulation. In the non-pregnant uterus the duration of stimulus required to trigger a spike was longer than

during pregnancy. It was 5 msec for non-pregnant uterus, 2–3 msec for 18–20th day of pregnancy, and 1–2 msec just after delivery (6–8 hr); stimulation was at 10 V, 0·5 c/s. The excitability also fluctuated with the periods of activity and inactivity in the same cell. Figure 4, recorded on the 20th day of pregnancy, shows that, during the inactive period, spikes could only be elicited by stimulation shortly before the spontaneous burst

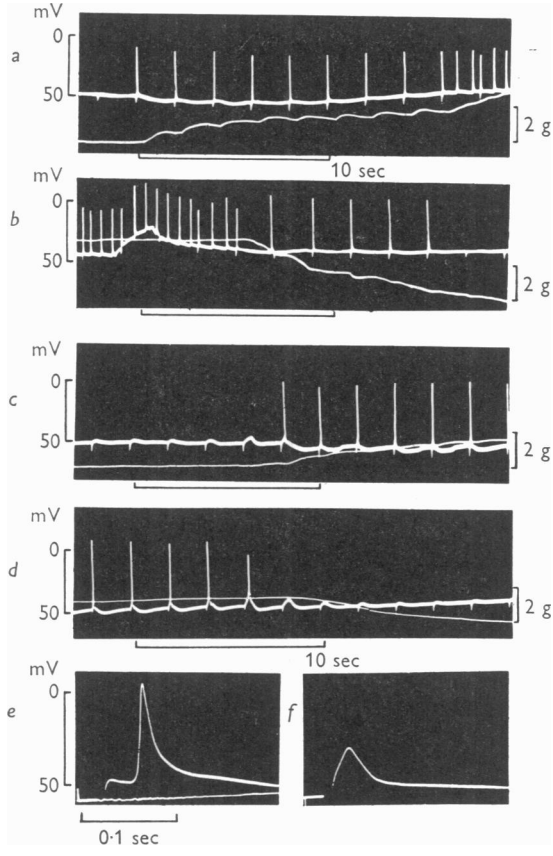

Fig. 4. Changes of excitability in the 20th-day pregnant uterus. (*a*) and (*b*) Fluctuation of the threshold to trigger the spike (0·5 msec, 10 V, 0·5 c/s). (*c*) and (*d*) Local potentials triggered by field stimulation (0·5 msec, 10 V, 0·5 c/s). (*e*) and (*f*) Shape of the spike and local potential recorded from the same cell as (*c*) and (*d*).

occurred (*a*), and immediately after the burst (*b*). In some cells repetitive stimulation caused the gradual development of a local potential, before spikes were triggered (Fig. 4*c*) and then, when spike generation failed, the amplitude of the local response gradually declined again (*d*). Spike and local potential are shown on a faster time base in Fig. 4*e*. The local responses may be due to electrotonic spread from neighbouring cells, since a

small tension change was sometimes recorded. It is unlikely that they are due to the release of chemical transmitter from nerve terminals by electrical stimulation, because they were not affected by simultaneous treatment with atropine sulphate (10^{-5} g/ml.) and phentolamine (10^{-5} g/ml.).

Further evidence for the fluctuating excitability is shown in Fig. 5. Five successive stimuli were chosen (1 msec, 10 V, 1 c/s) which, just before the spontaneous burst, triggered five spikes without failure. About 20 sec after the spontaneous burst, successive stimuli were still effective at a

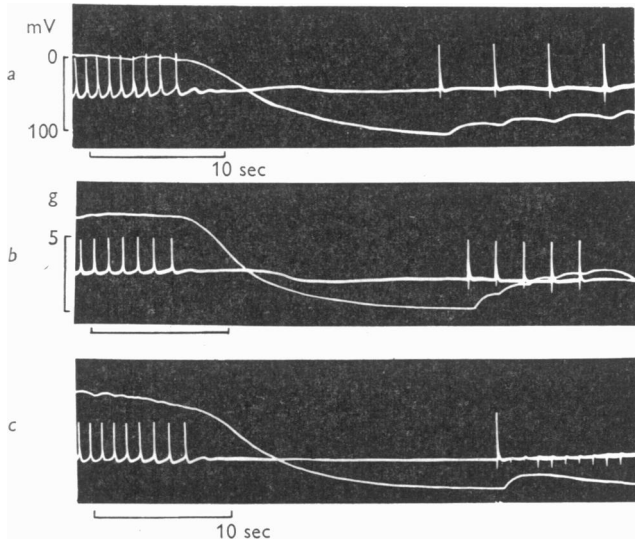

Fig. 5. Responses of the membrane on the 20th day of pregnancy to different stimulus frequency (1 msec, 10 V). (*a*) 0·25 c/s. (*b*) 0·5 c/s. (*c*) 1 c/s. The electrical stimulation was applied about 20 sec after the spontaneous burst had ceased.

frequency of 0·25 and 0·5 c/s (Fig. 5*a* and *b*). But at the higher frequency of 1·0 c/s the first stimulus alone triggered a spike (Fig. 5*c*). These observations were another indication that the fluctuation of excitability is connected with an intrinsic rhythm of the uterine muscle cell and is not explained by its refractory period.

Using two successive supramaximal stimuli the relative refractory period could be measured. If the pulse interval was 0·15–0·2 sec successive spikes could be triggered, but the second spike was smaller than the first. The same amplitude was obtained only when the pulse interval was 1 sec or more.

The ionic content of the myometrium

The total potassium, sodium, chloride and calcium contents, the inulin and the ethanesulphonate spaces of the tissues were measured during

TABLE 2. Ionic content (m-mole/kg wet wt. ± S.E.) and equilibrium potentials (mV) of the myometrium in different conditions (n = number of determinations)

	Non-pregnant		Pregnant		Post partum	
	Anoestrus	Oestrus	15 days	19–20 days	½ day	1 day
			Total ionic content			
K	63·1 ± 0·7 (n = 55)	60·7 ± 1 (n = 45)	59·4 ± 1 (n = 59)	62·3 ± 0·8 (n = 78)	54·2 ± 1·2 (n = 14)	50·8 ± 2 (n = 36)
Na	85·7 ± 1·3 (n = 54)	87·1 ± 1·3 (n = 45)	83·7 ± 1·7 (n = 55)	85·4 ± 0·9 (n = 78)	88·5 ± 1·7 (n = 14)	93 ± 1·7 (n= 36)
Cl	89·3 ± 1·1 (n = 52)	91·2 ± 1·1 (n = 45)	90·6 ± 1·1 (n = 55)	90·7 ± 1·1 (n = 77)	92·1 ± 1·7 (n = 14)	94 ± 1·5 (n = 39)
Ca	3·71 ± 0·1 (n = 27)	3·51 ± 0·11 (n = 18)	3·24 ± 0·07 (n = 41)	2·7 ± 0·05 (n = 68)	3·6 ± 0·11 (n = 14)	3·85 ± 0·08 (n = 35)
Inulin space (ml./kg wet wt.)	398 ± 10 (n = 30)	402 ± 11 (n = 28)	399 ± 7 (n = 26)	376 ± 6 (n = 38)	473 ± 12 (n = 13)	484 ± 15 (n = 17)
Ethanesulphonate space (ml./kg wet wt.)	564 ± 10 (n = 42)	(564)*	(560)*	557 ± 12 (n = 33)	—	616 ± 17 (n = 13)
			Intracellular ionic concentration calculated from the means of the total content and the extracellular spaces			
Inulin $[K]_i$	110	106	103	104	107	102
Ethanesulphonate $[K]_i$	154	148	143	149	—	140
Inulin $[Na]_i$	56	58	52	59	50	57
Ethanesulphonate $[Na]_i$	22	25	18	23	—	27
Inulin $[Cl]_i$	65	68	67	70	60	62
Ethanesulphonate $[Cl]_i$	35	40	40	41	—	33
			Equilibrium potentials calculated from the intracellular and extracellular ionic concentrations (mV)			
Inulin E_K	−78	−77	−76	−77	−77	−76
Ethanesulphonate E_K	−87	−86	−85	−86	—	−85
Inulin E_{Na}	+24	+23	+26	+23	+27	+23
Ethanesulphonate E_{Na}	+49	+45	+54	+48	—	+43
Inulin E_{Cl}	−19	-18	−18	−17	−21	−21
Ethanesulphonate E_{Cl}	−36	-32	−32	−32	—	−37

* Assumed value.

anoestrus, oestrus and pregnancy, and post partum. The mean values are summarized in Table 2. From these values and from the means of the inulin or ethanesulphonate spaces the internal potassium, sodium and chloride contents were calculated per cell volume assuming a constant tissue density of 1·05 (Goodford & Hermansen, 1961).

The changes in ion content observed during the different stages of pregnancy are not significant. Though the values for extracellular space differed by as much as 150 ml. per kg wet wt. according to the method used, the mean values for the intracellular ionic concentration shown in Table 2 were remarkably constant, and thus the theoretical equilibrium potentials did not change either. The only significant change found was a change of the total calcium content. During oestrus and anoestrus the calcium content was the same. During the progress of the gestation it declined and reached the lowest value of 2·7 m-mole per kg wet wt. at the last stage of pregnancy. The calcium content recovered rapidly to that before pregnancy within 12–14 hr after delivery.

These results indicated that the differences of the membrane potential in non-pregnant, pregnant and post-partum uterus are not caused by changes of internal ionic content, but that they might be due to permeability changes of the membrane to individual ions.

Effect of changing the external potassium concentration on the ionic content, the membrane potential and the spike activity

Three different solutions were used, as described in the Methods section, to investigate the effect of varying the external potassium concentration from 5·9 to 118 mM.

Solution I. In solution I, the external sodium and chloride concentrations were kept constant and excess-potassium solutions were prepared by adding potassium ethanesulphonate. These solutions were hyperosmotic. For potassium concentrations above 30 mM, the change of the membrane potential plotted against the logarithm of the external potassium concentration was linear. The maximal change in membrane potential produced by a tenfold change of the external potassium concentration was 39 mV on the 20th day of pregnancy. This finding confirmed previous observations made by Jung (1959), Goto & Csapo (1959), Marshall (1962) and Csapo & Kuriyama (1963) who used KCl.

Solution II. In solution II, the external chloride concentration was kept constant, while the external sodium concentration was reduced in proportion to the increase of the external potassium concentrations, so as to keep the solution isosmotic. Table 3 shows, for the 20th day of pregnancy, the total ion content, the internal ion content calculated from the total, and the inulin and ethanesulphonate spaces respectively. The theoretical

TABLE 3. Ionic content (m-mole/kg wet wt. ± S.E.) of the 20th-day pregnant uterus after immersion in different potassium concentrations (solution type II)

(n = number of determinations)

m-mole $[K^+]_o$	5·9	29·5	59	118
	Total ionic content in m-mole/kg wet wt.			
K	63·2 ± 1·7 (n = 12)	80·3 ± 1·5 (n = 9)	98·3 ± 1·5 (n = 10)	127·1 ± 0·8 (n = 10)
Na	89·2 ± 1·9 (n = 12)	76 ± 3·2 (n = 10)	54·6 ± 2·1 (n = 10)	23 ± 1·2 (n = 10)
Cl	94·6 ± 1·1 (n = 12)	97·3 ± 2·4 (n = 9)	95·2 ± 1·8 (n = 10)	100·5 ± 2·5 (n = 8)
Inulin space (ml./kg wet wt.)	365 ± 19 (n = 6)	379 ± 30 (n = 6)	342 ± 18 (n = 6)	371 ± 17 (n = 6)
Ethanesulphonate space (ml./kg wet wt.)	570 ± 20 (n = 8)	567 ± 20 (n = 10)	(570)*	598 ± 19 (n = 6)
	Intracellular ionic content calculated from the means of the total content and the extracellular spaces			
Inulin $[K]_i$	104	121	128	143
Ethanesulphonate $[K]_i$	156	165	169	160
Inulin $[Na]_i$	66	58	42	24
Ethanesulphonate $[Na]_i$	29	30	17	22
Inulin $[Cl]_i$	78	81	81	87
Ethanesulphonate $[Cl]_i$	48	55	49	58
	Equilibrium potentials calculated from the intracellular and extracellular ionic concentrations (mV)			
Inulin E_K	−77	−37	−21	−5
Ethanesulphonate E_K	−88	−46	−28	−8
Inulin E_{Na}	+19	+18	+18	+2
Ethanesulphonate E_{Na}	+41	+36	+42	+4
Inulin E_{Cl}	−15	−13	−13	−11
Ethanesulphonate E_{Cl}	−28	−24	−27	−23
Membrane potential	58 ± 0·8 (n = 30)	36 ± 0·6 (n = 30)	26 ± 0·4 (n = 30)	11 ± 0·3 (n = 30)

* Calculated value.

equilibrium potentials were calculated for both extracellular space measurements, to be compared with the measured membrane potentials. The change of the theoretical potassium equilibrium potential produced by a tenfold change of the external potassium concentration was 56 mV when inulin was used, and 60 mV when ethanesulphonate was used, while the maximal potential change measured in this tissue was 44 mV. The sodium equilibrium potential remained constant in external potassium concentrations up to 59 mM. The internal chloride concentration was not influenced and thus the chloride equilibrium potential also remained constant.

The differences between the maximal potential changes produced by a tenfold change of the external potassium concentration obtained in solutions I and II are probably mainly due to the loss of water. In solution I a shrinkage of the tissue was observed under the microscope at high potassium concentration and was probably due to the hyperosmoticity of this solution. In contrast, the tissue did not shrink in solution II, which is isosmotic. Absence of shrinkage was confirmed by the consistency of the dry weight/wet weight ratios of the tissues in the different potassium concentrations.

Solution III. This solution was prepared to determine whether the change of membrane potential followed the Boyle–Conway type of the Donnan equation, which has been found to apply to skeletal muscle by Hodgkin & Horowicz (1959). The product $[K]_o \times [Cl]_o$ was therefore kept constant and the sodium was reduced to keep the solution iso-osmotic. The total ion contents in the different potassium concentrations and the calculated internal ion contents are given in Table 4*a*, *b*. The effects of varying the external potassium concentrations on the measured membrane potential and on the calculated potassium and chloride equilibrium potentials are shown in Fig. 6 for the non-pregnant and the 20th-day pregnant uterus. The maximum change of the membrane potential per tenfold change of external potassium concentration was 32 mV in the non-pregnant and 51 mV in the pregnant uterus. The differences in slope of the two regression lines was highly significant ($P < 0{\cdot}001$). The change of the calculated potassium equilibrium potential per tenfold change of the external potassium concentration was 56 mV using the inulin space and 58 mV using the ethanesulphonate space, in both pregnant and non-pregnant uterus.

These results indicate, first, that the membrane potential of the pregnant and non-pregnant uterus is mainly a potassium potential, but that an influence of the sodium permeability on the membrane potential is probable since, in the lower range of external K concentrations, the relation becomes non-linear (Fig. 6). Second, over the whole range of external potassium concentrations, the potassium permeability of the non-

TABLE 4*a*. Ionic content (m-mole/kg wet wt.) in non-pregnant rat uterus after immersion in different potassium concentrations (solution type III) (n = number of determinations)

m-mole $[K^+]_o$	5·9	29·5	59	118
	Total ionic content in m-mole/kg wet wt.			
K	65·7 ± 1·3 (n = 15)	85 ± 2·1 (n = 10)	102·2 ± 1·3 (n = 10)	137·5 ± 1·7 (n = 13)
Na	90·4 ± 2·1 (n = 15)	81·5 ± 2 (n = 10)	66·6 ± 1·8 (n = 10)	24·7 ± 0·5 (n = 13)
Cl	92·5 ± 1·9 (n = 15)	27·6 ± 1·5 (n = 10)	18·5 ± 1·7 (n = 10)	14·7 ± 1·7 (n = 12)
Inulin space (ml./kg wet wt.)	387 ± 16 (n = 9)	(390)*	(390)*	397 ± 14 (n = 9)
Ethanesulphonate space (ml./kg wet wt.)	569 ± 16 (n = 15)	547 ± 17 (n = 17)	(547)*	547 ± 9 (n = 17)
	Intracellular ionic content calculated from the means of the total content and the extracellular spaces			
Inulin $[K^+]_i$	114	131	141	165
Ethanesulphonate $[K^+]_i$	163	171	173	180
Inulin $[Na^+]_i$	70	66	61	28
Ethanesulphonate $[Na^+]_i$	33	47	50	27
Inulin $[Cl^-]_i$	73	30	24	18
Ethanesulphonate $[Cl^-]_i$	43	32	28	27
	Equilibrium potentials calculated from the intracellular and extracellular ionic concentrations (mV)			
Inulin E_K	−80	−39	−23	−8
Ethanesulphonate E_K	−88	−47	−32	−11
Inulin E_{Na}	+17	+14	+9	−5
Ethanesulphonate E_{Na}	+38	+24	+14	−2
Inulin E_{Cl}	−16	+3	+14	+25
Ethanesulphonate E_{Cl}	−30	+5	+19	+37
Membrane potential	−38·5 ± 0·5 (n = 31)	−29·5 ± 0·4 (n = 32)	−19 ± 0·6 (n = 31)	−11·5 ± 0·3 (n = 29)

* Assumed value.

TABLE 4*b*. Ionic content (m-mole/kg wet wt.) in 20th-day pregnant rat uterus after immersion in different potassium concentrations (solution type III) (n = number of determinations)

m-mole $[K^+]_o$	5·9	14·8	29·5	59	118
	Total ionic content in m-mole/kg wet wt.				
K	66·8 ± 1·5 (n = 15)	72·4 ± 0·9 (n = 7)	84·1 ± 2·6 (n = 14)	103 ± 3 (n = 15)	136·6 ± 1·4 (n = 15)
Na	85·6 ± 1·2 (n = 15)	79·6 ± 3 (n = 7)	72·4 ± 2·1 (n = 14)	58 ± 1·7 (n = 15)	23·8 ± 1·4 (n = 15)
Cl	90·7 ± 1 (n = 11)	45·5 ± 2·8 (n = 4)	29·4 ± 2·3 (n = 10)	15·7 ± 1 (n = 10)	11·7 ± 1·5 (n = 11)
Inulin space (ml./kg wet wt.)	375 ± 20 (n = 7)	(385)*	395 ± 12 (n = 7)	(400)*	406 ± 9 (n = 5)
Ethanesulphonate space (ml./kg wet wt.)	561 ± 11 (n = 9)	(560)*	560 ± 25 (n = 9)	564 ± 30 (n = 5)	590 ± 33 (n = 8)
	Intracellular ionic content calculated from the means of the total content and the extracellular spaces				
Inulin $[K^+]_i$	113	118	132	144	162
Ethanesulphonate $[K^+]_i$	162	164	173	179	185
Inulin $[Na^+]_i$	59	53	48	44	27
Ethanesulphonate $[Na^+]_i$	22	19	22	27	24
Inulin $[Cl^-]_i$	66	43	33	19	17
Ethanesulphonate $[Cl^-]_i$	40	39	36	21	20
	Equilibrium potentials calculated from the intracellular and extracellular ionic concentrations (mV)				
Inulin E_K	−78	−56	−40	−24	−8
Ethanesulphonate E_K	−88	−64	−47	−29	−12
Inulin E_{Na}	+23	+24	+23	+17	−4
Ethanesulphonate E_{Na}	+48	+51	+44	+30	+1
Inulin E_{Cl}	−19	−6	+6	+9	+25
Ethanesulphonate E_{Cl}	−32	−9	+8	+12	+29
Membrane potential	−56·5 ± 0·4 (n = 31)	−47·1 ± 0·8 (n = 14)	−29·5 ± 0·5 (n = 34)	−16·0 ± 0·3 (n = 30)	−7 ± 0·3 (n = 15)

* Assumed value.

pregnant uterus appears to be lower than that of the pregnant uterus. In late pregnancy the maximum slope of the line relating the measured membrane potential to the log of the external K concentration is nearly parallel to that of the calculated potassium equilibrium potential.

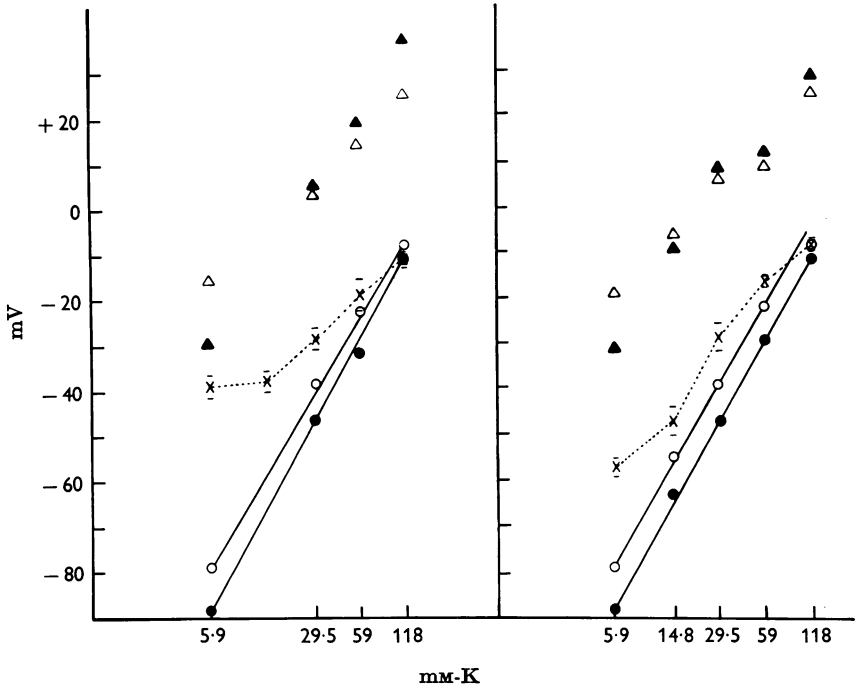

Fig. 6. The relation between the membrane potential, the calculated potassium and chloride equilibrium potential and the external potassium concentration. Ordinate = potentials in mV; abscissa = external potassium concentration in mM on a logarithmic scale. The crosses are the measured membrane potential (mean ± S.D.). The circles are the calculated potassium equilibrium potentials, (○) inulin, (●) ethanesulphonate, and the triangles the calculated chloride equilibrium potential, (△) inulin, (▲) ethanesulphonate.

In solution III, when the external chloride concentration was reduced with increasing $[K]_o$, the intracellular chloride concentration was also reduced, but to a smaller extent than in the external solution. The discrepancy may be due either to bound chloride or to a low chloride permeability of the cell membrane. The decrease of the intracellular chloride concentration was less when the ethanesulphonate space was used than with inulin. Consequently the change of the chloride equilibrium potential per tenfold change of the external chloride concentration was similar to that of the potassium equilibrium potential. It was the same in the

pregnant and non-pregnant uterus. Since the internal chloride concentration was higher than the external concentration when this was decreased below 27 mM, the chloride equilibrium potential became positive.

Figure 7 shows the effect of varying the external potassium concentration (solution III) on the membrane potential, and on the amplitude and shape of the spike of the non-pregnant uterus. The spikes were evoked by

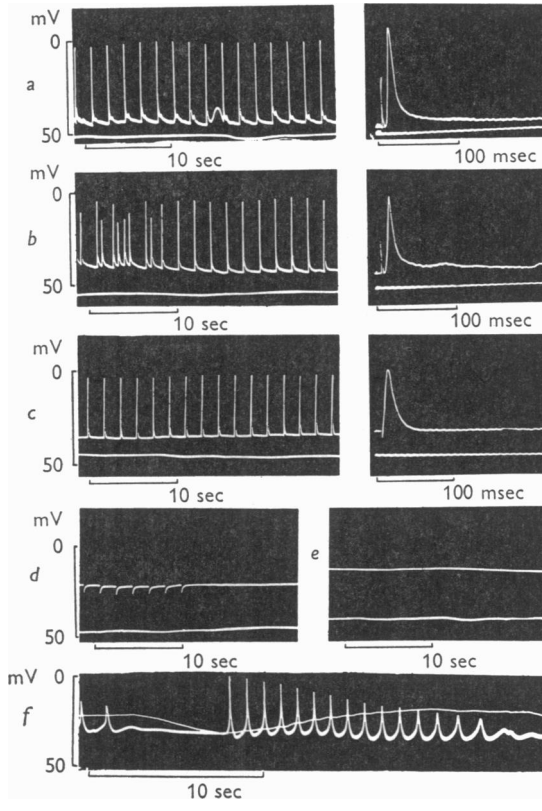

Fig. 7. Effect of increasing the external potassium concentration on the response of the non-pregnant (*a–e*) rat uterus to electrical stimulation (3 msec, 10V, 0·5 c/s). (*a*) 5·9 mM-K; (*b*) 14·8 mM-K; (*c*) 29·5 mM-K; (*d*) 59 mM-K; (*e*) 118 mM-K; (*f*) Effect of 29·5 mM-K on the spontaneous activity of the 20th-day pregnant rat uterus.

electrical stimulation of 3 msec pulse duration, 10V, and 0·5 c/s. Increasing the external potassium concentration reduced the spike amplitude and in 59 mM $[K]_o$ no more spikes could be triggered. A striking difference between the non-pregnant and the pregnant uterus was observed at an external potassium concentration of 29·5 mM. The membrane potentials of both tissues were nearly the same at this potassium concentration. In

the non-pregnant uterus, however, spikes could still be triggered by electrical stimulation (Fig. 7*c*) whereas in the pregnant uterus the shape of the spikes quickly deteriorated to oscillatory potentials (Fig. 7*f*). This result suggests that the inactivation process of the sodium carrier is more pronounced in the pregnant rat uterus than in the non-pregnant one.

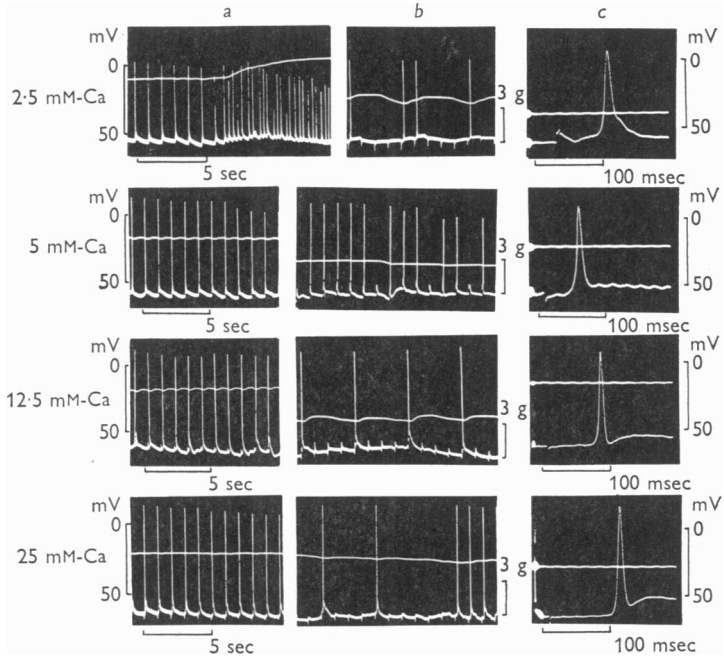

Fig. 8. Effect of varying the external calcium concentration (2·5–25 mM) on the response of the 20th-day pregnant rat uterus to electrical stimulation (1 msec, 10 V, 1 c/s), (*a*) during the spontaneously active state; (*b*) during the inactive state; (*c*) shape of the spike.

Effect of changing the external calcium concentrations (2·5–25 *mM*) *on the membrane potential of the non-pregnant and pregnant uterus*

Figure 8 shows the effect of varying the external calcium concentrations on the membrane potential, on the amplitude and on the shape of the spikes evoked by electrical stimulation (1 msec, 10 V, 1 c/s) during the active state (*a*), and the inactive state (*b*), of the membrane on the 15th day of pregnancy. As already described, each stimulus triggered a spike during the active state but not during the inactive state. An increase of the external calcium concentration up to 12·5 mM hyperpolarized the membrane and increased the amplitude and maximal rates of rise and fall of the spikes. A further increase (up to 25 mM) produced a smaller increase of the resting potential. When the external calcium concentration is

higher than 12·5 mM the membrane potential may rise to 75 mV and the overshoot potential to 20 mV. However, the maximum rate of rise never exceeds 30 V/sec.

Figure 9 shows the changes of the membrane potentials produced by varying the external calcium concentrations in the non-pregnant uterus, on the 15th and the 20th day of pregnancy. The 15th day was included in these experiments because the membrane potential is at its highest value

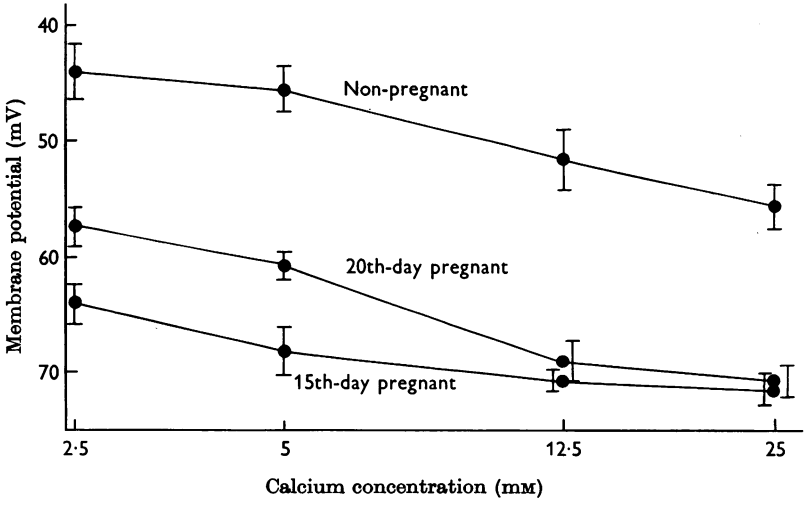

Fig. 9. Change of the membrane potential produced by varying the external calcium concentration in the non-pregnant, and 15th and 20th-day pregnant, rat uterus. Ordinate = membrane potential (mean ± S.D.), abscissa = external calcium concentration on logarithmic scale.

at this stage of gestation. In both stages of pregnancy the membrane potential rose, in 12·5 mM calcium, to more than 70 mV. In the non-pregnant uterus, however, the same calcium concentration increased the membrane potential only to 52 mV. The influence of raising the external calcium concentration to 12·5 mM was greatest during the last stage of pregnancy.

DISCUSSION

This discussion is based on many assumptions: for example, that the membrane potentials measured from cells whose diameter changes from 2–3 μ to 5–7 μ during pregnancy (Reynold, 1949) are the correct values of the membrane potential, and that the membrane potential measured during silent periods is comparable with the resting potential of skeletal muscle in the steady state. We have also to assume that the intracellular ions are distributed in a homogeneous system in the cell with the same activity coefficient in the intra- and extracellular solution.

The most important uncertainty concerns the extracellular space. It has been shown that, in mammalian smooth muscle, the inulin space is smaller than the ethanesulphonate space (Goodford & Lüllmann, 1962). In the rat uterus (20th day of pregnancy) we found that the extracellular space, determined with ^{35}S-labelled ethanesulphonate, was 557 ml./kg wet wt. which is much larger than 376 ml./kg wet wt. obtained for the inulin space. The possibility that the higher value is due to uptake of ethanesulphonate by binding-sites is improbable because an identical space is found in the presence of high concentrations of non-labelled ethanesulphonate. The results based on the inulin space, which has been generally used as an estimate of the extracellular space, are presented together with those based on the ethanesulphonate space. However, we consider the latter to be nearer to the true value in view of the recent findings of Goodford & Leach (1964) who showed that the presence of mucopolysaccharides prevents a homogeneous distribution of inulin in the extracellular space.

Our observations on the changes of the membrane potential during the progress of gestation are in agreement with previous observations by Thiersch *et al.* (1959), Kuriyama & Csapo (1961) and Jung (1964) in the rat uterus and Goto & Csapo (1959) and Kuriyama & Csapo (1961) in the rabbit uterus. Though the membrane potential rose from 42 to 61 mV on the 15th day of pregnancy and fell post partum to the original value, no significant change of the intracellular K, Na or Cl concentrations was detectable. This result indicates that the changes of the membrane potential might be due to changes of the membrane properties, since there was not only a change in membrane potential but also in spike amplitude and shape, in frequency and number of spikes per train, and in the frequency at which train discharges occurred. The observations made during the different conditions of the uterus suggest that the membrane in the non-pregnant uterus has a low potassium permeability which, during the progress of the gestation, gradually increases to a maximum on the 14th–15th day of gestation. It then remains high until the termination of pregnancy. A gradual increase of the sodium permeability of the membrane develops 3–4 days before delivery, and reaches a maximum at parturition. After delivery, both the sodium and potassium permeabilities of the membrane rapidly return to the level before pregnancy.

Supporting evidence for the above working hypothesis is found in the following experimental results. First, the maximum slope of the line relating the changes of membrane potential to the logarithm of the external potassium concentration in the non-pregnant rat uterus was much lower than that in the late pregnant uterus. Second, in a solution containing 5 times the calcium concentration of Krebs solution, the degree of the

hyperpolarization of the membrane was much greater in the late pregnant and in the non-pregnant uterus than in early pregnancy.

Since the membrane potential of the uterus is low in all conditions, we may assume a relatively high sodium conductance of the membrane in the resting state. The relative sodium permeability was calculated from the equation (Hodgkin & Horowicz, 1959) in the presence of the physiological external potassium concentration (5·9 mM)

$$E = \frac{RT}{F} \ln \frac{[K]_i + \alpha[Na]_i}{[K]_o + \alpha[Na]_o}, \quad (1)$$

$$\alpha = \frac{P_{Na}}{P_K} \quad (2)$$

in which E is the measured membrane potential during the silent period, $[\ ]_i$ and $[\ ]_o$ the internal and external ion concentrations, and α the ratio of sodium permeability coefficient (P_{Na}) to the potassium permeability coefficient (P_K). Recently Noble (1962) modified the Goldman equation (Hodgkin & Katz, 1949) to derive the membrane potential for the spontaneously active cardiac muscle cell. However, in contrast to cardiac muscle in which the interval between action potentials is only about 500 msec, uterine muscle has silent periods between bursts of activity which last from 10 to 180 sec. Therefore, for the uterine muscle cells, we preferred to use the simplified equation used by Hodgkin & Horowicz (1959) for the quiescent skeletal muscle.

In the normal potassium concentration (5·9 mM) α was found to have a value of 0·13 when the inulin space was used, and 0·20 when the ethanesulphonate space was used, in the non-pregnant rat uterus. α was 0·03 and 0·06 respectively on the 15th day, and 0·06 and 0·10 respectively on the 20th day of pregnancy. These values are much higher than that found in skeletal muscle (0·01) (Hodgkin & Horowicz, 1959). The high value in the non-pregnant uterus may be due not only to the high membrane permeability to sodium but also to the low permeability to potassium. Thus the increase of the membrane potential during the progress of gestation may be due not only to an increase of the potassium permeability but also to a reduction in the sodium permeability.

In skeletal muscle (Hodgkin & Horowicz, 1959; Hutter & Noble, 1960), the chloride permeability of the membrane is higher than the potassium permeability. In the myometrium this does not seem to be so, since the reduction of the external chloride concentration (in experiments with solution III) does not produce a comparable reduction of the internal chloride concentration.

Membrane activity. Changes of spike amplitude in different conditions of the uterus follow the changes of the membrane potential. In the non-

pregnant uterus overshoot potentials were seldom seen but after the 12th–14th day of gestation they appeared consistently. The maximum overshoot potential was 15 mV. The maximum rate of rise of the spike also increased until the 12th–14th day of gestation; it then remained constant but fell again rapidly after delivery. The maximum rate of rise of the spike never exceeded 16 V/sec in the physiological solution. The above results indicate that the sodium carrier system is poorly developed in this tissue as it is in other smooth muscles (taenia coli: Holman, 1958; Bülbring & Kuriyama, 1963*a*; uterus: Marshall, 1959; Kuriyama & Csapo, 1961; vas deferens: Burnstock & Holman, 1961; Kuriyama, 1963*b*; longitudinal muscle of intestine: Gillespie, 1962). This value of 16 V/sec is less than 1/20th of the maximal rate of rise of the spike of the skeletal muscle and cardiac muscle which have the same sodium equilibrium potential (see reviews by Hodgkin, 1951; Shanes, 1958; Weidmann, 1956).

An increase of the calcium concentration up to 12·5 mM increased the maximal rate of rise of the spikes, but with a further increase to 25 mM the maximal rate of rise of the spike remained the same or was slightly lowered. This might be due to the inhibition of the sodium-carrier mechanism caused by a more stable binding of the carrier with calcium ions, a mechanism shown in skeletal muscle by Ishiko & Sato (1957).

The present experiments provide no data for a precise interpretation of the automaticity in uterine muscle cells. The results simply indicate that the individual cells have a cyclic variation of their excitability and that this cycle is synchronous for all cells of the tissue even in the presence of cholinergic or adrenergic blocking agents. This indicates that the excitability cycle is myogenic and that it may be related to the permeability of the membrane to sodium, as postulated for the taenia coli (Bülbring & Kuriyama, 1963*a*). Bursts of spontaneous spike discharges are more frequent in late pregnancy and this would correspond to the increased sodium permeability of the membrane at this stage.

Marshall (1959) observed that *in vivo* treatment of rats with oestrogen increased the resting potential of the myometrium up to 58 mV (oestrogen dominated) and that additional treatment with progesterone produced a further increase to 64 mV (oestrogen and progesterone dominated). Similar observations were made by Jung (1961) in the rat uterus, and by Goto & Csapo (1959) in the rabbit uterus. *In vivo* and *in vitro* treatment with progesterone blocked the spike generation and the propagation of excitation in rat and rabbit uterus (Marshall, 1959; Kuriyama & Csapo, 1961; Kuriyama, 1961; Jung, 1964). Several authors (Csapo, 1959, 1961; Bengtsson, 1957; Schofield, 1960) discussed the possibility that during the last stage of gestation in rabbit uterus, a diminution of the progesterone effect appears before a decline of the oestrogen effect.

In the present experiments, it was found that the myometrium on the 15th day of pregnancy resembled an oestrogen and progesterone-dominated uterus, with an increased potassium permeability and a reduced sodium permeability. The myometrium at the end of the gestation resembled an oestrogen-dominated uterus with an increased sodium permeability caused by the withdrawal of progesterone.

SUMMARY

1. The membrane potential and the activity pattern of rat uterus muscle was recorded during the oestrus cycle, pregnancy and the post-partum period. In pieces from the same tissues the ionic content, and the inulin and ethanesulphonate spaces were determined, in order to compare the measured values of membrane potential with the theoretical ionic equilibrium potentials.

(*a*) The membrane potential of the non-pregnant uterus was $42 \pm 0{\cdot}7$ mV (S.E. of mean, $n = 48$). It increased during the progress of the gestation to $60{\cdot}5 \pm 0{\cdot}5$ (S.E. of mean, $n = 95$) on the 15th–16th day. After the 19th day the membrane potential declined slightly to 54 mV ($54{\cdot}5 \pm 0{\cdot}5$, S.E. of mean, $n = 45$) and after delivery it fell rapidly to the value before pregnancy.

(*b*) The spike amplitude increased throughout pregnancy. An overshoot potential was rarely seen in the non-pregnant uterus, but was seen consistently after the 14th day of pregnancy.

(*c*) The threshold of excitation by electrical stimulation was higher in the non-pregnant than in the pregnant uterus. In all conditions the excitability of the cells showed periodic fluctuations.

(*d*) The intracellular potassium, sodium and chloride concentrations calculated from the total contents and the inulin or ethanesulphonate space did not change in the different conditions of the uterus. Thus the theoretical potassium, sodium and chloride equilibrium potentials remained constant in all conditions.

(*e*) The calcium content fell during the progress of gestation and reached its lowest value just before parturition.

2. The change of membrane potential produced by varying the external potassium concentration was investigated in the range of 5·9–118 mM. On the 20th day of pregnancy the maximum change of the membrane potential produced by a tenfold change of the external potassium concentration was 39 mV in the hyperosmotic solution I, and 43 mV in the isosmotic solution II. The maximum change of the membrane potential produced by a tenfold change of the external concentration with solution III ($[K]_o \times [Cl]_o = k$) was 51 mV. In the non-pregnant uterus it was

38 mV. The change of the calculated potassium equilibrium potential was 58 mV for both conditions.

3. Excess calcium (12·5 mM) hyperpolarized the membrane and increased the amplitude and the maximum rate of rise of the spikes. The effect was greatest on the 20th day of pregnancy.

4. The above results suggest that the non-pregnant rat uterus has a low membrane permeability to potassium. During pregnancy the potassium permeability increases gradually until the 14th–15th day of gestation and remains high until delivery. During the last 3–4 days of pregnancy the sodium permeability increases slightly. After delivery both return rapidly to the levels of non-pregnant uterus. This working hypothesis is discussed in relation to the actions of hormones on the uterus.

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