THE ROLE OF SODIUM IN ACTIVATION OF UTERINE SMOOTH MUSCLE

A Thesis

Submitted in Partial Fulfilment
of the Requirement for the Degree of
Master of Arts

in

the Department of Pharmacology

We accept this thesis as conforming
to the required standard

by

HARCHARAN SINGH, B. Pharm.,
Panjab University, India, 1956.

THE UNIVERSITY OF BRITISH COLUMBIA
October 1958

ABSTRACT

Extracellular action potentials and isometric contractile tension have been recorded simultaneously in vitro from uterine longitudinal smooth muscle of the pregnant cat, pregnant rabbit, estrogen-treated rabbit, and estrogen-treated rat. Action potentials were recorded from the surface of the muscle strips with glass electrodes having a large tip diameter. Tension was recorded with an RCA transducer.

Spontaneous contractions are associated with a series of action potentials. During relaxation no electrical activity is observed. Electrical and mechanical activities were first recorded in Kreb's Ringer medium and then in sodium-poor media (replacement of sodium chloride with choline chloride or sucrose). Sufficient reduction in the external sodium concentration resulted in increased amplitude (peak to peak) of the biphasic action potential spikes. The duration of the peak to peak deflection and the maximum rate of potential change remained unchanged. However, decrease in the external sodium concentration reduced the frequency of the action potentials, considerably in the cat, and less so in the rabbit and rat.

The external sodium concentration was reduced in stepwise fashion to $\frac{1}{2}$, $\frac{1}{4}$ and $\frac{1}{8}$ its initial value. Each successive decrease in the external sodium concentration was accompanied by a prompt initial contraction, followed by very slow relaxation and subsequent resumption of spontaneous contractions accompanied by action potentials. With cat uteri reduction of the sodium concentration of the medium to a level of 15-20 mEq/l resulted in a greatly prolonged contraction with eventual relaxation when tissues failed to contract. This paralysis was associated with cessation of action potentials.

The electrical responses of uteri of the other two species (rabbit and rat) during exposure to sodium-poor media were similar to those observed

with the cat uterus. However, the mechanical activity of rat and rabbit uteri in sodium poor media was different from that of the cat uterus. Decrease in the external sodium concentration below 25–30 mEq/l usually resulted in prolonged contractions, and finally to complete failure of the tissue to relax (even after 2–2½ hours). Outbursts of action potentials at irregular intervals were seen in the initial stages of this persistent contraction but eventually action potentials also disappeared.

It was difficult to reconcile these facts with the "Sodium Hypothesis". A selective inward flow of sodium ions probably cannot account for the initiation of action potentials in uterine smooth muscle since considerable reduction of the external sodium concentration (down to 15–20 mEq/l in cat and 25–30 mEq/l in the other two species) did not effect the characteristics of the action potentials in the expected manner. However, further reduction in sodium did result in electrical and mechanical inactivity. The view that an outward flow of intracellular anions might be responsible for depolarization (14) receives further support from the present studies. In addition to many differences from other types of excitable tissue (nerve, cardiac and skeletal muscle) uterine smooth muscle also shows considerable intra- and inter-species variation.

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representative. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Pharmacology

The University of British Columbia,
Vancouver 8, Canada.

Date Sept 24, 1958

TABLE OF CONTENTS

LIST OF TABLES

TABLE OF FIGURES

Table of Figures (cont'd)

ACKNOWLEDGMENTS

The author wishes to express his profound appreciation to Dr. E.E. Daniel and Dr. J.G. Foulks for their keen interest, encouragement and helpful criticism during the course of investigations and the preparation of this report.

Thanks are also due to Mr. P.P. Singh of the Department of Physics, U.B.C., Vancouver and Dr. G.E. Dower for the helpful discussions. Financial assistance provided by the Banting Research Foundation is also gratefully acknowledged.

INTRODUCTION

The action potentials of nerve and skeletal muscle are currently considered to be initiated by a selective increase in the permeability of the cell membrane to sodium ions (1). This is believed to be followed, after a lag period, by a similar increase in the permeability of the membrane to potassium ions (2). Other sequential events in the later phases have been described which permit the maintenance of electrical neutrality, and bring about recovery of the resting membrane potential. (1,2,3,4).

In many tissues, a change in the concentration of sodium in the external solution elicits marked changes in the trans-membrane potential recorded with intra-cellular micro-electrode (the amplitude of the action potential, the rate of rise of the upstroke of the action potential, and the rate of conduction of an impulse). For example, in the case of (a) some types of cardiac muscle (5,9); (b) Frog's sartorious muscle, (6); (c) myelinated nerve (7); and (d) giant axon of squid (8), a decrease in the external sodium concentration results in marked decreases in the height of action potentials. In cardiac muscle decrease in the external sodium concentration by 2/3 of the initial value greatly decreased the positive phase of the injury potential (9). In some cases (5,6) the rate of rise of the upstroke of the action potential was reported to have decreased with decrease in the external sodium concentration. Similar decreases in external sodium concentration also were shown to cause disappearance of spontaneous activity of Purkinje Tissue (5). Some types of cardiac muscle show little alteration in the depolarization phase of action potential in this type of experiment, (9a).

The rate of conduction in nerve tissue decreases as the salinity of the external medium is reduced. Eventual blockade of nerve conduction occurred on further lowering of the external concentration (6,10). However, the response of some excitable tissues is not in accord with the hypothesis that sodium ions carry the depolarization membrane currents. Fatt and Katz (11) have reported an increase in the amplitude of action potentials in isolated crustacean muscle fibre when sodium chloride in the perfusion medium was replaced bycholine chloride or certain quarternary ammonium ions. Wood (12) in a study of neuromuscular transmission in herbivorous insects, reported non-specificity of cations in the external solution as carriers of the action currents. However, relatively few such studies have been made on smooth muscle. Holman (13) has shown that action potentials in guinea pig taenia coli are to a great extent independent (down to 17 mEq/l) of the external sodium concentration.

In the present in vitro investigation the effects of a low external sodium concentration on the electrical and mechanical activity of uterine smooth muscle have been studied. The tissues used include uterine myometrium of pregnant cat, pregnant rabbit, estrogen-treated rabbit and estrogen-treated rat. The uterine smooth muscle in these species maintained its electrical as well as its mechanical activities in rather low concentrations of external sodium (15-25 mEq/l). However, when sodium was reduced to extremely low concentrations (less than 15-20 mEq/l), the action potentials disappeared and mechanical "paralysis" ensued.

METHODS

(a) Recording Systems

Isometric contractile tension and action potentials were recorded simultaneously during spontaneous contractions of isolated strips of uterine longitudinal smooth muscle. Tension was recorded with an RCA transducer (#5734). For recording the action potentials, signals were picked up from the surface of the myometrium. This was accomplished by using a platinum wire lead inserted into a capillary glass electrode with a tip diameter of about 300 μ (i.d.) and filled with the same solution as the external medium. The exploring electrode was arranged to record with respect to a grounded platinum electrode which also was inserted into the bathing medium. The signals thus picked up, were fed into the input of a pre-amplifier. The pre-amplifier in turn was connected to a conventional DC-coupled push-pull amplifier to drive the pen motor of a Sanborn-Twin-Viso Recorder. Amplifier tubes were heated by an electronically regulated direct current.

(b) Solutions

The various perfusion solutions employed consisted of:

(1) Krebs Ringer - prepared by diluting Deca Krebs 1:10 with double distilled water and adding 160 ml of 2.6% $NaHCO_3$ solution and 100 ml of 20% freshly prepared glucose solution per liter of diluted solution:

Deca Krebs consisted of:

50 parts 9% NaCl, 4 parts 5.75% KCl, 3 parts 8.06% $CaCl_2$
1 part 19.1% $MgSO_4$, 1 part 10.55% K_2PO_4

(2) Sodium-free solutions - Due to lack of any suitable substitute for $NaHCO_3$, this salt was omitted in the sodium free solution.[1] Hence the solution was slightly hypotonic as compared with Krebs Ringer.

The chief sodium free solutions employed were (1) choline Ringer and (2) sucrose Ringer. These solutions were identical with Krebs Ringer except that NaCl was replaced by isomotic equivalents of choline chloride or sucrose respectively. These solutions are referred to in the text as Na-poor media.

The composition of the three principle solutions used is given in Table I. All the perfusion media were constantly aerated with carbogen (95% O_2 and 5% CO_2) and were maintained at 34-35° in a thermostatically controlled water bath.

(c) Hormonal Treatment of the Animals:

Young rabbits and rats were given 100 mcg of estrogen per day for six days before the uteri were removed.

(d) Analytical Methods:

(1) For Perfusion Solutions: Na and K were determined by a method previously described for plasma using a Jahnke flame photometer and employing lithium nitrate as an internal standard (15,16). Chloride (Cl) was determined by direct titration with $Hg(NO_3)_2$ using diphenylcarbazone solution as an indicator (17).

(2) Tissues: The analytical procedures employed for tissues have been described in detail elsewhere (15). Endometrium was scraped from the tissues chosen for electrolyte analysis. The tissue was carefully blotted dry of external solution, weighed immediately in a previously weighed beaker, and carefully dried at 95-105° for five days. After re-weighing, the tissues were powdered and finally digested with HNO_3 (15). Chloride was determined by potentiometric titration with 0.01N $AgNO_3$ after preliminary cold extraction of chloride with 0.1N HNO_3 as outlined by Whittam (18).

---

[1] (Footnote from page 3). Recently a suitable method of sodium bicarbonate substitution has been reported (28).

TABLE I

	Krebs Ringer mM/L	Choline Ringer mM/L	Sucrose Ringer mM/L
Na or Choline or Sucrose	137.4	125.9	248.5
K	5.79	6.23	6.23
Ca	2.47	2.65	2.65
Mg	1.16	1.25	1.25
Cl	125.1	137.4	11.5
HCO_3	21.9	0.0	0.0
PO_4	1.16	1.25	1.25
SO_4	1.16	1.25	1.25
Glucose	49.2	52.9	52.9

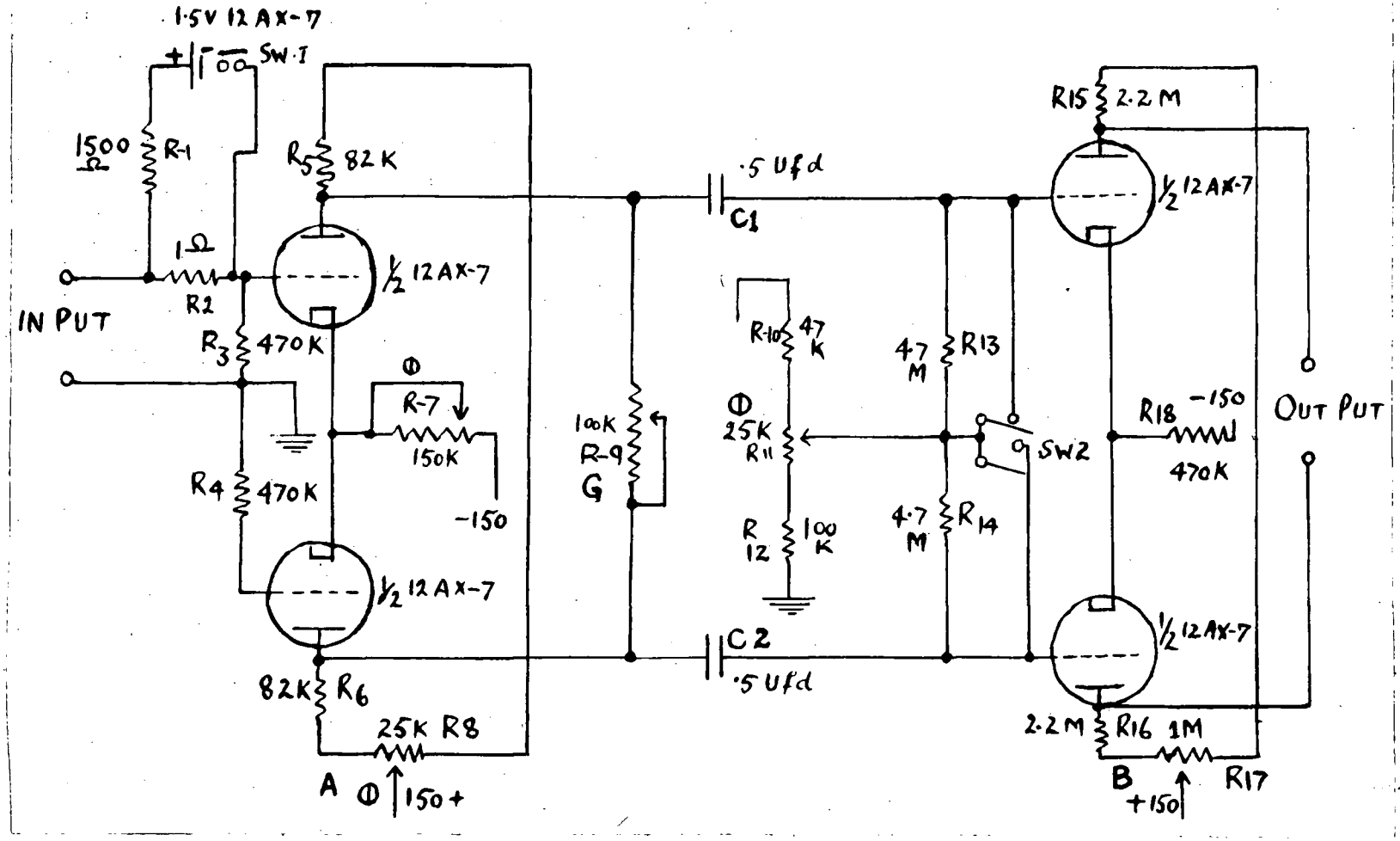


Figure I. This figure shows the salient features of the preamplifier assembly. It consists of (1) AC-coupled push-pull amplifying circuits and (2) C_1 and C_2 coupling condensers which in conjunction with resistances R_{13} and R_{14} givenan input time constant of 2.5 seconds. The maximum sensitivity of this system is 1 mv = 4 cm. SW I is the calibration switch, which gives 1 mv calibration signals when the circuit through the 1.5 V 12Ax-7 battery (B1) and resistances R_1 and R_2 is closed. A is the knob for fine screw adjustments to balance the gain. B is the knob for fine screw adjustments of the base line. C is the gain control knob. SW 2 is the double-pole single-throw (instomatic) switch. When the circuit through it is closed the current can leak rapidly through the path of least resistance.

(e) Calculations

Electrolyte concentrations in solutions were expressed as mEq/kg fresh weight of the tissue. Na and Cl spaces were calculated on the basis of formulae described by Manery (19).

Results treated statistically were expressed in terms of ± the standard error of the mean. If a sample varied by more than 2 x the standard deviation it was rejected and the results re-calculated omitting that particular sample.

(f) Criteria of Selection of Action Potentials for Statistical Treatment

Each spontaneous contraction was accompanied by a series of biphasic action potentials. With such a series, variation in amplitude[1] in Krebs Ringer was very large in rat and rabbit uteri and less so in the pregnant cat uterus. In rabbit and rat uteri the amplitude of a considerable proportion of action potentials in a series was too small to be estimated accurately. Consequently in each series potential with maximum amplitude, and those appearing close to this value, were taken for statistical evaluation. The values obtained under one condition (Krebs Ringer) were compared with the results similarly obtained under other conditions (low external sodium). This provided a uniform though arbitrary basis for a statistical evaluation of the characteristics of action potentials in various external sodium concentrations.

In sodium-poor media the amplitude of the action potentials was increased considerably. In pregnant cat uteri the variation in amplitude during a series was much reduced whereas with uteri from the other two species variations in amplitude, though reduced still persisted. If the

[1] In the text amplitude refers to the peak to peak amplitude of biphasic action potentials and the duration and rate of change of potential referes to the duration and the rate of change of the peak to peak deflection respectively, unless mentioned otherwise.

average amplitude is calculated for all of the action potentials in a series the increase in the mean amplitude of action potentials in sodium-poor medium is far greater than that calculated from selected action potentials of near maximum amplitudes. This is due to the fact that only a small proportion of action potentials were near maximum amplitude in Kreb's solution, but a large proportion of action potentials were near maximum value after reduction of the external sodium concentration. Variation in the duration of action potentials was not significant and the original configuration of the action potentials observed in Kreb's Ringer was maintained in sodium-poor media. Thus the amplitude was the only major variable.

(g) Experimental Procedure

Uteri were removed from pregnant cats and rabbits under barbiturate anesthesia (35-40 mg/kg), Estrogen treated rabbits and rats were killed by a blow on the head and the uteri rapidly removed.

For experimental purposes, 2 or 3 longitudinal uterine strips, each about 3 cm. in length, were excised and one of them was mounted in the medium bath so as to allow the tension to be recorded isometrically with the RCA transducer. Initially, the medium bath contained Krebs Ringer maintained at 34°- 35° , and aerated with a 95% O_2 + 5% CO_2 mixture.

Temperature control of the medium was achieved by pumping prewarmed water through the double walled medium bath. The temperature of the circulating water was controlled thermostatically. The tissue was mounted and placed under a basal tension of 4-5 gm. Under these conditions the tissue generally resumed spontaneous contractions after about 30 minutes. At this stage both action potentials and contractile tensions were recorded. At the end of a satisfactory control record, a sample of the bathing medium was taken for electrolyte analyses. The bath was then drained and sodium-poor solution

(previously warmed to 35°) was poured into the bath after a preliminary rinse with this solution. After allowing an equilibration period of about 20 minutes the activity was recorded as usual. The total period for which the tissue was left in a particular sodium-poor solution varied but in most cases the minimum duration of exposure was one hour. After satisfactory recording, samples of the medium was taken for electrolyte analyses and the bathing solution was replaced with a medium in which the sodium concentration was further reduced. In some instances, the tissue was exposed to a medium completely free of sodium in the final step. After the final step, the tissue mounted for recording and the solution bathing it were preserved for subsequent electrolyte analyses. Preliminary observations indicated that mounting per se did not contribute to alteration in tissue electrolyte composition in vitro. Unmounted uterine strips were kept in various media which were warmed and aerated in the same fashion as the experimental tissues. These unmounted segments kept in Krebs Ringer served as controls for the estimation of tissue electrolyte changes of the pieces which were subjected to sodium-poor media, control and experimental tissues being removed from this respective media for analysis simultaneously.

For the estimation of the tissue electrolyte changes in uterine segments which had been exposed to sodium-poor solutions, but were to be subjected to further experiments in other media, unmounted segments which had been immersed in similar solutions for the same period of time were employed. The small amounts of tissue available in estrogen-treated rabbits and rats often precluded the possibility of analysing unmounted tissue samples for each step in the reduction of the external sodium concentration.

RESULTS

(a) Pregnant Cat Uterus

The results obtained from typical experiments are summarized in Table II. Specimen records of action potentials and contractile tension in media with various external sodium concentrations are presented in Figures 2-6.

In any particular series of repetitive action potentials some degree of variation usually was observed in the amplitude of action potentials (Figures 2 and 3). These variations in amplitude were somewhat greater in Krebs Ringer than in sodium-poor media. Because of the large variation in the amplitude of the action potentials from one experiment to another in Krebs Ringer, each piece was employed as its own control for comparison with the results obtained in sodium-poor media. The results from four typical experiments are arranged in Table II. The following findings were consistently by observed in satisfactory experiments.

(i) In a given series of repetitive action potentials, an initial action potential prededed contraction and the rest of them occurred during contractions.

(ii) The amplitude of the action potentials was always increased in sodium-poor media (Figures 4,5 and Table II).[1]

---

[1] In one of the experiments only slight electrical and mechanical activity were observed in Krebs Ringer medium. Keeping in view the fact that activity generally improved in sodium-poor solutions, Krebs Ringer was replaced in part by sucrose Ringer. On reducing the external sodium concentration the muscle contracted and then relaxed gradually. Rhythmic contractions associated with typical biphasic action potentials then appeared. In fact the peak to peak amplitude of the action potentials in this series was the highest that we have recorded (up to 5 mv. Table II series 4 & Fig. 6).

TABLE II

PREGNANT CAT UTERUS

No.	Bath. Medium	Basal ϕ Tension Gm	Maximum Tension Rise Gm	Minimum Time-1Gm Tension Rise ϕϕ msec.	Duration of Contraction secs. xx	Maximum Amplitude of Action Potential mv/ x	Maximum Rate of Change of Action Potential mv/100 msecs.	Duration of Action potential msecs.	Minimum Interval Between two successive Action Potent. msecs.
1.A	Krebs Ringer	4	11.5	60–65	35–40	0.8± 0.01 (9)	3.4	28–30	300–340
.B	Na-poor medium X Na = 60 mEq/l	4	11.5	55–60	75–80	1.2± 0.92 (9)	5.0	22–25	2400–2500
C	Na-poor medium X Na = 18 mEq/l	4	11.5	50–55	90–95	1.0± 0.02 (10)	5.0	20–24	2400–2500
2.A	Krebs Ringer	4	11.6	110–115	57–60	2.6 ± 0.03 (9)	5.3	26–30	1225–1255
B	Na-poor medium X Na = 55 mEq/l	4	11.6	80–85	65–70	2.9 ± 0.08 (9)	5.8	30–34	3500–4000
C	Na-poor medium X Na = 19 mEq/l	4(+1)	11.6	-	very prolonged	2.9 ± 0.1 (9)	6.5	30–35	3700–4000
3.A	Krebs Ringer	5	12.5	375	60–70	1.7 ± 0.6 (9)	9.6	22.24	500–550
B	Na-poor medium X Na = 25 mEq/l	5(+3)	12.5	250	30–35	2.3 ± 0.14 (9)	8.3	24–28	800–900
4.A	Krebs Ringer	6	Poor Contractions (less than 1 Gm)			Occasional action potentials of less than 0.2 mv amplitude			
B	Na-poor medium Na = 23 mEq/l	6(+2)	13.5	250	15–20	3.1 ± 0.18 (12)	11.5	30–32	10,000–12,000
C	Na-poor medium Na = 18 mEq/l	6(+4)	13.5	-	14–16	5.0 ± 0.08 (9)	16.5	32–36	10,000–12,000

TABLE II (Cont'd)

x The action potentials had biphasic configuration.

X Substitution with choline ringer medium.

/ Substitution with sucrose ringer medium.

xx Tissue showed contraction with each reduction in external sodium, concentration, gradual relaxation and eventual resumption of spontaneous contractions. The duration of contractions in sodium-poor solution denoted in the table represents the duration after resumption of spontaneous activity. Unless otherwise mentioned these conditions hold true for subsequent tables.

ø Figures in parenthesis indicate the change in basal tension during the course of an experiment.

øø The time (in msecs) during which tension rise was fastest was taken. This duration was divided by the number of Gms by which the tension rose to get the value for the Gm maximum tension rise as represented in the tables.

(iii) The duration of peak-to-peak deflections (20-35 msec.) remained statistically unchanged in sodium-poor solutions.

(iv) The maximum rate of change of peak-to-peak deflections either remained unchanged or increased (Table II).

(v) The minimum interval between two successive action potentials (frequency) was increased considerably in sodium-poor media, sometimes as great as 8 times that observed in Krebs Ringer.

(vi) The duration of spontaneous contractions in sodium-poor media was more prolonged than in Krebs Ringer although exceptions sometimes were observed.

(vii) The same results were obtained irrespective of whether choline chloride or sucrose was substituted for Na. (Figures 5 and 6, and series 4 in Table II).

(viii) In very low concentrations of external sodium (less than 10-15 mEq/L) both electrical and mechanical activities disappear (Fourth tracing from the left in Figure 5).

When the external medium is changed to a sodium-poor solution, a sudden contraction took place. The muscle gradually relaxed after a relatively long period of time, (about 15-20 mins.) and spontaneous activity eventually was resumed. However, when the external sodium concentration was lowered to less than 15-20 mEq/l, the muscle showed an even more prolonged initial contraction with intermittent outbursts of action potentials. The tissue then relaxed gradually but failed to contract again. At this stage the complete cessation of action potentials also occurred.

A tendency for basal tension to be increased commonly was observed on exposure to moderately sodium-poor solutions, and this affect often became

more marked in media with lower concentrations of sodium. The effect of reduction in the external sodium concentration on the rate of rise of contractile tension was rather variable, although the maximum rate remained fairly constant.

(b) Pregnant Rabbit Uterus

The results obtained from a single pregnant rabbit uterus are summarized in Table III.

Specimen records are presented in Figures (7 to 10). The results obtained were qualitatively the same as in pregnant cat uterus. Action potentials were complex rather than biphasic and tended to be more variable in shape and magnitude than in the pregnant cat uterus. Action potentials were associated with contraction but they did not always precede the contraction (unlike pregnant cat uterus) The amplitude of action potentials was increased in sodium-poor media (Table IV and Fig. 10) (from 0.74 + 0.03 in Krebs Ringer to 1.03 + 0.07 in Na-poor media). While the rate of change of potential was increased, its duration remained practically unaltered (17-20 msec.). The basal contractile tension was increased as the sodium concentration of the external solution was lowered. When the external sodium concentration was reduced from 138.5 mEq/l to 29 mEq/l (Table V) the basal tension rose from 5 Gm. to 11 Gm. The concentration of sodium was not decreased any further in this experiment to avoid the sustained contraction which develops in very low external sodium concentrations (below 20-25 mEq/l). The maximum rate of tension rise remained unaltered but the duration of contraction increased to about 8 to 10 times that observed in Krebs Ringer medium. The frequency of action potentials was slightly decreased.

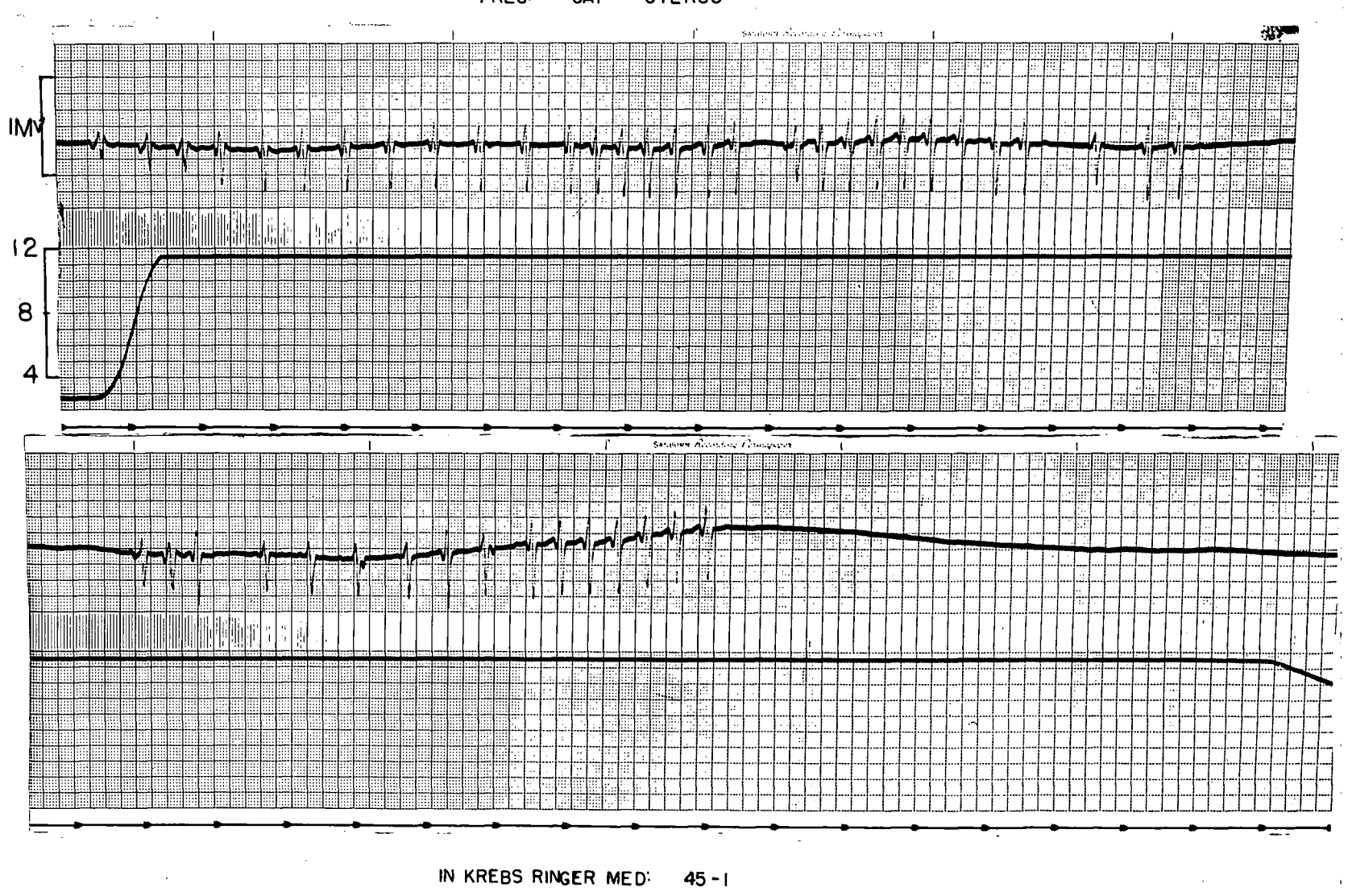


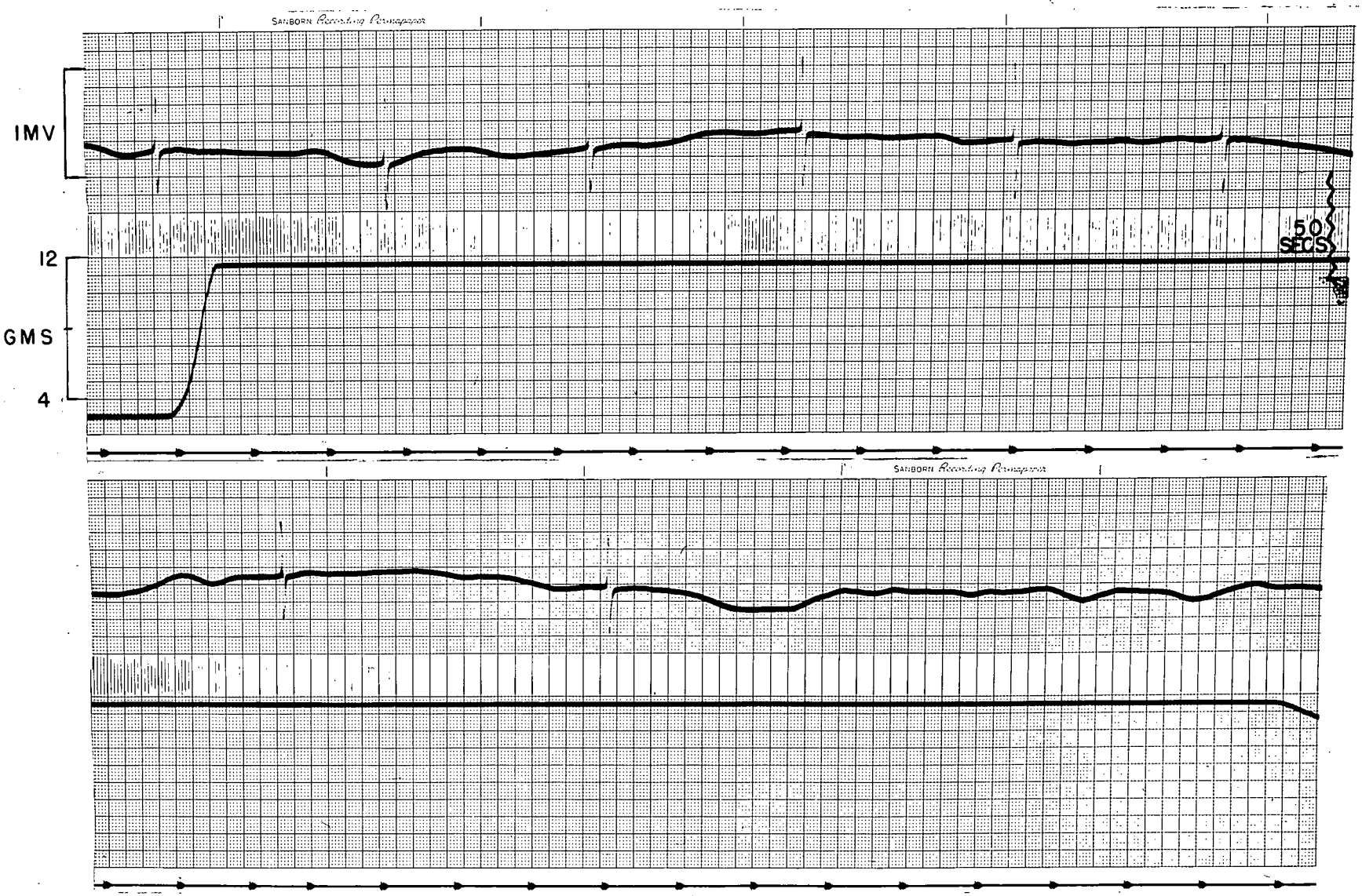
Figure 2. This figure shows spontaneous action potentials and isometric contractile tension from the pregnant cat uterus in Krebs Ringer. Note that the initial action potential preceeds the contraction. The amplitude of the action potentials in this series varied from 0.2 to 0.7 mv. Action potentials ceased to occur before the tension began to fall. During the interval of maximum tension, the action potentials occurred at a frequency of about 2/sec.

* In the figures to follow isometric contractile tension is referred to simply as contraction.

Figure 3. This record illustrates a spontaneous contraction and accompanying action potential in a medium in which sodium was replaced by choline. Sodium concentration in the medium as subsequently determined was 60 mEq/l. This record should be compared with the control record presented in Figure 2 which was obtained from the same preparation. Note the increased amplitude of action potentials (0.9 to 1.1 mv) their almost unaltered peak to peak duration, their decreased frequency (1 every 2.5-4.5 seconds) and the very much prolonged duration of contraction. This contraction continued over an interval of 70 seconds. The general pattern of action potentials and contraction during this 70 second interval was the same as that in the portion shown in this figure.

(To follow page 16)

PREG. CAT UTERUS



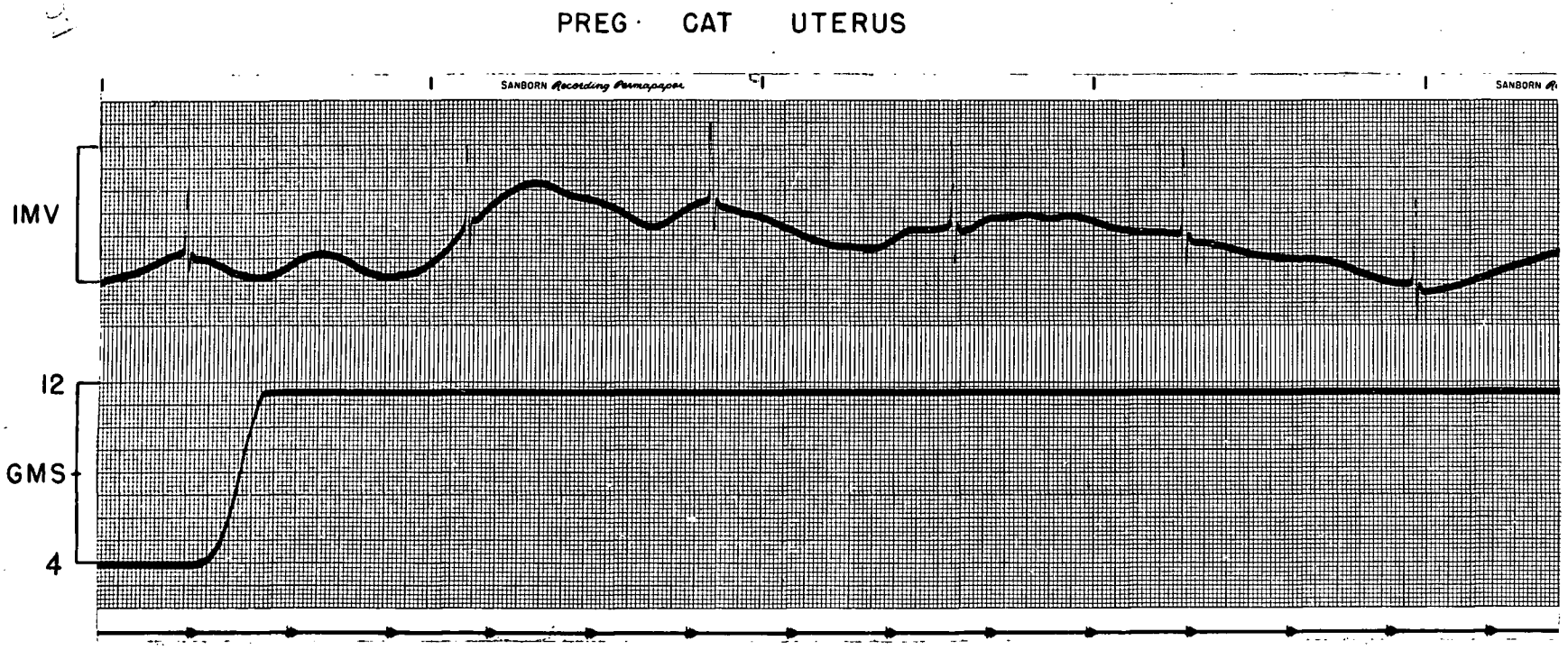


Figure 4. This record also is from the same experiment as the one represented in Figures 2 and 3. The sodium concentration in the medium was lowered by further replacement with choline. Final sodium concentration in the medium was 18 mEq/l. The amplitude of action potentials is still increased as compared with the control record (0.6 to 0.8). The unaltered duration and pattern and decreased frequency of action potentials also is evident (compare to the control records in Figure 2).

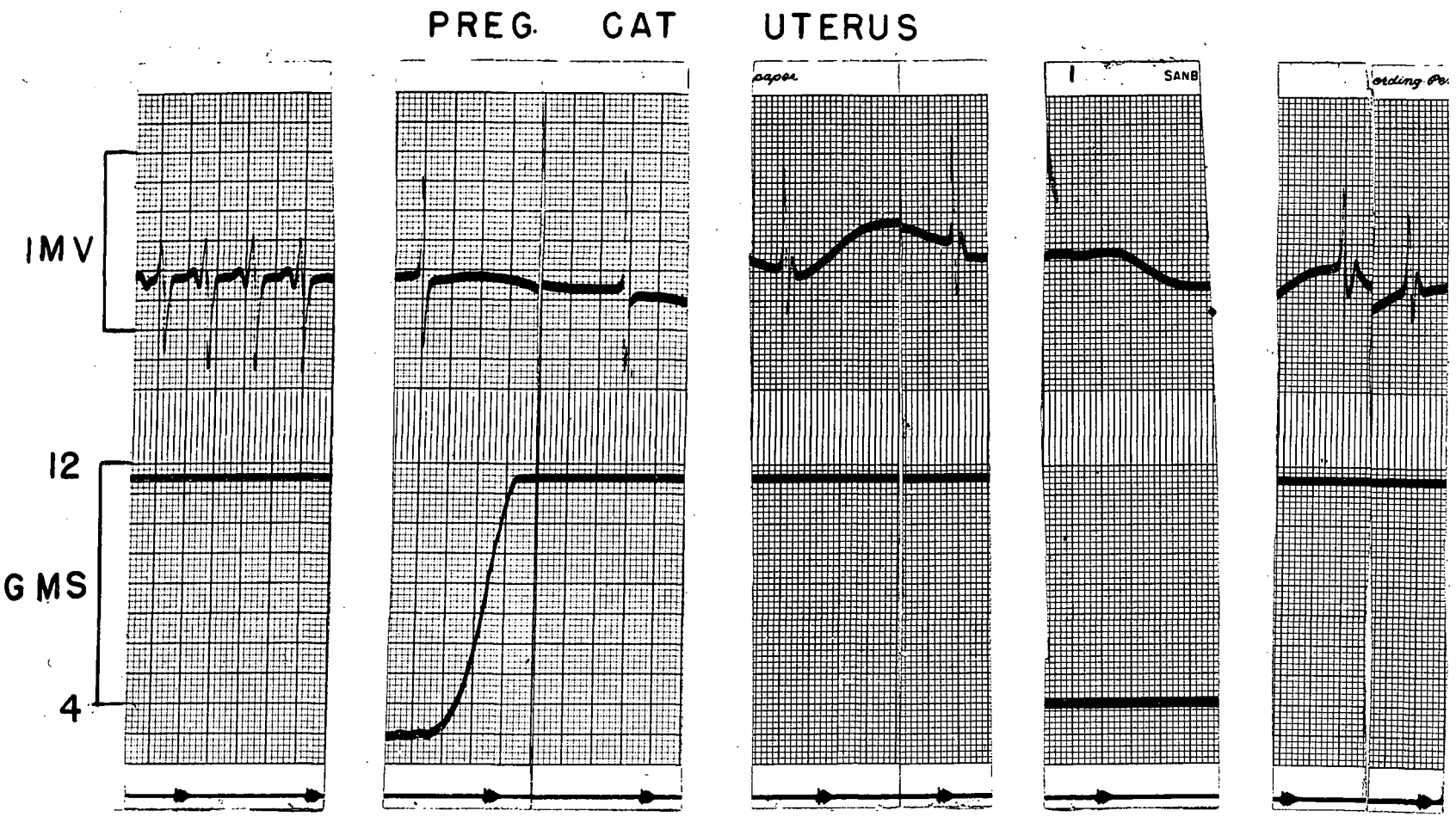


Figure 5. This figure summarizes the effects of different external sodium concentrations on the amplitude of action potentials in the pregnant cat uterus. For effects of different external sodium concentrations on other characteristics of action potentials see Figs. 2, 3 and 4). The fourth segment from the left shows the disappearance of action potentials and contractile tension (which fell to the control level under which the tissue was originally placed) when external sodium concentration was approximately 1 mEq/l. The fifth portion of the record indicates the reappearance of spontaneous contractions and action potentials five minutes after the sodium concentration was raised from 1 to 14 mEq/l.

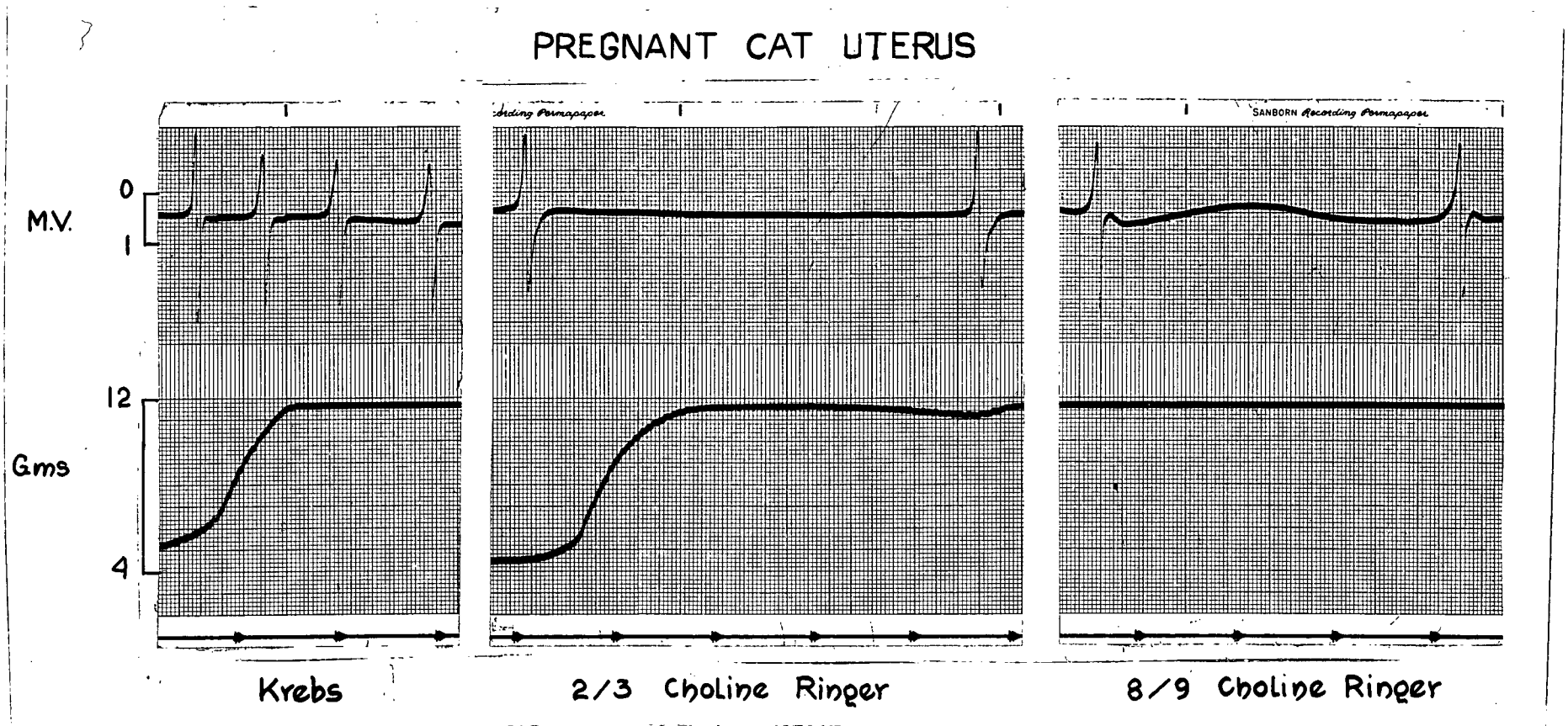


Figure 5a This record summarizes the effect of different external sodium concentrations on action potentials and contractions in pregnant cat uterus from another experiment. Note the similarity to the records presented in Figs. 2, 3, 4 and 5.

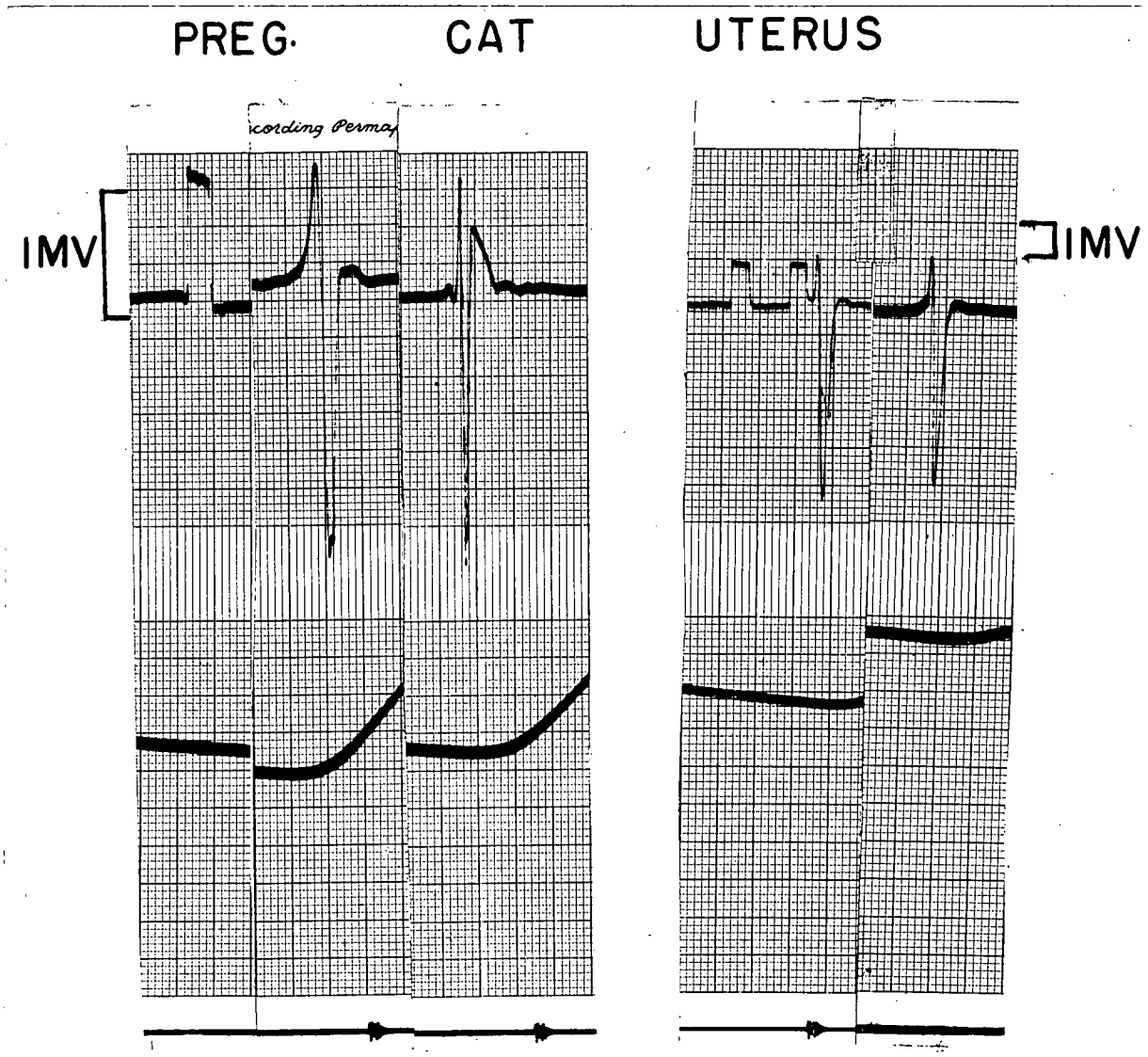


Figure 6 - This figure shows results from another experiment with pregnant cat uterus in which the effects of lowering external sodium were analyzed. This record lacks the control portion because spontaneous action potentials during a contraction failed to appear in this preparation when Krebs Ringer was used as the medium. Replacement of sodium by sucrose initiated spontaneous action potentials and contractions. Action potentials of high amplitude developed when the sodium concentration was lowered to 35 mEq/l (in the first portion on the left) and to 16 mEq/l (in the second portion of the figure). This record however, lacks the typical representation of effects of sodium depletion in the external medium on the frequency of action potentials and the contractile responses of the tissue.

TABLE III

PREGNANT RABBTT UTERUS

No.	Bath. Medium	Basal Tension Gm. ø	Maximum Tension Rise Gm	Minimum Time-1Gm Tension Rise øø msec.	Duration of Contration secs. xx	Maximum Amplitude of Action Potential mv x	Maximum Rate of Change of Action Potential mv/100 msecs.	Duration of Action potential msecs.	Minimum Interval Between two successive Action Potent. msecs.
A	Krebs Ringer	5	12.5	125	14-16	0.7 ± 0.03 (9)	6.0	13.-16	75-100
B-Na-poor medium Na = 60 mEq/l X		5(+2)	12.5	250	35-40	0.8 ± 0.01 (7)	6.0	15-17	250-300
C-Na-poor medium Na = 29 mEq/l X		5(+6)	12.5	---	90-120	1.0 ± 0.07 (9)	8.0	17-21	300-350

x, xx, X, ø, øø = See Table II.

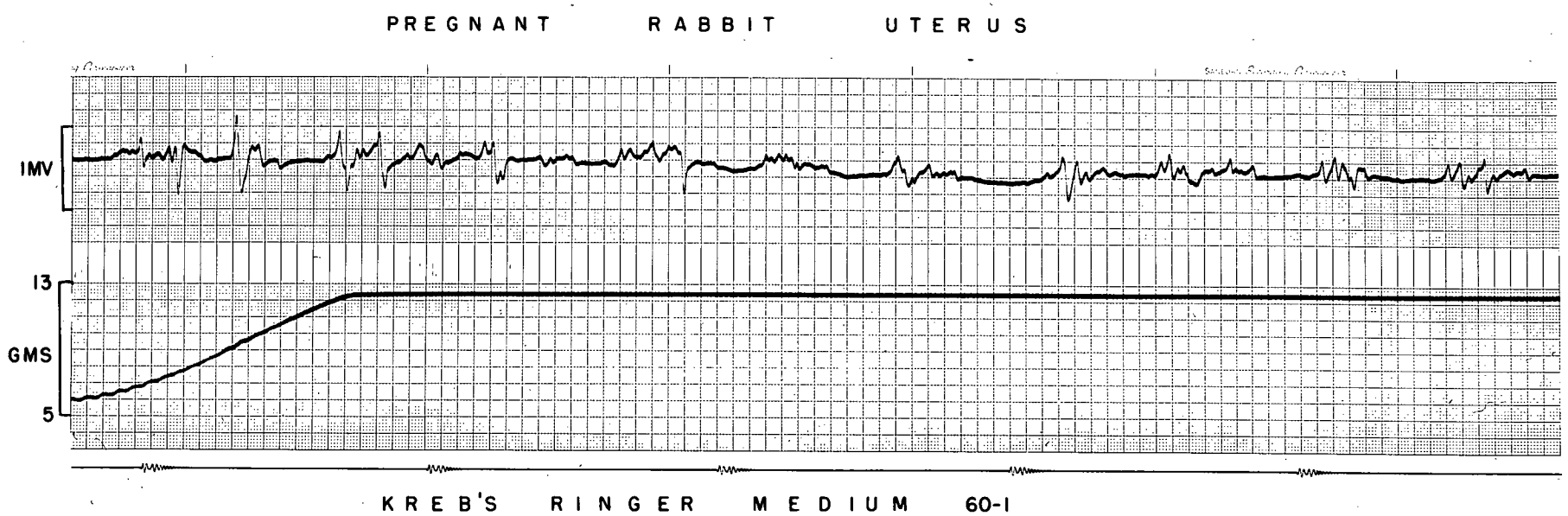


Figure 7 . This figure illustrates spontaneous action potentials during the contraction of pregnant rabbit uterus in Krebs Ringer medium. Note the variability in the amplitude of action potentials and the presence of a number of small wavy electrical fluctuations (compare with figure 2). The first action potential coincided with the initial rise of tension (note the similarity to cat uteri in figure 2).

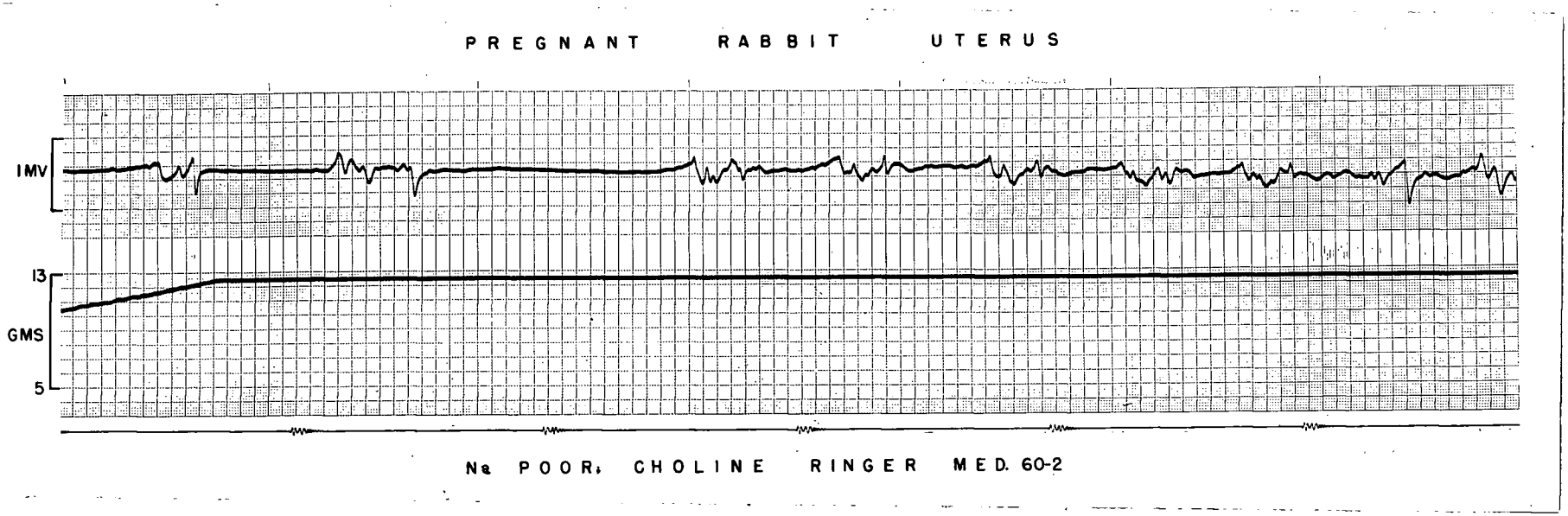


Figure 8. This record illustrates action potentials during uterine contraction in a sodium-poor medium (in the same preparation as that illustrated in Figure 7). Choline partially replaced sodium, the concentration of sodium in the medium as determined subsequently being 60 mEq/l. Note the rise in basal tension on exposure to the sodium-poor medium (compare with Figure 3).

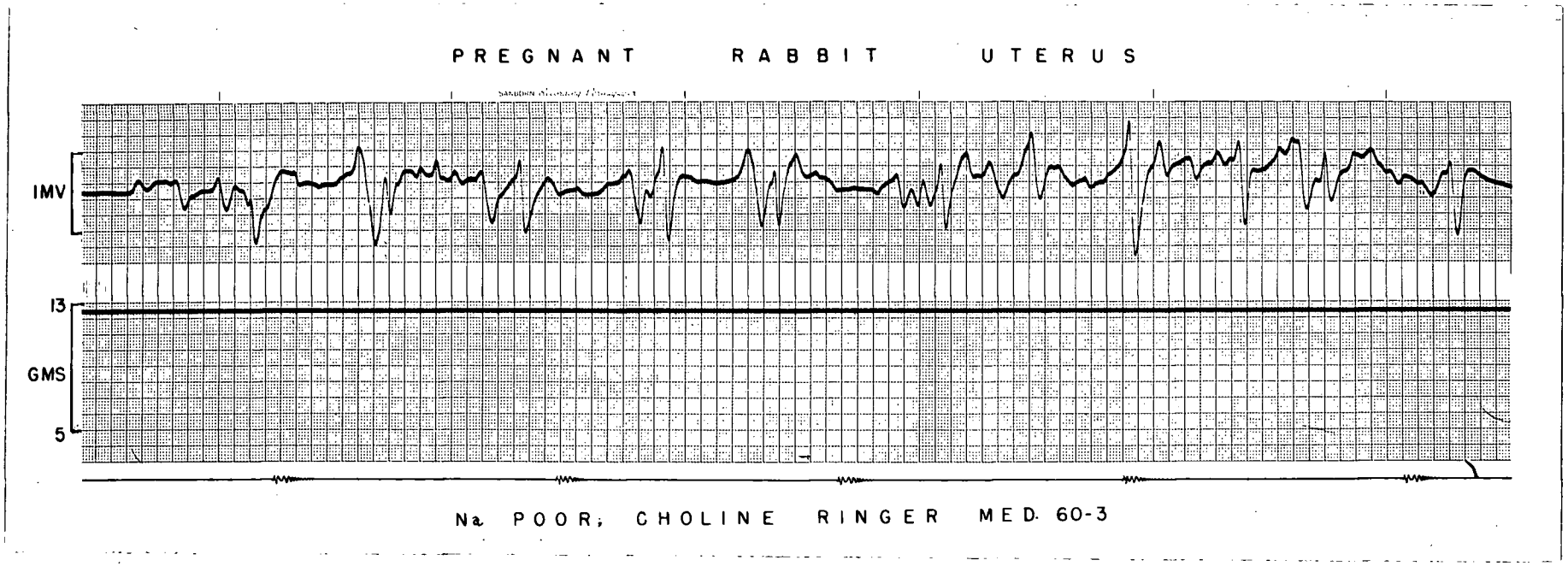


Figure 9. This record illustrates spontaneous action potentials during contraction (in the same preparation furnishing the records shown in figures 7 and 8) after further reduction in external sodium concentration (sodium concentration as determined subsequently was 29 mEq/l). Note the marked increase in the amplitude of action potentials, their unaltered frequency and the persistance of small wavy electrical fluctuations (also 8). Though not very clearly seen in the figure, basal contractile tension was increased from 5 to 11 gm during exposure to this medium. The maximum tension rise was 12 - 12.5 gm. External sodium concentration was not lowered any further to avoid the persistant contracture which was observed in some of the similar experiments done previously in the non-pregnant rabbit uterus.

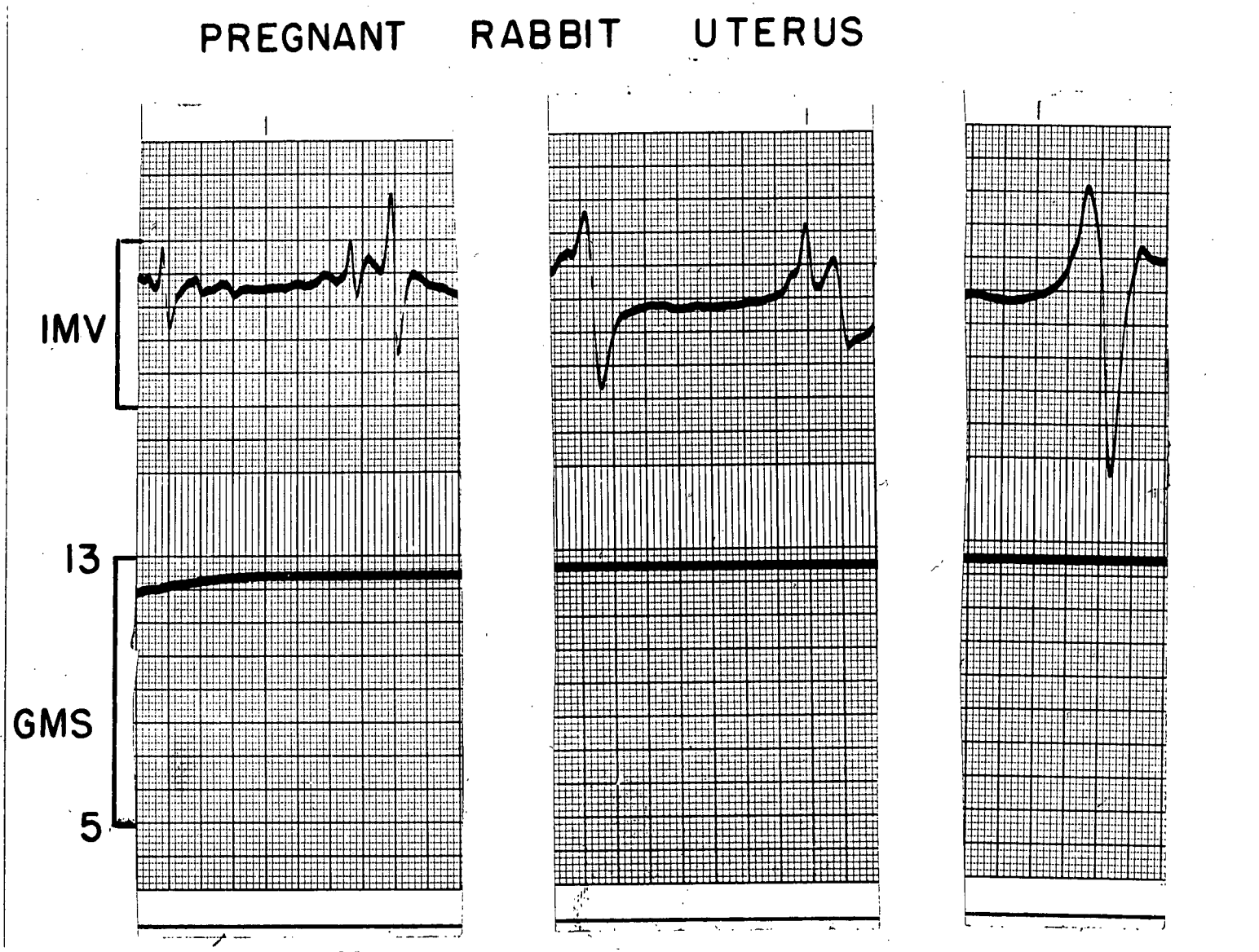


Figure 10. This figure summarizes some of the typical features of the records presented in figures 7, 8 and 9. Note the increased amplitude of action potentials and their unaltered frequency in sodium-poor media.

(c) Estrogen-Treated Rat Uterus

The results obtained from estrogen-treated rat uteri are shown in Table IV.

Various specimen-tracings of action potentials and contractile tension are shown in Figures 11 to 14. As in pregnant cat and rabbit uteri, the reduction in the sodium concentration of the external medium resulted in an increased amplitude of action potentials. The duration of action potentials remained almost unaffected. The maximum rate of change of potentials was variable in different experiments, being increased in some cases (series 1 and 2 in Table VI), but decreased slightly in others (series 4 in Table VI). The frequency of action potentials was not changed significantly in sodium-poor solutions. In a given chain of repetitive action potentials variations were considerable and the frequency of spikes also was irregular (Figures 12-14). Thus the properties of rat uteri resembled those of the pregnant rabbit uterus rather closely.

Spike discharges (action potentials) always were related to mechanical activity in some manner. They usually occurred during the rising phase, on the plateau of contraction, However, in the rat uterus, groups of action potentials sometimes occurred when the muscle had just started to relax. The exact significance of the relationship between the occurrence of action potentials and the phase of contraction is not clear. Reduction in the external sodium concentration produced very marked effects on the contractile function of this tissue. With each step in lowering the external sodium concentration there was a tendency toward more and more prolonged contractions (lasting several minutes as compared with the usual 15-20 secs. in Krebs Ringer Medium) and incomplete relaxation was common. Spastic contraction, or even complete inability to relax, developed when the external

TABLE IV

ESTROGEN-TREATED RAT UTERUS

No.	Bath Medium	Basal Tension Gm ø	Maximum Tension Rise Gm	Minimum Time-1Gm Rise msecøø	Duration of contraction secs. xx	Maximum Amplitude of Action Potential mv x	Maximum Rate of Change of Action Potential mv/100 msecs.	Duration of Action potential msecs.	Minimum Interval Between two successive Action Potent. Msecs.
1.A	Krebs Ringer	4	9.5	90-100	10-12	2.0± .08 (9)	9.7	15-17	350-375
	B-Na-poor medium^{X}	4 (+3)	11.0	500	35-45	1.9± 0.04 (9)	9.8	18-20	275
	C-Na-poor medium^{X}	4 (+5.5)	11.0	—	120	2.2± 0.07 (9)	11.1	20-22	280-295
2.A	Krebs Ringer	4	11.0	300	15	1.6± .06 (9)	12.5	20-24	385-295
	B-Na-poor medium^{X} 25 mEq/L	4 (+3)	12.0	400	50-52	2.1± .13 (9)	16.0	25-30	380
	C	In media of lower external sodium concentration, the muscle went into sustained concentration and the action potentials disappeared.							
3.A	Krebs Ringer	2.5	9.5	—	15	0.7± 0.01 (9)	—	16-20	—
	B-Na-poor medium$^{X\not,X}$	2.5	9.5	—	300-1800	0.8± 0.23 (9)	—	18-22	—
	C	Further muscles passes into sustained contraction with disappearance of AP's. Low Na Concentration.$^{X\not,X}$							
4.A	Krebs Ringer$^{\not}$	4	8	650	20	1.2	6.0	22-24	—
	B-Na-poor medium$^{\not}$ 28 mEq/l	4 (+4.5)	9.5	—	300-1800	1.0	5.0	20-22	—

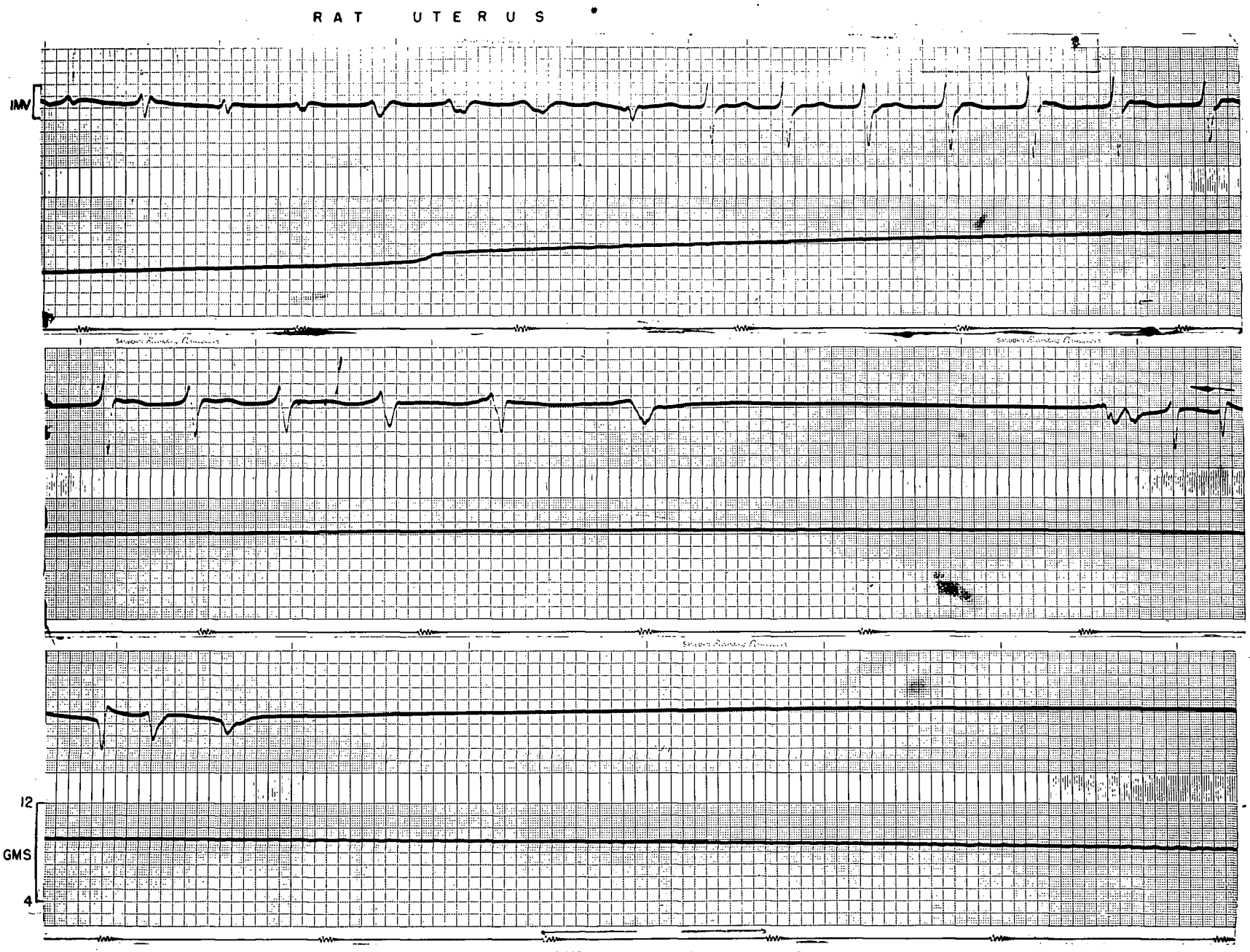
Further decrease in Na Concentration of the external medium caused sustained spastic contraction in the muscle with disappearance of action potentials.

X$\not$ Action potential had nomophasic ($\not\Lambda$) configuration.

$\not$,X,ø,øø,x,xx = See Table II.

Figure 11: This record illustrates spontaneous action potentials during a contraction from an estrogen treated rat uterus in Krebs Ringer medium. Note the gradual onset of action potentials and their relation to contraction. Also note the variability in the amplitude and configuration of the action potentials.

(To follow page 28)


RAT UTERUS
1MV
12
GMS
4

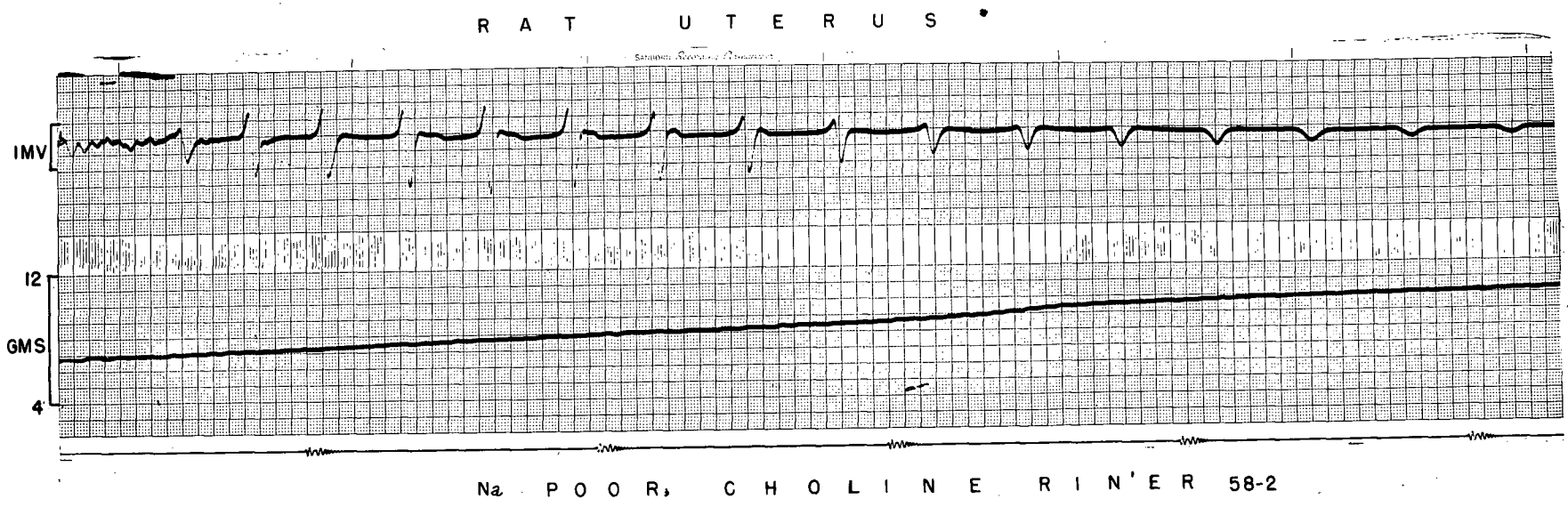


Figure 12. This record illustrates spontaneous action potentials during a contraction in a sodium-poor medium in the same preparation from which the record shown in figure 11 was obtained. Choline was substituted for sodium. The final concentration of sodium in this experiment was 43 mEq/l. Note the gradual onset of the action potentials and of contraction (compare with figure 3 and 4). The variability in the configuration of the action potentials still persisted (compare with figure 11). Note that the action potentials tended to disappear when the contraction reached its peak. The frequency of action potentials was not affected significantly in this medium as compared to the control in Krebs Ringer (see figure 11, and compare with figure 3 and 4).

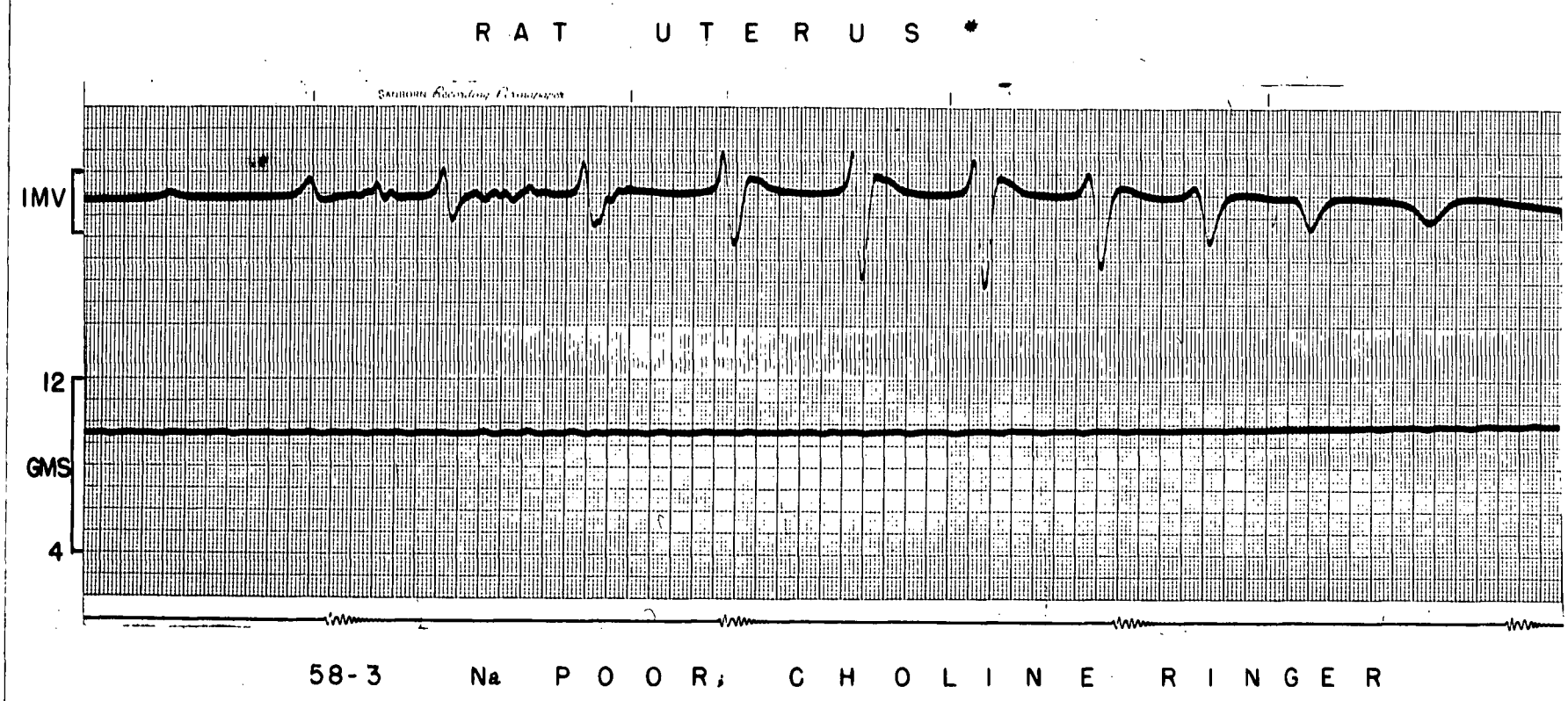


Figure 13. - This record illustrates the changes in action potentials and contractions in the same experiment as that represented in figures 12 and 13 during further lowering of the external concentration of sodium. The sodium concentration of this medium was 27 mEq/l. Note the increase in basal tension after exposure to the sodium-poor medium (compare with the control in figure 11). The variations in the configuration of the action potentials persisted even under these conditions. Note the contrast to the cat uteri under similar external sodium concentrations (figures 3 and 4).

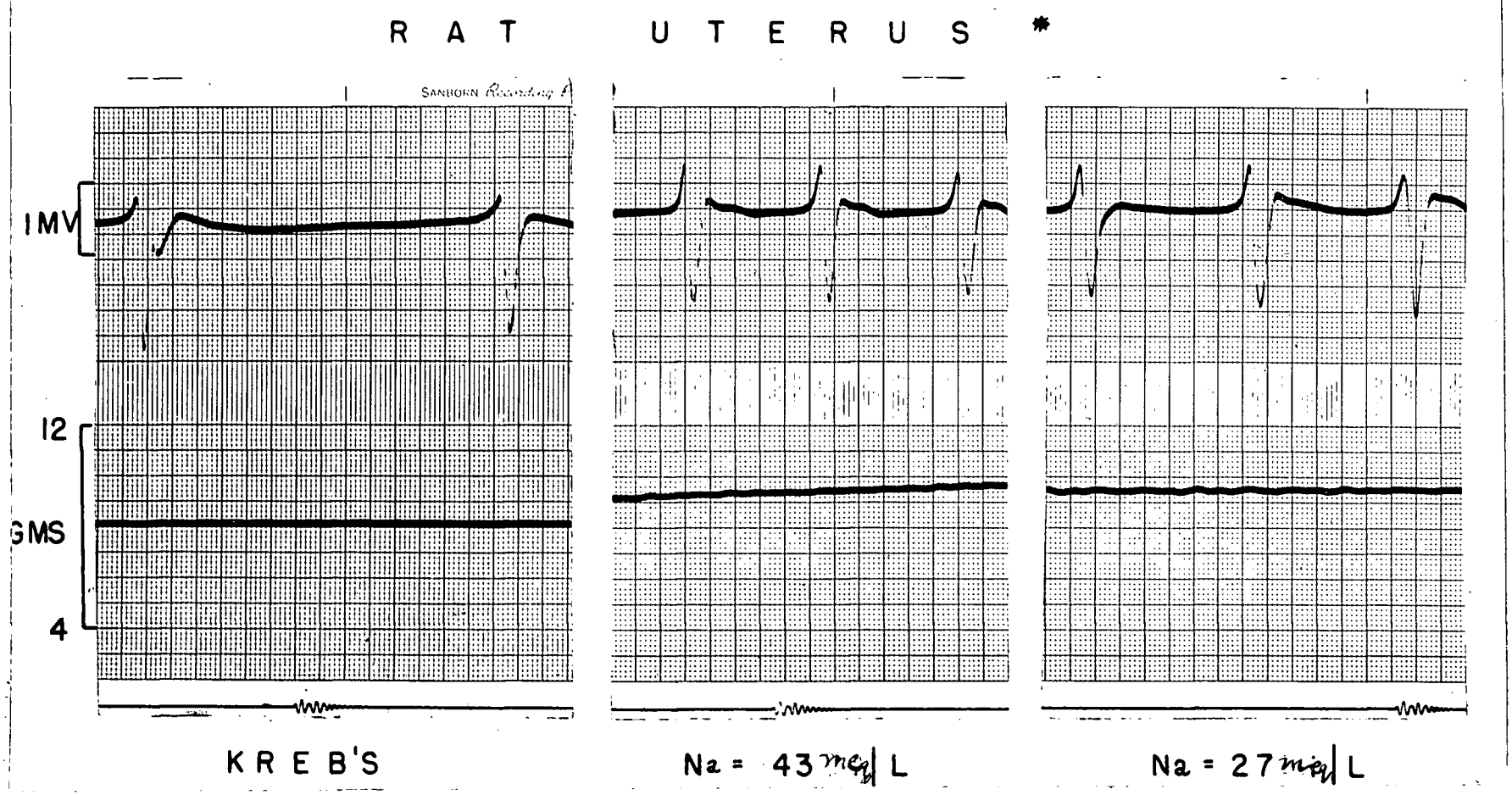


Figure 14: This figure summarizes the records presented in figures 11, 12 and 13. Portion 1 on the left shows action potential at the peak of contraction while in the remaining two portions of the record (in sodium-poor medium) the increase in basal tension should be noted. The amplitude of action potentials is not so large as that observed in other experiments with uteri of the same species. The frequency of action potentials was almost unaltered.

sodium concentration was still in the vicinity of 25 mEq/l. This behaviour precluded the possibility of any further reduction in the external sodium concentration if sustained contracture, and subsequent mechanical and electrical inactivity was to be avoided. The contraction which developed in sodium-poor solutions was found to persist for as long as 2-2½ hours. During the first stage of such contracture intermittent outbursts of action potentials were recorded, but as the contracture became more prolonged, all electrical activity disappeared. Possibly this behaviour represents another facet of the same paralytic phenomenon which is seen in cat uteri in very low concentrations of external sodium. However, cat uteri ultimately relaxed after the initial prolonged contracture in sodium-poor solutions, and failed to contract again, while rat uteri became incapable of relaxing after persistent contraction developed in sodium-poor media. This type of contraction could not be antagonized by the conventional pharmacological smooth muscle relaxants. (Sodium-nitrite failed to initiate relaxation of this type of contracture.)[1]. Some of the inhibitory sympathomimetic amines also failed to bring about relaxation.

(d) Estrogen-Treated Rabbit

Results obtained with estrogen-treated rabbit uteri are summarized in Table V. Specimen recordings are presented in Figure 15. Only two experiments were carried out. The results obtained were qualitatively

---

[1] This persistent increase in tension probably should be classified as contracture since eventually it was not accompanied by electrical activity.

TABLE V

ESTROGEN TREATED RABBIT UTERUS

No.	Bath Medium	Basal Tension Gm ϕ	Maximum Tension Rise Gm	Minimum Time-1Gm Tension Rise ϕϕ msec.	Duration of Contraction secs. xx	Maximum Amplitude of Action Potential mv x	Maximum Rate of Change of Action Potential mv/100 msecs.	Duration of Action potential msecs.	Minimum Interval Between two successive Action Potent. msecs.
1. A	Krebs Ringer	6	7	1000	7-8	0.3 ± 0.02 (9)	2.0	16-18	450-500
B	Na-poor medium X	6 (2.5)	9	—	6-7	0.3 ± .01 (10)	1.7	18-20	600-650
C	Na-poor medium X	6 (5.5)	12	—	6-7	0.4 ± 0.03 (9)	2.4	18-22	650-700
2. A	Krebs Ringer	5 (+2)	11.5	700	20-25	0.3 ± 0.01 (10)	2.2	18-20	Irregular
B	Na-poor medium X	6 (+4)	11.0	1000		0.4 ± 0.03 (9)	2.7	18-22	600-650

ϕ, ϕϕ, x, xx, X, ∠ = See Table II.

the same as in other species. The amplitude of action potentials was very small (0.1-0.3) in Krebs Ringer medium. In fact, earlier attempts to record action potentials from this tissue with electrodes having a smaller tip diameter had always ended in failure. The increase in maximum amplitude of action potentials in sodium-poor media was slight[2], but the duration and rate of change of the peak to peak deflection remained unchanged.

This tissue showed a tendency toward incomplete relaxation with each decrease in the external sodium concentration, resembling rat uteri in this respect. At a sodium concentration of about 25-30 mEq/l, the basal tension had increased to approximately the value observed with maximal contractions in Krebs Ringer.

(e) Tissue Electrolytes

Tables VI, VII, VIII and IX summarize the electrolyte analyses of the tissues, the corresponding perfusion fluids and the functional states of the tissues in these media. Tissue sodium tended to be lost in sodium-poor media. The greater the reduction in the concentration of external sodium, the greater was the tissue sodium loss until a point was reached when further reduction in the external sodium concentration no longer affected the concentration of tissue sodium to an appreciable extent. Even when the sodium concentration in the external medium was almost nil (0.2-1.0 mEq/l), and the tissues were inactive, electrolyte analysis revealed a tissue sodium concentration which was not significantly

---

[2] Action potentials within the maximum range of amplitude were considered for statistical representation in the table. However, the average of all action potentials was markedly greater in amplitude in sodium-poor media because the proportion of action potentials with maximum amplitude was markedly greater in sodium-poor media.

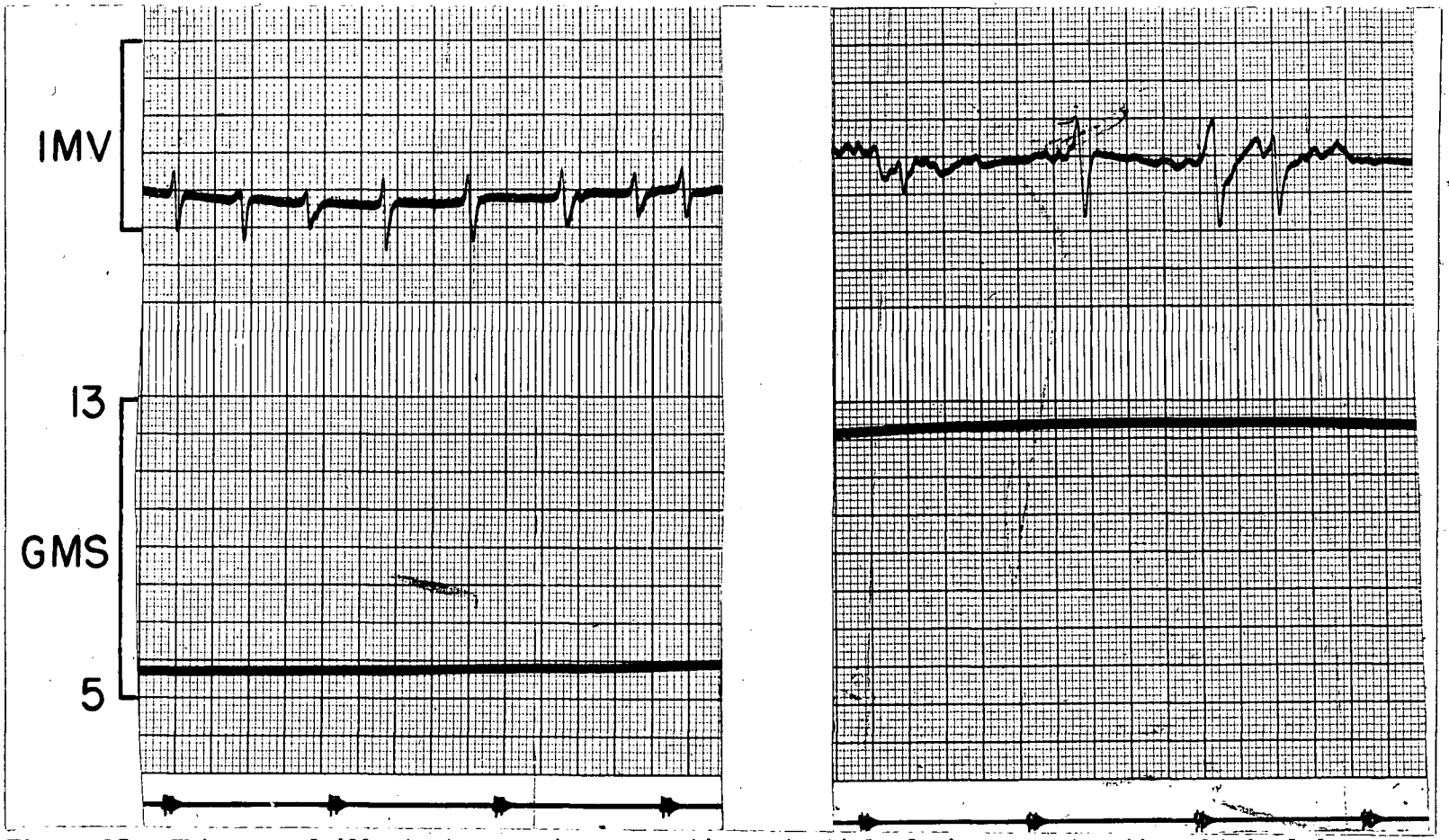


Figure 15. This record illustrates spontaneous action potentials during a contraction obtained from an estrogen-treated rabbit uterus, first in Krebs Ringer and then in a sodium-poor medium (The sodium concentration of the sodium-poor medium 29 mEq/l). Note the increased amplitude of action potentials, their unaltered frequency, and the persistance of small wavy electrical fluctuations even during exposure to the sodium-poor medium. The increase in basal tension on exposure to a sodium-poor medium also should be noted.

TABLE VI

PREGNANT CAT UTERUS

Electrolyte Composition of Media, Tissues and Their Functional State

Treatment	Functional state	Electrolyte Composition of media mEq/l			Electrolyte Composition of Tissues mEq/kg fresh weight			G/Kg fresh weight
		Na	K	Cl	Na	K	Cl	H_2O
Direct Control	—	—	—	—	86.7	51.2	82.0	806
"	—	—	—	—	85.8	68.8	82.8	820
Krebs Ringer	Active	151.2	5.0	122.5	127.8	51.3	95.2	818
" "	"	138.5	4.8	128.0	92.2	56.7	101.5	814
Partial Choline Ringer	Active	60.0	5.5	137.5	79.1	51.2	78.8	834
" "	"	54.4	5.4	129.4	32.9	14.8	104.4	853
" "	"	25.0	5.2	126.3	28.7	15.3	107.5	762
" "	"	19.0	5.5	133.8	16.7	9.5	112.2	834
100% Choline Ringer	Inactive	6.0	5.0	123.0	26.2	51.2	95.7	—
" "	"	3.0	5.5	130.6	19.8	19.0	90.0	837
Partial Sucrose Ringer	Active	70.0	5.1	88.4	44.2	33.3	44.9	—
" "	"	23.2	5.8	35.9	15.5	4.8	57.9	801
" "	"	18.5	5.3	24.5	12.3	3.0	45.5	779
Sucrose Ringer	Inactive	0.2	5.7	8.2	17.5	25.5	21.8	818
" "	"	1.0	5.8	10.9	18.9	43.5	25.0	760
Left in Isotonic K_2SO_4	—	0	167.0	0	12.5	174.5	10.2	845

* Action potentials and contraction always occurred together although the sequence of occurrence of one with respect to another was variable sometimes (details in the text). However in the absence of contraction action potentials were not recorded.

TABLE VII

Pregnant Rabbit Uterus

Electrolyte analysis of tissues and perfusion fluids at different functional states of the tissue.

Treatment	Functional* state	Electrolyte Composition of media mEq/l			Electrolyte Composition of Tissues mEq/kg fresh weight			G/Kg fresh weight
		Na	K	Cl	Na	K	Cl	H_2O
Direct Control	—	(148)	(4.5)	(110)	71.27	66.35	53.73	817
" "	—	—	—	—	81.04	29.89	78.94	837
A Krebs Ringer	Active	138.5	4.8	128.07	90.22	31.86	117.80	827
B Choline Ringer	Active	60.0	4.8	131.57	—	—	—	—
C Choline Ringer	Active	29	5.8	127.63	31.74	30.30	94.50	798

* See Table VI

TABLE VIII

Estrogen Treated Rat Uterus

Electrolyte analysis of tissues and corresponding perfusion solutions at different functional states of the tissues

Treatment	Functional state*	Electrolyte Composition of media MEq/l			Electrolyte Composition of Tissues mEq/kg fresh weight			G/Kg fresh weight
		Na	K	Cl	Na	K	Cl	H_2O_4
Control	—	—	—	—	74.5	43.0	68.3	798
Krebs Ringer	Active	136.5	5.0	130.1	105.1	40.5	86.6	804
Part Choline Ringer**	Active	43.0	5.4	126.3	40.6	21.0	87.7	818
" "	"	30.2	5.7	125.6	43.1	54.4	78.9	818
" "	+	25.0	5.2	126.3	19.5	35.1	103.3	783
100% Choline Ringer ≠	Inactive	1.2	5.8	123.0	19.0	43.2	74.7	809
" "	"	4.0	7.0	122.0	22.8	61.2	99.5	818
Sucrose Ringer**	Active	28.0	6.5	32.5	—	—	—	—
Sucrose Ringer ≠	Inactive	16.0	6.0	19.5	14.9	18.0	19.1	802
"	"	2.0	6.5	9.7	15.7	20.9	16.5	806
"	"	0.5	5.7	7.8	27.5	57.5	14.0	819

* See Table VI

** Tissues showing incomplete relaxation in spite of increased amplitude of action potential after changing to sodium-poor medium.

≠ Tissue showing indefinitely prolonged contraction and eventual arrest of action potentials.

TABLE IX

Estrogen Treated Rabbit Uterus

Electrolyte Analysis of Tissues and Perfusion Fluids at Different Functional States of the Tissues

Treatment	Functional State *	Electrolyte Composition of media mEq/l			Electrolyte Composition of Tissues mEq/kg fresh weight			G/Kg fresh weight
		Na	K	Cl	Na	K	Cl	H_2O_4
Krebs Ringer	Active	138.5	4.8	128.0	75.8	62.3	72.1	864
" "	"	140.5	5.0	130.2	81.3	66.3	76.9	866
Choline Ringer	Active	31.5	5.4	122.8	28.2	56.5	75.0	871
" "	"	29.0	5.4	125.4	24.5	60.3	77.4	860
Choline Ringer	Inactive	1.0	5.3	112.0	29.9	61.1	52.8	850
Left in isotonic Choline Chloride	—	0	0	167.0	22.7	44.4	101.1	860

* See Table VI

different from that which was found in functionally active tissues in somewhat higher sodium concentrations (20-25 mEq/l). Even when tissues were left in isotonic solutions of K_2SO_4, choline chloride or sucrose, over a prolonged period (3-4 hours and sometimes even over night) considerable quantities of sodium (about 15-20 mEq/kg) were still found in the tissues. Such residual tissue sodium has been reported previously from this laboratory (15).

After exposure to sodium-poor media, tissue potassium concentrations also were lower than the control values. The low concentration of tissue potassium in the pregnant cat uteri was very striking. Values as low as 4-5 mEq/kg were obtained while the tissue still exhibited full mechanical and electrical activity. In other species, the levels of tissue potassium in sodium-poor media were never as low as in the cat although a decrease sometimes was noticed.

The alteration in tissue chloride in sodium-poor media was not significant when choline chloride was used to replace sodium chloride. However, tissue chloride was decreased in sodium-poor media employing sucrose as a substitute for sodium chloride. The decrease in tissue chloride in the latter case probably was due primarily to the decrease in chloride concentration in the extracellular space.

DISCUSSION

I. Recording Techniques

The microelectrode technique has been employed extensively to record transmembrane action potentials from many excitable tissues (1-4). In this laboratory, attempts to apply this technique to one of the tissues (uterus of rabbit) employed in the present investigation have met with considerable difficulty, presumably due to small cell size and failure of microelectrodes to penetrate the cells without appreciable damage. However, action potentials could be recorded with ease from such tissues using extracellular surface electrodes. The limitations of such recordings in failing to give exact information regarding transmembrane potentials and rates of depolarization and repolarization were recognized.

Concomitant recordings with microelectrodes and extracellular electrodes have been made by other workers (20) from isolated single fibres of skeletal muscle. A comparison of the simultaneous biphasic extracellular action potentials and monophasic intracellular action potentials showed that the positive peak of the biphasic action potentials occurred at the same time as the onset of the rising phase of the monophasic action potential, and the negative peak of the biphasic action potential coincided with the peak depolarization of monophasic action potentials. The peak to peak deflection of biphasic extracellular action potentials thus corresponds in time with the upstroke of monophasic action potentials. However, the precise relationship between transmembrane potentials and extracellular recordings made from multicellular units (such as the tissues we have studied) has not been clarified. Various factors can influence the extracellularly-recorded action potential from multicellular units which have little effect on intracellularly recorded action potentials. These factors

may be described as follows:

An extracellular electrode near an electrically active region in the tissue records a voltage which is different from that of a distant electrode because the two electrodes are at different isopotential lines emanating from the electrical source and sink. In practice the distant electrode can usually be regarded as at zero potential. In these studies alterations in the position of the distant electrode did not noticeably alter the records obtained, so that it could be effectively regarded as indifferent.

A recordable change exists somewhere in the medium throughout the entire period that an impulse is present in any part of the tissue (21). Further studies (23) have shown that in uterine muscle using the present recording technique, activity at one electrode had no recordable effect on the second electrode which was 1-2 mm away from it. Therefore the different electrode could be regarded as recording only from the cells which were very close to its tip. According to Lorente De No (23) the tracings obtained from a single cell with an external electrode are related to the transmembrane potential curve as its second derivative, i.e. amplitude of extracellularly recorded action potentials related to the rate of change of the slope of the transmembrane potential curve.

In multicellular units, extracellularly recorded potentials are related to the sum of the potentials generated by all of the cells in the immediate vicinity of the recording electrode tip. The net potential difference recorded might be influenced by the rapidity with which these cells are activated and by sequential pattern of activation as well as by the magnitude of the potentials across the individual cells. If the cells are activated sufficiently close together (synchronously) and in proper sequence, the sum of their potentials will allow the recording of larger extracellular action potential. With

relatively asynchronous or nonsequential activation, potential produced in different cells may partly or completely nullify one another. It appears obvious from the considerations of the size of the electrode tip used and of the uterine muscle cells that some summation of potentials must occur to permit the recordings made extracellularly.

Many factors can increase the relative synchrony of the activation of the cells and in turn might contribute towards an increased amplitude of recorded potentials.

1. The rate of conduction of action potentials and the pattern of activity over the entire multicellular area from which the electrode records:

A fast rate of spread of activation will increase the degree of synchrony of activation of fibres, provided that the pathway of spread of activity is regularly and linerly maintained throughout the series of action potentials. Moreover, the regularity and symmetry of the pattern of spread of an impulse into the area which contributes the major potentials may determine the extent to which the potentials generated by asynchronously activated cells will tend to cancel one another. If the pattern of spread of activity varies during a series of impulses, considerable variability in amplitude of the extracellular potentials may result.

2. The number of cells responding in the area from which the electrode records:

Out of those cells which are close enough to the electrode (and can influence the recordings) the proportion of cells which are actually activated might determine the magnitude of extracellularly recorded action potentials. The factors which might determine the proportion of the responding cells in the close vicinity of the electrode are difficult to define in the absence of conclusive evidence as to whether or not a particular smooth muscle is electrically excitable(24). If there is an

absolute and relative refractory period as would be expected in an electrically excitable tissue or if excitability is directly related to the recovery of the transmembrane potential following an action potential, then the rate of repolarization and the frequency of action potentials might influence the magnitude of action potentials recorded by the technique used in this study. Thus an increase in the rate of repolarization might increase the relative number of cells capable of responding during each of a series of action potentials provided that the time interval between each action potential remains constant. Similarly an increase in the time interval between two successive action potentials (decreased frequency) might increase the proportion of cells capable of responding by allowing enough time for even those cells which normally recovered slowly to regain their excitability more completely in the longer period available.

In addition to these factors which indirectly influence the recorded amplitude of extracellular action potentials (by effecting the synchrony of activation of the cells) the change in the resistance between the electrode and the medium might also influence the amplitude of extracellularly recorded action potentials. Changes in the composition of the medium may produce changes in the resistance between the electrode and the medium which would affect the current flow through the medium and the potential occurring at the near electrode and would change the recorded amplitude of action potentials. The resistance between the electrode and medium is not expected to change significantly when sodium chloride in the medium is replaced by another electrolyte, e.g. choline chloride. However, the resistance might increase when a sucrose containing medium is used.

The implications of these principles are applicable to the observed changes and are discussed in further detail in the following sections.

II. Results

(1) Changes in Action Potentials in Reduced External Sodium Concentration:

The amplitude of action potentials was increased in sodium-poor media (Tables II-V and Figures 2-15). The slope of the major deflection was slightly increased or appeared unchanged and the original biphasic configuration of action potentials was maintained at all stages in sodium-poor media. However, cessation of action potentials occurred (along with mechanical inactivity) when the sodium concentration was reduced to the vicinity of 15-20 mEq/l. Independent studies on the conduction of action potentials revealed no decrease in the conduction rate in sodium-poor media until just before mechanical and electrical inactivity developed (22). The frequency of action potentials in the cat uterus was decreased considerably in sodium-poor media (to about 3-5 times less frequent than in Krebs Ringer). In the uteri of other species the decrease in frequency in sodium-poor media was not marked. The variation in amplitude between individual members of a series of action potentials was quite large in Krebs Ringer. In cat uteri, such variations were considerably decreased in sodium-poor media but were little affected in the uteri of other species.

(2) Explanation of Observed Changes in Action Potentials:

Microelectrode studies (14) of uterine smooth muscle in sodium-poor media have shown no change in the rate of depolarization, while the rate of repolarization was actually increased. The amplitude of the transmembrane action potential was not appreciably altered in sodium-poor media and even overshoot sometimes was observed. This indicates that in sodium-poor media there was no decrease in the transmembrane potential of individual cells. It also suggests that the increased amplitude of extracellularly recorded action potentials in sodium-poor

media must be due to increased synchrony of response of multicellular areas and/or altered pattern of spread of activation. In a given series of repetitive action potentials uniformly increased synchrony during each of the member impulses might have been brought about by the faster rate of repolarization (14) which presumably decreased the period of refractoriness of the cells. In cat uteri the decreased frequency (3-5 times less than that in Krebs Ringer medium) of action potentials might have further contributed to an increased in the degree of synchronous activation of cells in such a way as to produce action potentials which were of relatively uniform high amplitude (as discussed in first section). In the other two species almost unaltered high frequency (150-300 msecs. between two successive impulses) of action potentials in sodium-poor media might have accounted for the persistence of many action potentials of low and variable amplitude (as every new impulse in a series might find a variable number of cells which were completely excitable).

In choline chloride sodium-poor media the slight change in conductivity between medium and electrode presumably did not play a significant role in modifying the amplitude of action potentials. However, in sucrose containing media the increased resistance could have been sufficient to decrease the amplitude of the action potentials. This tendency would be opposed by the reduced electrolyte concentration of the medium which would retard short circuiting through the medium and thus enhance the recorded amplitude of the action potential. Actually, no decrease in the amplitude of action potentials ever was recorded in sucrose-containing sodium-poor media. The similarity between the changes observed when sucrose or choline chloride was used to replaced sodium chloride suggest that altered conductivity of the external medium played little role in producing the observed changes.

III. The implications of current observations for the "Sodium Hypothesis" in Smooth Muscle.

According to the sodium hypothesis (1) a selective increase in the permeability of the cell membrane to sodium ions is responsible for depolarization and the initiation of action potentials in many tissues (1-10), If this theory were applicable to uterine smooth muscle, the reduction in the external sodium concentration should have produced a decrease in the amplitude of transmembrane action potentials (1) and the rate of depolarization should have increased (1). However, no such changes were observed (14). This indicates that the sodium hypothesis cannot be applied to uterine smooth muscle without serious modifications. Our own observations have shown that action potentials recorded extracellularly persist at remarkably low external sodium concentrations. The possible reconciliation of these findings with the "Sodium Hypothesis" and an explanation of the relative independence of the presence of action potentials and the external sodium concentration could be based on three main arguments.

(1) Non-equilibration of external sodium in the medium with extracellular sodium in the tissue

The available evidence is not in harmony with this explanation for the relative independence of this presence and amplitude of action potentials and the external sodium concentration. If equilibration between the medium and the extracellular space of the tissue were incomplete, it would be difficult to account for other change in function which are observed in sodium-poor media after a few minutes (5-10 min.). The disappearance of action potentials in tissues whose sodium concentrations were very similar to those of other tissues which under the same circumstances remained active also indicates that the persistence of action potentials in sodium-poor media is not due to incomplete equilibration (retention of extracellular sodium). The tissues immersed

in solutions with negligible external sodium for prolonged periods of time retained considerable amounts of residual sodium (Tables VI-IX). The sodium space under these conditions was enlarged significantly as compared with the control. This suggests the possibility that a part of the residual sodium was bound in a non-diffusable form. The degree of such binding, however important , cannot be determined satisfactorily until suitable direct methods are used.

(2) Maintenance of Constant Ratio Between the cellular and extracellular concentrations of sodium (Na_o/Na_i) in various concentrations of external sodium.

This explanation of the relative independence of the magnitude of action potentials and the external sodium concentration also appears implausible. Maintenance of a fixed ratio Na_o/Na_i at various external sodium concentrations would only be possible if tissue sodium losses from outside and inside of the cells occurred at a constantly proportionate rate. The available evidence indicates (15) that extrusion of cellular sodium in sodium-free media is met with certain barriers tending to hinder the cellular sodium loss. Sodium loss is rapid from the extracellular space. Thus, the ratio Na_o/Na_i must be decreased at lowered external sodium concentration, at least in the initial stages. Hence, the amplitude of intracellularly recorded action potentials should have decreased and some reduction in magnitude of extracellular action potentials might have been expected. However, no decreases in transmembrane action potentials were observed in low external sodium solutions (14) and extracellularly recorded potentials actually manifested an increase in their amplitude very soon after exposure to such media.

(3) Saturation of sodium "carrier-system".

It has been suggested that the mechanism whereby excitation is accomplished by membrane depolarization may involve a sodium "carrier system" which is made

available by excitatory depolarizing processes, permitting an inward current to be carried by sodium ions moving along their concentration gradient. It might be possible that such a sodium "carrier-system" is super-saturated with sodium in smooth muscle so that reduction of external sodium concentration has little effect on the quantity of carrier combined with sodium until very low levels of external sodium are reached. Evidence has been presented previously (14) which almost excludes this hypothesis as an explanation of the relative independence of the presence, amplitude and pattern of action potentials and the external sodium concentration.

Selective sodium currents do not appear adequate to account for the depolarization of uterine smooth muscle. The possibility that other ions may carry the action potential currents in these tissues should be considered. However, potassium and chloride ionic currents flowing as a result of a generalized increased in membrane permeability would cause hyperpolarization (14). An increased permeability to all ions as a cause of depolarization does not adequately account for the experimental results (14). Under these conditions, an hypothesis based on an outward flow of intracellular anions other than chloride as a possible cause of depolarization and action currents must be considered. This hypothesis could explain the various findings without leading to any glaring discrepancies.

IV. Eventual Mechanical and Electrical Inactivity in Extremely Low Sodium Concentration.

It is difficult to advance an explanation of eventual mechanical and electrical inactivity in extremely low concentrations of external sodium if the sodium hypothesis is discarded. However, the fact remains that mechanical activity was affected in some important way in sufficiently sodium-poor media.

Sudden contraction, gradual relaxation and resumption of spontaneous activity occurred when the external sodium concentration was only moderately lowered. When the external sodium concentration was lowered beneath 15-20 mEq/l, the pregnant cat uterus usually showed sudden contraction, gradual relaxation, and failure to resume spontaneous activity. The action potentials disappeared at this stage. In the other two species, the inital sudden uterine contraction on transfer to sodium-poor media was usually followed by incomplete relaxation. In sodium concentrations as low as 20-25 mEq/l persistent contraction occurred and the tissue failed to relax even over a long period (over 3 hours). Action potentials were recorded in the initial stages but they disappeared in the later stages.

It is possible that certain metabolic pathways associated with electrical and mechanical activity were inhibited at extremely low levels of tissue sodium. It has been suggested that contraction in smooth muscle is associated with break-down of high-energy phosphates (25) as in other types of tissue (26,27,28). Whether or not sodium in optimum concentrations plays a role in the breakdown of high-energy phosphate fractions or their re-synthesis is a matter of speculation. If the break-down of certain high-energy phosphate bonds were inhibited, or if their synthesis were retarded, the energy required to produce mechanical activation might not be available and consequently inactivity would ensue. Differences of inhibition in the site of these energy sources (during the cyclic phases of contraction and relaxation) might possibly account for the two types of eventual mechanical inactivity observed in different species in extremely low external sodium concentrations: (inactivity without exertion of tension in the cat uterus; and inactivity with exertion of tension in rabbit and rat uteri).

Such an inhibition of metabolism might hinder the repolarization of the cells after excitation and thus could account for secondary electrical inactivity. If electrical activation is necessary to elicit contraction, then mechanical inactivity might follow secondarily. Thus it might be that inhibition of metabolism directly affects the electrical activity and only secondarily the mechanical activity.

V. Tissue Potassium and Chloride Distribution After Exposure to Low Sodium Solutions:

Quantitatively, the distribution of uterine tissue potassium following immersion in sodium-poor media was inconsistent, as was noted earlier for brain slices (29). Tissue potassium tended to be lost more rapidly in sodium-poor media, although the extent of the loss was variable. Greater quantities of potassium were lost in solutions poorer in sodium content, and losses appeared to be greater when choline chloride rather than sucrose was used to replace sodium chloride (see 15). In pregnant cat uteri tissue potassium concentrations as low as 4-5 mEq/kg fresh weight were obtained in some cases (Table VI). Evidently potassium gradients across the cellular membranes were greatly altered from the normal value in these cases.

The altered potassium gradient in these tissues raised important questions regarding the mechanism of potassium distribution in smooth muscle and its relationship to resting and action potentials. It was of interest to determine if loss of electrical activity had any relationship to potassium loss in Na-poor media. If potassium were freely diffusable across the cell membrane and at equilibrium, its distribution would be expected to be related approximately to the resting potentials by the relationship $E_{RP} = RT/F \log Kc/Ke$. This would be approximately true irrespective of whether or not equilibrium was obtained if its permeability were much greater than that of other ion species. In calculated Kc, an estimated value for the extracellular space (ECS) must be

used. The chloride space was often larger than the sodium space and even larger than the ECS conceivably could be. Therefore, an ECS value was calculated assuming Kc = 150 mEq/liter for the controls and this value for the ECS was applied to the experimental pieces to calculate their Kc. This in turn was used to calculate the potassium equilibrium potentials, which are close to the resting transmembrane potential in these cells. These values indicated that neither the level of tissue potassium nor the magnitude of the resting membrane potentials calculated from potassium distribution have any direct relationship to the presence or absence of electrical activity.

Actually there was no correlation between the extent of tissue potassium loss and the incidence of mechanical and electrical inactivity in the various species studied. Some uteri with tissue potassium levels close to their controls became inactive in low external sodium concentrations while other uteri with a much lower tissue potassium concentration under similar conditions remained active (Table VI-IX). Moreover, no relationship seemed to exist between the degree of cellular potassium depletion in the uteri of various species studied and the two different types of mechanical inactivity which were observed at extremely low levels of external sodium.

In the potassium depleted cells, electrical neutrality must be maintained in some fashion. This could be accomplished by gain of cations, e.g., sodium, hydrogen, calcium, and/or magnesium. Alternately loss of intracellular anions could maintain the ionic balance. However, the available data (30) do not support the view that sodium could substitute for the lostcellular potassium. A compensating gain of hydrogen would change the cell pH to highly acidic levels. While the possibility of calcium and/or magnesium uptake to offset cellular potassium loss cannot be ruled out, the small concentrations in which these ions exist in tissues suggest that these

ions probably are not involved in an exchange for lost cellular potassium.

The loss of intracellular anions accompanying potassium loss would maintain electrical balance but would considerably decrease the intracellular osmolarity. Under these conditions water must be lost from within the cell. If so, the ratio E.C.S./I.C.S. might change under different conditions. However, the answer to these problems must be awaited till further evidence bearing upon these questions is available.

Values for tissue chloride and chloride spaces in sodium-poor media containing choline chloride were comparable to control samples left in Kreb's Ringer solution. However, when sodium-poor media containing sucrose were used, the tissue chloride levels decreased (Table VI-IX). Such losses probably could be accounted for by decreased chloride in the extracellular space. However, the estimated chloride space in sodium-poor media containing sucrose were enlarged, indicating that some of the chloride probably was bound within the tissue or equilibrated very slowly with the external medium.

SUMMARY

1. Extracellular action potentials and isometric contractile tension have been simultaneously recorded in vitro from uterine longitudinal smooth muscle of the pregnant cat, pregnant rabbit, estrogen treated rabbit and estrogen treated rat.

Recordings were at first made in Krebs Ringer and then in sodium-poor media (sodium chloride replaced by choline chloride or sucrose).

2. Decrease in the external sodium concentration resulted in increased peak to peak amplitude of action potentials. The rate of change and the duration of peak to peak deflection of action potentials remained almost unchanged. The frequency of action potential was reduced in pregnant cat uteri but remained almost unchanged in the uteri of other species.

3. In sufficiently reduced external sodium concentrations, the muscle went into sudden initial contraction, gradual relaxation and resumption of spontaneous contraction and action potentials. However, in rabbit and rat uteri, the relaxation after an initial contraction was usually incomplete and even a persistant contracture developed at extremely low external sodium concentrations (15-20 mEq/l).

4. In extremely low sodium concentrations (below 15-20 mEq/l) action potentials eventually disappeared along with mechanical inactivity. In cat uteri mechanical inactivity occurred when the uteri were in the relaxed phase after contraction while in other species the tissue had failed to relax after initial contraction at such low external sodium concentrations.

5. Evidence is presented to indicate that selective inward flow of sodium ions probably cannot account for initiation of action potentials in

(Summary (cont'd)

5. uterine smooth muscle since considerable reduction of the external sodium concentration (down to 15-20 mEq/l in cats and 25-30 mEq/l in other species) did not affect the characteristics of action potentials in the expected manner.
6. The view that an outward flow of intracellular anions might be responsible for depolarization in these tissues receives further support from the present studies.
7. The observed changes in action potentials in sodium-poor media and also intra and inter species variations are discussed.
8. It is suggested that the eventual inactivity (electrical as well as two different types of mechanical inactivity, i.e. without exertion of tension as in cats and with exertion of tension as in rabbits and rats) might be due to altered biochemical processes related to the electrical and/or mechanical phenomenon in these tissues.

BIBLIOGRAPHY

1. Hodgkin, A.L. — The Ionic Bases of Electrical Activity in Nerve and Muscle. Biol. Rev. 26: 339–409, 1951.

2. Keynes, R.D. — The Ionic Movements During Nervous Activity. J. Physiol. 114: 119–150, 1951.

3. Hodgkin, A.L. and Huxley, A.F. — Currents Carried by Sodium and Potassium Ions Through the Membrane of Giant Axon of Loligo J. Physiol. 116: 449–472, 1952.

4. Desmedt, J.E. — Electrical Activity and Intracellular Sodium Concentrations in Frog Muscle. J. Physiol. 121: 191, 1953.

5. Draper, M.H. and Weidman, S. — Cardiac Resting and Action Potential, Recorded with an Intracellular Electrode. J. Physiol. 115: 74–93, 1951.

6. Nastuk, W.L. and Hodgkin, A.L. — The Electrical Activity of Single Muscle Fibre. J. Cell. Comp. Physiol. 35: 39–74, 1950.

7. Huxley, A.F. and Stampfli, R. — Effect of Potassium and Sodium on Resting and Action Potential of Single Myelinated Nerve Fibre. J. Physiol. 112: 496–508, 1951.

8. Hodgkin, A.L. and Katz, B. — The Effect of Sodium on Electrical Activity of Giant Axion of Squid. J. Physiol. 108: 37–77, 1949.

9. Cranefield, P.F., Eyster, J.A.E., and Gilson, E. — Effect of Reduction of External NaCl on Injury Potential of Cardiac Muscle. Am. J. Physiol. 166: 269–272, 1951.

9a. Cranefield, P.S., and Hofman, B.F. — Electrophysiology of Single Cardiac Cells. Physiol. Rev. 38: 41–77, 1958.

10. Katz, B. — The Effect of Electrolyte Deficiencies on the Rate of Conduction in Single Nerve Fibre. J. Physiol. 106: 411 et seq, 1947.

11. Fatt, P., and Katz, B. — The Electrical Properties of Crustacean Muscle Fibre. J. Physiol. 120: 171–204, 1953.

12. Wood, D.W., — The Effect of Ions upon Neuromuscular Transmission in a Herbivorous Insect. J. Physiol. 138: 119–139, 1957.

Bibliography (Cont'd)

13. Holman, M.E. — The Effect of Changes in Sodium Chloride Concentration on Smooth Muscle of Guinea Pig Taenia Coli. J. Physiol. 136: 569-584, 1957.

14. Daniel, E. and Singh, H. — The Electrical Properties of Smooth Muscle Cell Membrane. Can. J. Biochem. & Physiol. 36: 959-975, 1958.

15. Daniel, E., and Daniel, B. — Effects of Ovarian Hormones on the Content and Distribution of Intact and Extracted Rabbit and Cat Uteri. Can. J. Biochem. & Physiol. 35: 1205-1223, 1957.

16. Spencer, A.G. — Flame Photometry. The Lancet, November, 623, 1950.

17. Asper, S.P., and Schales, S.C. — A Simple and Accurate Method for Determination of Chloride in Biological Fluids. J. Biol. Chem. 140: 879, 1941.

18. Whittam, R. — A Convenient Micro-method for Estimation of Tissue Chloride. J. Physiol. 128: 65 P, 1955.

19. Manery, J.F. — Water and Electrolyte Metabolism. Physiol. Rev. 34: 335-417, 1954.

20. Hakansson, C.H. — Action Potential Recorded Intracellularly and Extracellularly from isolated frog muscle fibre in Ringer Solution and Air. Acta. Physiol. Scand. 39: 291-312, 1957.

21. Fulton, J.F. — Text Book of Physiol. 17th Edition, 519 et seq, 1957.

22. Daniel, E.E. — Unpublished results. Department of Pharmacology, U.B.C. Vancouver

23. Lorente De No — A Study of Nerve Physiology, Stud. Rockefeller Institue of Medical Research 132: 394-477, 1947.

24. Grundfest, H. — Electrical Inexcitability of Synapses and Some Consequences in Central Nervous System. Physiol. Rev. 37: 337, 1957.

25. Born, G.V.R. — The Relation Between the Tension and the High Energy Phosphate Content of Smooth Muscle. J. Physiol. 131: 704-711, 1956.

26.	Perry, S.V.,	Relation Between Chemical and Contractile Function and Structure of Skeletal Muscle Cell Physiol. Rev. 36: 1, 1956.
27.	Fritz, B., Svensmark, A, and Rosenfalck, P.	Mechanical and Chemical Events in Muscle Contraction. Physiol. Rev. 36: (4) 503, 1956.
28.	Szent-Gyorgyi, H.	Chemical Physiology of Contraction in Body and Heart Muscle. 2nd Edition, Academic Press, N.Y., 1951.
29.	Pappius, H.M., Rosenfeld M., Johnson, D.M., and Elliot, K.A.C.	Effects of Sodium-free Media Upon the Metabolism and the Potassium and Water Content of Brain Slices. Can. J. Biochem. & Physiol. 36: 217-226, 1958.
30.	Daniel, E.E.	Unpublished Results. Department of Pharmacology, U.B.C.