The Effect of Oxytocics on the "Ca-Deficient" Uterus

A measure of oxytocic potency

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ABSTRACT If the excised, parturient rabbit uterus is repeatedly treated with a Krebs solution free from Ca, its tension in a tetanus drops gradually, and in 15 to 30 minutes becomes zero. We call such a uterus "Ca-deficient." The uterus in this condition has a high threshold, it is non-propagating, "inexcitable," fails to respond to oxytocics in a characteristic fashion, but retains maximum contractility. As Ca is gradually restored to the Krebs, these lost qualities return in a graded fashion and tension of the tetanized uterus becomes a log function of the [Ca]. If the [Ca] is kept low, *i.e.* 1/10 to 1/20 of the normal, tetanic tension is small but steady, and the preparation offers a full scale of tension increment for the measurement of oxytocic potency. Keeping the stimulus and the [Ca] constant, excitability (measured by tension increment) is a log function of the drug concentration. The recovery of excitability by restoring Ca to the Ca-deficient uterus is strongly temperature-dependent. The Ca-deficient uterus is a useful preparation for the study of the mechanism of regulation. When its excitability is partially recovered by Ca, the electrically stimulated uterus becomes an excellent tool for the quantitative measurement of oxytocic potency.

INTRODUCTION

Significant conclusions are based on the effect of "oxytocics" on the isotonic shortening of the spontaneously contracting excised uterus (reviewed recently by Fitzpatrick, 1957). Some investigators claim that oxytocics directly influence the contractile system, and they call these pharmacological agents uterine "stimulants." Others use the increment in shortening of the excised uterus as a quantitative measure of oxytocic concentration. The loss of oxytocic potency, determined by the same method after treatment with human preg-

From The Rockefeller Institute.
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Received for publication, January 16, 1959.

nancy serum, is taken as evidence for the existence, and as a measure of activity, of a specific enzyme, "oxytocinase."

This technique, although subject to slight modifications from one laboratory to the next, can be misleading if regarded as quantitative. The method is inadequate because of (1), the use of unloaded or slightly loaded isotonic muscle levers; (2), the recording of the first muscle response only (upon the administration of the tested drug); and (3), the registration of the spontaneous mechanical activity alone.

In considering this technique from a theoretical as well as practical viewpoint, one indeed appreciates the remark of A. V. Hill, who stated (1956): "The mechanical response of active muscle is easy to record, indeed too easy, for very inadequate methods have been used and the older physiological literature is burdened with the results of experiments which depended more on the properties of levers than on those of muscle."

The first spontaneous shortening of the unloaded uterus is an inadequate measure of oxytocic potency because of the non-linearity between dose and response. If the load is small or absent, the uterus can shorten maximally if only one fibre bundle out of ten or twenty is activated (Csapo, 1954, 1955). Thus non-linearity arises from the lack of a quantitative relationship between shortening and the extent of activation. The correlation is linear if force or work (with optimum load) is measured. For a 1 gm. segment of the estrogen-dominated, non-pregnant rabbit uterus, the optimum load (yielding maximum work) is about 16 gm., one-half of the maximum isometric force. This force equals the maximum load which can be held but not lifted by the active muscle. Shortening of the optimally loaded uterus is as good a measure of activation as is isometric tension.

The first spontaneous response of the uterus to an oxytocic, even when isometrically recorded, is, however, an inadequate measure of the effect, since it is unpredictable whether it is going to be the smallest or the largest in a train of subsequent responses. If the spontaneous mechanical activity of the uterus is used to determine oxytocic potency, the increase in frequency is a more reliable indication of the effect than is the increment in tension.

The spontaneous activity of the uterus, however, is seldom regular enough to serve as a quantitative measure of pharmacological effect. Thus the electrical excitability of the uterus, *in vitro* as well as *in situ*, was examined and described (Csapo and Goodall, 1954; Csapo, 1955; Schofield, 1955; Bengtsson, 1957), in experiments which showed that the uterus driven by electrical stimuli performs regular and predictable mechanical activity, subject to modification by pharmacological agents. The techniques presented here in detail appeared to be adequate for most experiments. For a reliable estimation of pharmacological potency they had to be modified, however. The intact estrogen-dominated uterus is not quite suitable as a recording instrument because

its threshold response is 50 per cent or more of its maximum tension, leaving only a narrow range of potential tension increment for the measurement of pharmacological effect.

This range can be widened by the use of the "Ca-deficient" uterus, a preparation which is non-propagating and "inexcitable," but which responds in a graded fashion to a stepwise increasing longitudinal electric field. When such a uterus is exposed to a Krebs solution of increasing [Ca], it increases its tetanic response, and its dose-response curve shows a logarithmic relation when tension increment (on electrical stimulation) is plotted as a function of the oxytocic concentration.

The experiments to be described may be more appreciated if certain properties of the myometrium are considered first. A truly resting uterus does not exist under physiological conditions; only the pattern of uterine activity varies. The intrinsic forces which drive the uterus are largely unknown, but some relevant information about these forces was obtained (Csapo, 1956), by studying the "threshold relation" of the myometrium. This study revealed that the threshold of the uterus, like that of the frog sartorius (Jenerick and Gerard, 1953), increases with hyperpolarization of its excitable membrane, whereas it decreases with depolarization. Spontaneous mechanical activity can be abolished if the membrane potential is sufficiently increased. As the membrane potential is gradually lowered (for example, by increasing the $[K]_0$), spontaneous activity increases until at a "critical" value ($\sim$30 mv.) maximum tension with high repetition frequency is recorded. If this "critical potential" is overstepped, the uterus goes into contracture.

This relationship suggests that the contractile system of the uterus is balanced in such a way that activity prevails unless rest is enforced by an excess of membrane potential of about 30 mv. over a critical value. Since the membrane potential of this muscle, as well as of other smooth muscles (Bülbring, 1954, 1955, 1957; Woodbury and McIntyre, 1954; Goto and Csapo, 1958) is generally less than 60 mv., activity is favored and no external stimulus is required for mechanical activity. The uterus, it is important to realize, need not be "stimulated" if its membrane potential excess is small, which is the case when the myometrium is dominated by estrogen.

Oxytocics do not directly stimulate the contractile machinery, but only regulate membrane function (Csapo, 1954, 1959). The uterus immersed in a Krebs solution of high external [K] = 120 mM/liter is rendered reversibly "inexcitable" and non-propagating. Oxytocics fail to elicit a characteristic response on such a depolarized muscle, in spite of its intact contractility. That contractility is intact can be demonstrated by exposing the depolarized uterus to a strong longitudinal electric field, capable of triggering close to maximum tension.

A distinction between a stimulating and regulating effect of oxytocics, *i.e.*

an effect on contractility or excitability respectively, is of significance. Since results different from our own, concerning the pharmacological reactivity of the depolarized smooth muscle, have been reported (Evans, Schild, and Thesleff, 1958), in the present article we shall provide independent evidence in support of the conclusion that the uterus rendered temporarily and reversibly inexcitable and non-propagating, yet of intact contractility, fails to respond in a characteristic manner to oxytocics.

It is well known that treatment with Krebs' solution of low [Ca] suspends spontaneous mechanical activity in the uterus. Pharmacologists have taken advantage of this effect by using low Ca environment in their current method of testing oxytocic potency (Holton, 1948). What has not been examined, however, is whether such treatment affects excitability only, or contractility as well. In order to make this distinction we have studied the effect of "Ca-deficiency" on the excitability, contractility, and pharmacological reactivity of the myometrium. We call a uterus, repeatedly treated with Ca-free Krebs, Ca-deficient, although our measurements of muscle Ca are as yet incomplete, and thus we do not know whether Ca is actually lost or is only displaced from "strategic sites." The measure of this deficiency is the loss of normal "excitability" of the uterus when tetanized.

Methods

New Zealand White *rabbits* are used exclusively, and the uterus is taken for experiment only under well defined endocrine conditions, either by employing adults in natural estrus determined by successful mating, or at known stages of pregnancy dated from the day and hour of mating; or hormone-treated immature rabbits. The "estrogen-dominated" animal receives 10 to 25 micrograms per day estradiol, in oil, intramuscularly for 7 to 10 days, whereas the "progesterone-dominated" animal is given an additional 5 mg. progesterone on the last 3 days of treatment.

The *uterus* is removed from the anesthetized animal (sodium nembutal, 40 mg./kg. body weight) after quick laparotomy and is placed in a preoxygenated mammalian Krebs' solution at room temperature. Two segments or strips are removed from the uterine horn, about 4 cm. long and weighing about 0.5–1.0 gm., so as to give 25 to 50 gm. tension when tetanized. The experiment begins a few minutes after the uterus is removed from the living animal. The myometrium can be stored for certain experiments, but special procedures must be observed during storage which need not be detailed here.

The experimental strips, one of which may serve as a control, are mounted in oxygenated Krebs' solution in a *chamber* consisting of a pyrex glass tube 14 cm. long, inside diameter 17 mm., and which has two open ends, the lower being closed by a rubber stopper. During the experiment the tube is fixed in a vertical position inside an automatically controlled water bath. A polyethylene tube passing through the stopper serves as an inlet for the $O_2 + CO_2$ mixture. A platinum hook built into the stopper holds the lower end of the uterine segment. The upper rim of the stopper is

occupied by a platinum ring electrode. The other ring electrode is immersed in the tube from above, and adjusted to an electrode distance of 10 cm. or less. The upper end of the strip is connected by a platinum hook to a light silver chain which connects the uterus to the recording strain gauge. Thus the muscle is in a vertical position between the two platinum wire rings of adjustable distance and it is in the middle of the electric field which is generated by passing current between the two electrodes. The strain gauge is in a fixed position directly above the tube which is mounted on a rack and pinion device. When the tube is moved up or down the "*resting length*" of the muscle changes. The resting length is defined as the maximum length of the uterine strip at which it develops no resting tension. This length is determined by a stepwise increase in the length of the uterine segment until resting tension is recorded.

The *mammalian Krebs solution* is freshly prepared every day from stock solutions of 0.154 M/liter (except $CaCl_2$, which is 0.110 M/liter). Its composition in mM/liter is: NaCl, 118.46; $NaHCO_3$, 24.87; KCl, 4.74; $CaCl_2$, 2.54; KH_2PO_4, 1.18; $MgSO_4$, 1.18; and 100 mg. per cent dextrose. The solution is oxygenated with 95 per cent O_2 and 5 per cent CO_2 before and during the experiment by a continuous gas flow which also serves as a means of mixing. The Krebs solution is identical in its composition with the ultrafiltrate of mammalian serum, and any modification of it ought to be justified by the special problem of the experiment.

The *strain gauge* has a maximum deflection of only a fraction of a millimeter, and thus the recording is practically isometric. The deflection of the gauge is magnified and recorded by an electroencephalograph. The excursion of the writing pen, which records the deflection of the gauge, has a linear relationship with the load applied on the gauge.

The *electric field* set up in the Krebs solution between the electrodes is controlled by an automatic timer (tandem, type A), and by a powerstat. The duration, repetition frequency, and strength of the stimuli can be set by these devices. The stimulus is 60 c/s, A.C., of different strength, duration, and repetition frequency. To tetanize the uterus we generally use a stimulus of 1.0 to 1.5 v./cm. of 5 seconds' duration.

As a basis for comparison, before each experiment, we determine in each specimen the maximum isometric force (maximum working capacity) in a tetanus, the threshold stimulus and tension, and the spontaneous mechanical activity.

A Ca-deficient uterus is obtained by washing the uterus repeatedly, at 6 minute intervals, with a Ca-free Krebs solution. Between washings the uterine strip is tetanized at 3 minute intervals to test excitability. Recovery from Ca-deficiency is studied by restoring Ca stepwise to the Krebs solution, and at each $[Ca]_0$ determining excitability, by tetanizing the muscle.

RESULTS

The Effect of Repeated Washing with Ca-Free Krebs on the Excitability of the Uterus

When the excised uterus is repeatedly tetanized in normal Krebs under the conditions described above under Methods, it develops maximum tension in

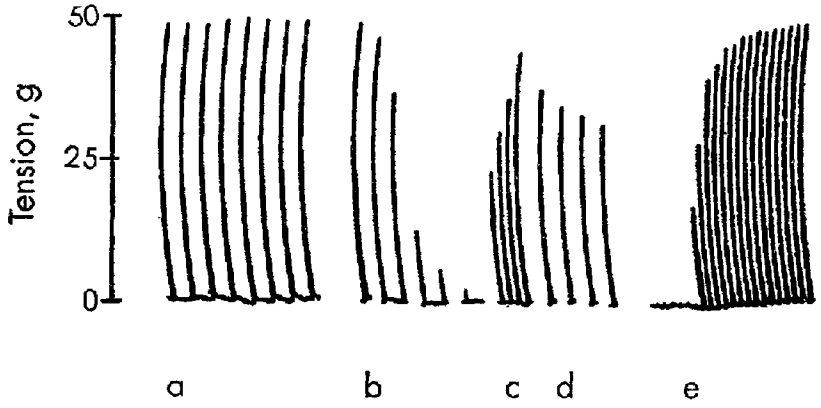

FIGURE 1. Parturient rabbit uterus at 37°C., original record. (*a*) Isometric tension of the uterine strip, tetanized at 3 minute intervals (1.5 v./cm.) in normal Krebs solution. (*b*) The same, but the strip is washed with Ca-free Krebs at 6 minute intervals. (*c*) The field strength is increased in steps up to 8 v./cm. (*d*) The field strength is 8 v./cm. and the strip is washed again in Ca-free Krebs at 3 minute intervals. (*e*) The normal [Ca] of the Krebs and the normal tetanic stimulus (1.5 v./cm.) are restored. Note the disappearance of the tetanus in Ca-free Krebs and the reappearance of tension at higher field strength. Note the complete recovery in normal Krebs.

a steady state for a period of several hours (Fig. 1 *a*). However, if the estrogen-dominated uterine strip is repeatedly washed with a Ca-free Krebs (for example, once every 6 minutes) its tension drops and becomes zero within 15 to 30 minutes (Fig. 1 *b*).

A significant question is whether excitability, contractility, or both, have failed under these conditions. The experiment shows (Fig. 1 *c*) that only normal excitability fails (stimulus = 1.5 v./cm.), while contractility is unchanged, and close to maximum tension is recorded at a field strength of 8 v./cm. This stimulus is about 5 to 8 times stronger than that required to tetanize the untreated uterus. If the uterine strip is further and repeatedly washed with Ca-free Krebs, tension gradually decreases in spite of the strong stimulus (Fig. 1 *d*). The loss of excitability, however, is considerably more rapid than that of contractility. Restoration of Ca to the Krebs results in complete recovery as indicated by the return of normal excitability after some initial latency (Fig. 1 *e*). Thus no irreversible changes occur either in excitability or contractility during the experiment.

These results may be explained by assuming two labile Ca fractions in muscle, one of them in the sarcoplasm, the other in the myoplasm, the former controlling excitability, and the latter contractility. When the uterus fails to respond to a normal tetanic stimulus in Ca-free Krebs it is inexcitable, perhaps because the membrane becomes Ca-deficient. However, it remains contractile, probably because myoplasmic Ca is not yet seriously affected.

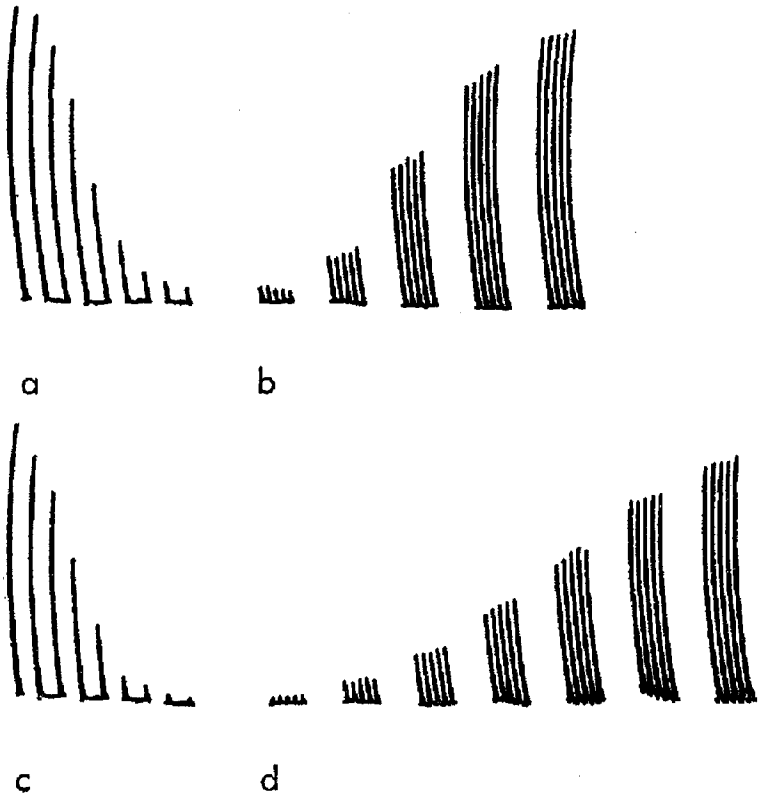

FIGURE 2. Parturient rabbit uterus, original record. (*a*) The uterine strip is tetanized once every 3 minutes at 35°C. and washed with Ca-free Krebs at intervals of 6 minutes until excitability is lost. The muscle in this condition is termed Ca-deficient. (*b*) The [Ca] is restored gradually in steps (0.25, 0.50, 0.75, 1.00, 1.25 mM/liter) while the strip is tetanized once every minute (1.5 v./cm.). A period of 5 minutes is allowed for each additional [Ca] to exert its effect. (*c*) The experiment of (*a*) is repeated. (*d*) The experiment of (*b*) is repeated at 25°C., and two additional [Ca] = 1.75 and 2.5 mM/liter are included in the series. Note that the recovery of excitability of the Ca-deficient uterus is a function of the external [Ca]. Note that recovery is also a function of temperature.

Recovery of Excitability of the Ca-Deficient Uterus as a Function of the External [Ca]

When Ca is restored to the Ca-free Krebs, not only does normal excitability of the uterus recover fully, but it can be graded at will by a gradual increase of the Krebs Ca (Fig. 2 *b*). When the normal tetanic stimulus is kept constant (1.5 v./cm., 5 second duration) throughout the experiment, tension becomes a measure of excitability. Excitability, on the other hand, is a function of the log [Ca] in the perfusion Krebs (Fig. 2 *b*, Fig. 3).

In contrast to the intact uterus whose threshold response (both spontaneously and electrically induced) is 50 per cent, or more, of its maximum, and thus its threshold pharmacological response is practically maximal, the Ca-deficient uterus offers a full scale tension increment for the gradation of excitability and pharmacological response. This quality of the Ca-deficient uterus can be utilized in measuring oxytocic potency.

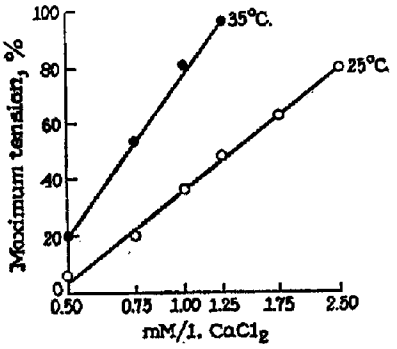

FIGURE 3. The results illustrated in Fig. 2 are plotted in order to show that excitability of the Ca-deficient uterus, as measured by tension, is a log function of the external [Ca]. Note this relationship and the effect of temperature.

The Effect of Temperature on the Ca-Deficient Uterus

The [Ca] in the Krebs at which the Ca-deficient uterus develops a certain value of tension depends on temperature. At lower temperatures a higher [Ca] is required by the Ca-deficient uterus for the development of the same tension (Fig. 2 *d*). Thus excitability of the Ca-deficient uterus not only depends on the external [Ca], but also on temperature, with a $Q_{10} \cong 2$. This suggests that the readsorption of Ca by "strategic sites" is an active process which depends on metabolism. This property of the Ca-deficient uterus allows a further refinement of the method used for the measurement of oxytocic potency.

The Effect of Oxytocics on the Ca-Deficient Uterus

One of the striking features of oxytocic potency is that it is not linked to a specific chemical structure. A variety of agents possess this potency, whose effects on the myometrium are indistinguishable from one another. In the present experiments we studied oxytocin, acetylcholine, and serotonin, compounds of sufficiently different chemical structure, justifying a certain degree of generalization concerning their effects.

It has been shown (Csapo, 1954, 1959), that these so-called uterine (or other smooth muscle) "stimulants," do not affect the myometrium in their characteristic fashion, if excess K treatment suspends temporarily and reversibly normal membrane function. Excess K treatment renders the uterus inexcitable and non-propagating, but leaves contractility unchanged. An abortive con-

tracture, of fractional tension, unlike the normal response of the intact uterus, is observed when the depolarized uterus is treated with oxytocics. By recording only the first phase of the initial response of the depolarized muscle to an oxytocic and by recording it isotonically or semi-isometrically, one completely misses this significant distinction between normal, abortive, and abnormal response.

We have restudied this question, this time on the Ca-deficient uterus, taking advantage of the fact that this muscle, like the K-depolarized uterus, is also temporarily and reversibly inexcitable and non-propagating, without loss of contractility. We obtained the same results (Fig. 4); namely, that the inex-

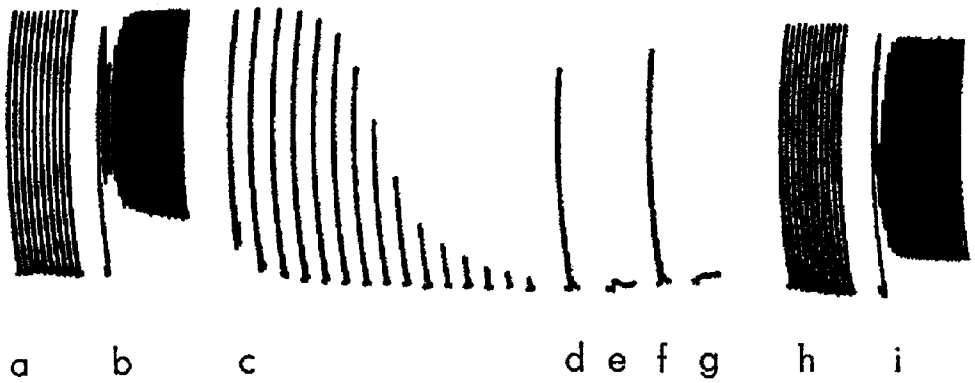

FIGURE 4. Rabbit uterine strip, 31 days pregnant, 37°C., original record. (*a*) Maximum tension of the strip tetanized once every minute (1.5 v./cm.). (*b*) Spontaneous mechanical activity of the strip after treatment with 10 mU/ml. oxytocin. (*c*) The strip is tetanized once every 3 minutes (1.5 v./cm.), and washed with Ca-free Krebs at 6 minute intervals, until excitability is abolished. (*d*) The field strength is increased to 8 v./cm. (*e*) The same as (*b*), but the uterus is Ca-deficient. (*f*) The same as (*d*). (*g*) The same as (*e*), but the oxytocin concentration is 100 mU/ml. (*h*) The same as (*a*), but after recovery from Ca-deficiency in normal Krebs. (*i*) The same as (*b*), but after recovery in normal Krebs. Note that the Ca-deficient uterus of intact contractility does not respond in a characteristic fashion to oxytocin.

citable and non-propagating, yet fully contractile Ca-deficient uterus shows no characteristic oxytocic effect. This finding would seem to strengthen the conclusion that oxytocics are not direct stimulants of the contractile system, but regulators of the triggering machinery.

The Effect of Oxytocics after Partial Recovery from Ca-Deficiency

We have shown that excitability is restored to the Ca-deficient uterus in a graded fashion by a gradual increase of the Krebs Ca. If the [Ca] is low, (for example, 0.3 mM/liter for the estrogen-dominated uterus), the threshold is

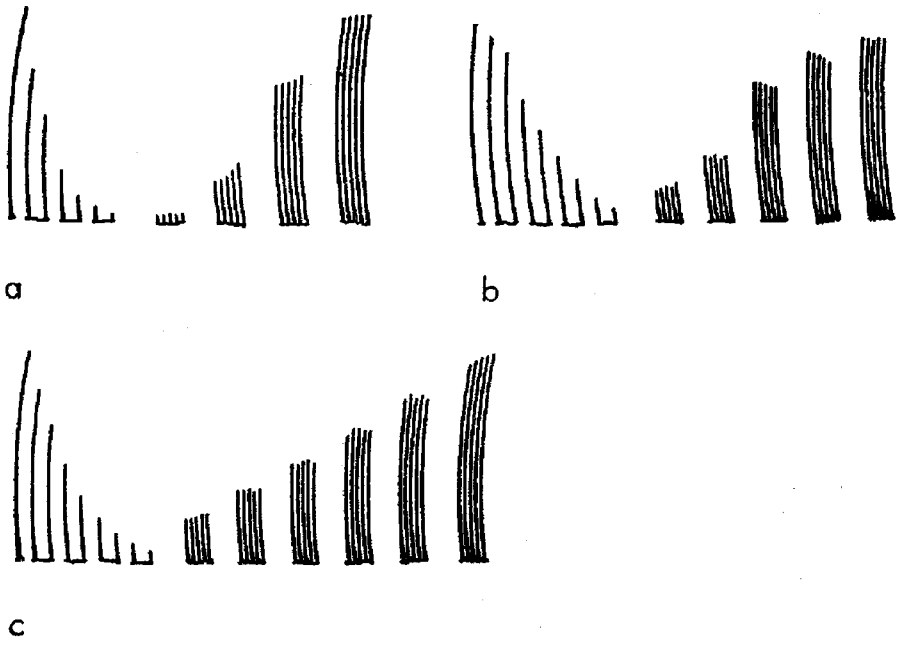

FIGURE 5. Uterine strips from parturient animals, 37°C., original records. In all three cases the uterine strip is rendered Ca-deficient by repeated washing with Ca-free Krebs. When excitability is lost, Ca is partly restored so as to elicit fractional tension in the tetanized strip, and to render the uterus responsive to pharmacological agents. (*a*) [Ca] = 0.07 mM/liter; the oxytocin concentrations = 0.2, 0.4, 0.8 mU/ml. (*b*) [Ca] = 0.15 mM/liter; the acetylcholine concentrations = 1, 10, 20, and 40 μg/ml. (*c*) [Ca] = 0.15 mM/liter; the serotonin concentrations = 0.1, 0.2, 0.5, 1.0, and 2.0 mg./ml. Note the gradual increment of tension (excitability) as a function of the oxytocic concentration.

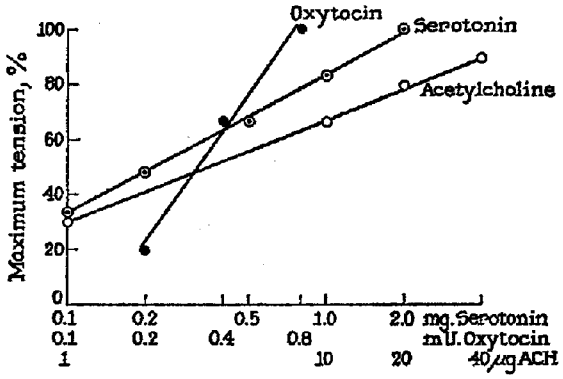

FIGURE 6. Experiments of the type illustrated in Fig. 5 are plotted in order to show that tension (excitability) of the Ca-deficient uterus, partially recovered by low external Ca, is a log function of the oxytocic concentration.

high, and thus tension in a tetanus is small but constant. This preparation offers full scale tension increment for the measurement of oxytocic potency. Fig. 5 illustrates the effect of oxytocin, serotonin, and acetylcholine. Since the tetanic stimulus is kept constant throughout the experiment, the increase in excitability (decrease in threshold) is manifested by tension increment. With all three compounds studied, tension (excitability or threshold) is a log function of the drug concentration (Fig. 6). Maximum tension can be recorded, for example, in the presence of 0.8 mU/ml. oxytocin, when the [Ca] is as low as 0.07 mM/liter, $\frac{1}{40}$ of the normal. This method, *i.e.* measurement of the tension increment of the Ca-deficient uterus (when tetanized repeatedly once every minute in a Krebs of low [Ca]), offers a reliable measure of oxytocic potency.

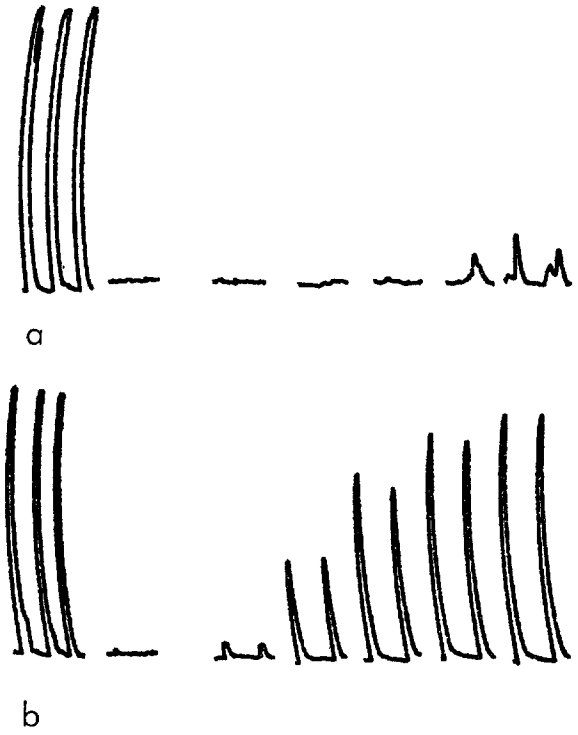

FIGURE 7. Rat uterus, estrogen-dominated, 25°C., original record. The isometric tension developed by the two horns of the same uterus is studied side by side. (*a*) Spontaneous mechanical activity is shown, whereas at (*b*) the horn is tetanized electrically (1.5 v./cm.). When a Krebs solution of low [Ca] = 0.1 mM/liter is introduced both spontaneously (*a*) and electrically induced (*b*) tension fail. A gradual increase in oxytocin concentration (ranging from 0.1 mU/ml. to 0.5 mU/ml.) results in gradual increment of tension in the stimulated (*b*) horn, while the unstimulated one (*a*) develops fractional tension only at an oxytocin concentration at which the stimulated horn responds maximally.

A Comparison of Oxytocin Effects on the Spontaneously and Electrically Driven Uteri

In the following we provide experimental evidence rather than theoretical considerations in support of the conclusion that spontaneous activity, as commonly used, is an inadequate measure of oxytocic potency. In order to eliminate the possibility that the different findings of different laboratories are due to species differences, we have used in these experiments the rat uterus, preferred by some investigators.

When the two horns of the same (estrogen-dominated) rat uterus are studied side by side at 25°C., and at a [Ca] = 0.1 mM/liter, both spontaneous and electrically induced tensions are abolished (Fig. 7). Upon treatment with 0.1 mU/ml. oxytocin, threshold tension develops in the tetanized muscle (Fig. 7 *b*), but no spontaneous activity is recorded (Fig. 7 *a*). As the oxytocin con-

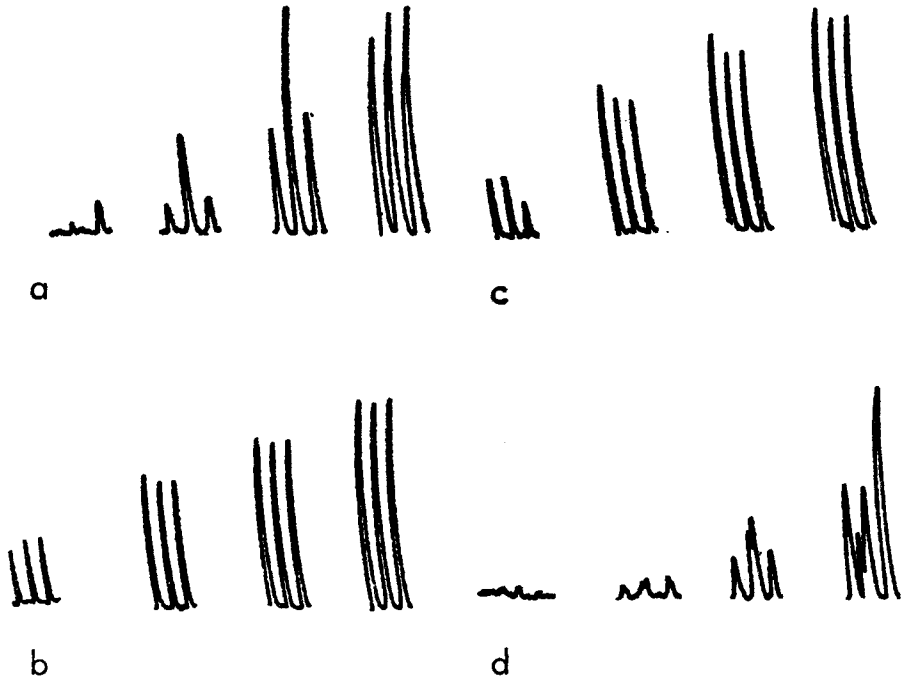

FIGURE 8. Rat uterus, estrogen-dominated, 25°C., original record. Two horns of the same uterus (*a*) and (*b*) are studied side by side to compare the spontaneous and electrically induced mechanical activity, respectively. In all cases the [Ca] = 0.1 mM/liter, oxytocin concentration is increased gradually in steps = 0.1, 0.2, 0.4, 0.8 mU/ml. (*a*) The uterus is tetanized (1.5 v./cm.). (*b*) Spontaneous activity, (*c*) and (*d*) the procedure is reversed. Note the striking irregularity of the spontaneously driven horn as compared with the electrically stimulated one.

centration is increased, the tetanic tension increases gradually as a function of the oxytocin concentration. On the other hand, in the spontaneously contracting muscle, tension of varying amplitude appears only at an oxytocin concentration which results in practically maximum tension in the tetanized uterus.

But it is not only the lower oxytocin sensitivity of the spontaneously driven uterus which is apparent. More significant is the irregularity of the spontaneous contractions as illustrated by Figs. 8 *a* and 8 *d*. At a given oxytocin concentration the magnitude of spontaneous tension, within the first three contractions, may show a variation as large as 100 per cent. This large scatter is a warning against the use of spontaneous contractility as a quantitative measure of oxytocic potency. On the other hand, the relationship between stimulated tension and oxytocin concentration is as good as it is in experiments with the rabbit uterus (Figs. 8 *b* and 8 *c*).

DISCUSSION

The experiments described propose a method, or rather a variety of methods, for the estimation of oxytocic potency. We refined the former routine of our laboratory by the use of the Ca-deficient uterus. This preparation allows us to accurately grade the mechanical response of the uterus, and thereby widen the range in which regulatory effects are measured quantitatively.

Ca-deficiency is an operational definition. The term describes a uterus treated repeatedly with Ca-free Krebs, until the loss of propagation, normal excitability, and pharmacological reactivity signals Ca-deficiency. Such a uterus retains its maximum contractility. Whether the myometrium in this condition has in fact lost a measurable fraction of its Ca is not yet certain. But measurements of muscle Ca, in progress, suggest that no substantial loss occurs. The observed drastic changes in function are perhaps due only to Ca displacement at strategic sites; or there may be a large concentration change, but in a structure whose volume is small in relation to the total volume of the muscle. The rapidity with which Ca deficiency develops (and is corrected when Ca is restored to the Krebs) favors the view that the affected structure is located at the surface of the muscle cell.

Oxytocic effects also develop rapidly. No effect is observed when normal membrane function is suspended temporarily and reversibly by treatment with excess K or by Ca-deficiency. Thus the evidence is strong in support of the conclusion that oxytocics are regulators of the "triggering" mechanism, rather than stimulants of the contractile machinery (Csapo, 1954, 1959). This distinction is significant if one considers the great differences in experimental approach and techniques, depending on whether one assumes an oxytocic effect in the membrane or in the myoplasm.

When the estrogen-dominated uterus is exposed to Ca-free Krebs its excitability decreases, and if this treatment is repeated normal excitability completely disappears. If the frog sartorius or toe muscle is exposed to Ca-free Ringers, excitability increases and in an extreme condition smooth muscle-like spontaneous activity develops (Bülbring *et al.*, 1956; Kernan and Csapo, 1957). This difference in behavior between the two types of muscle is, however, not a genuine discrepancy, but seems to be the consequence of the original state of their regulatory "tuning." These cross-striated muscles have a large excess potential and considerable stability. When treated with Ca-free Krebs the membrane potential drops only moderately (Jenerick and Gerard, 1953; Bülbring *et al.*, 1956), but sufficiently to lower the threshold. As their membrane potential approaches that of smooth muscle, they assume smooth muscle-like properties. But if the membrane potential of these cross-striated muscles is lowered (by excess K) so as to simulate the conditions normally existing in smooth muscles, and they are then treated with Ca-free Ringer, their excitability, like that of the uterus, decreases and eventually disappears (Csapo, 1959). Whether treatment with Ca-free solution produces the symptoms of Ca-deficiency or not, seems to depend not so much on the type of muscle, as upon the magnitude of the excess potential.

Observations on cross-striated muscles lead to the conclusion (Csapo, 1959), that Ca-deficiency "uncouples" the link between membrane and myoplasmic events. If indeed a discrete link exists between the triggering and contractile machinery, the possibility should be considered that regulation exerts its controlling influence on this target.

Observations (Coutinho and Csapo, 1958) show that the ovarian steroids, estrogen and progesterone, strikingly affect the Ca balance of the myometrial cell. Another set of experiments (Goto and Csapo, 1958; and data to be published) using the intracellular microelectrode technique, provides evidence that the myometrium becomes depolarized when Ca-deficient and that estrogen and progesterone control the magnitude of the membrane potential. This can be explained by the stabilizing effect of Ca on cellular structures.

Although much independent evidence points to the significance of Ca as a key ion in regulation, it is not possible as yet to state how this ion is used in imposing regulatory effects on muscle function. The difficulties lie in the complexity of function itself, about which we know very little. Muscle has been the favorite experimental object of classical physiology for centuries, and more recently of molecular physiology. However, it still remains largely a mystery, although perhaps less so than other tissues. What really happens when a resting muscle is transformed into an active one is unknown, and as long as it is unknown, there is little justification for elaborate theorizing as to how any of the principal events leading to function are regulated.

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