Brit. J. Pharmacol. (1962), **19**, 129–135.

MEASUREMENT OF UTERINE ACTIVITY IN VITRO BY INTEGRATING MUSCLE TENSION

BY

P. R. STYLES AND T. J. SULLIVAN

From the Departments of Medical Electronics and Pharmacology and Therapeutics, St. Thomas's Hospital Medical School, London

(*Received March* 29, 1962)

Spontaneous or electrically stimulated activity of the uterus is measured isometrically *in vitro* by integrating tension against time. Uterine contractions move the operating rod of a potentiometer transducer, the output voltage from which is coupled to an electrical integrator motor and a servo recorder. Several parameters of uterine activity can be expressed in a single measurement, and a record of isometric contractions is obtained simultaneously. Oxytocin can be assayed accurately and the effect of drugs on uterine motility can be measured.

To measure uterine activity *in vitro*, and the response of the isolated uterus to pharmacologically active agents, there are advantages in using a technique which measures as many parameters of uterine activity as possible over the chosen period of time. Spontaneous activity, however, involving both activating and contractile mechanisms (Schofield, 1954), is so variable that when comparing the oxytocic potency of drugs on the isolated uterus, it is customary to suppress the activating mechanism in order to obtain consistent results. This can be done either by lowering the temperature and calcium content of the Ringer solution used (Garcia de Jalon, Bayo Bayo & Garcia de Jalon, 1945) or by substituting electrical stimulation for spontaneous activation (Coutinho & Csapo, 1959). With such methods, however, the response of the contractile system to the drugs tested is studied only by measuring the amplitude of individual contractions.

In an attempt to measure several parameters of spontaneous activity in the isolated uterus despite its irregularity, Sandberg, Ingelman-Sundberg, Lindgren & Rydén (1958) devised an arbitrary scale for measuring variations in tone, frequency and amplitude of contractions. With this complex system, however, these parameters are measured as separate entities.

In the method to be described, the activity of the isolated uterus is measured by integrating the tension developed in the contracting uterus against time. The integral expresses the resultant of several parameters of uterine activity in a single measurement. Simultaneously, a kymograph tracing of isometric contractions is obtained with a servo recorder. The two methods may be used independently. The spontaneously acting or electrically stimulated uterus can be studied over any period of time chosen.

METHODS

Description of the apparatus. A block diagram of the apparatus is shown in Fig. 1. The uterine strip was set up in a perspex organ bath of 10 cm length and 10 ml. volume. Inside the bath, two silver wire electrodes were fixed into grooves in the perspex 6.5 cm apart, and connected to leads from a stimulator. At the lower end, the wall of the bath was pierced by two perspex side tubes, through which Krebs solution entered to wash the preparation

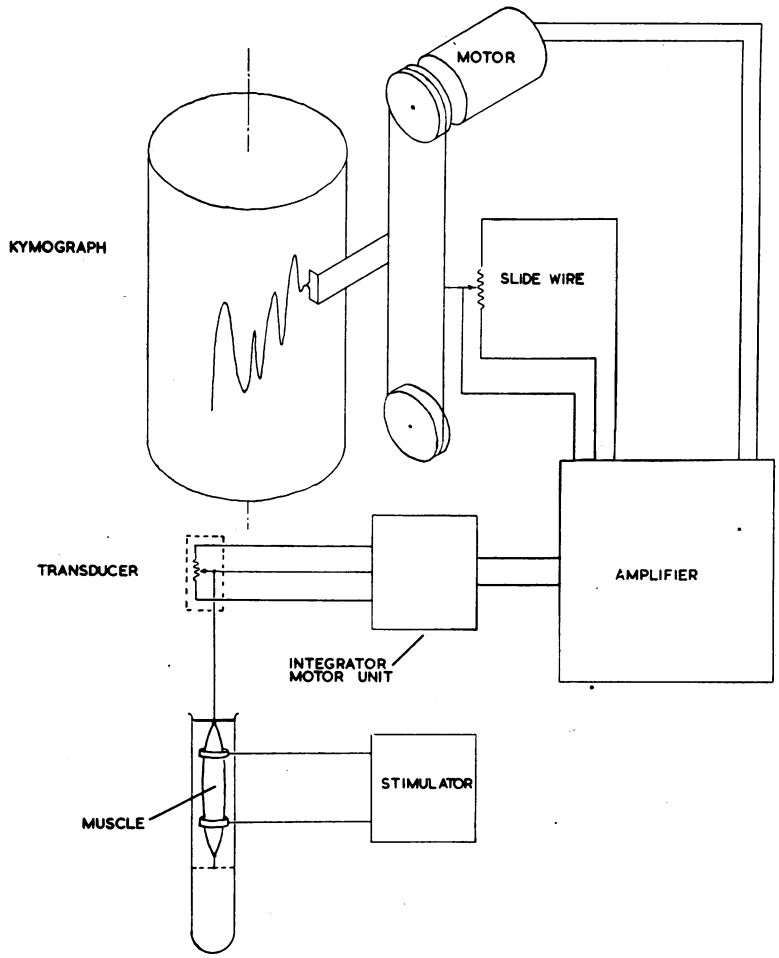

Fig. 1. Block diagram of the apparatus.

and flow out over the top. The base of the bath, which could be unscrewed to set up the uterine strip, had a hook for attaching the lower end of the uterus and was perforated by a perspex tube for bubbling gas into the bath. The organ bath was kept at constant temperature in a thermostatically controlled water bath. Krebs solution was warmed by passing through glass coils in the water bath before entering the organ bath.

The upper end of the uterine strip was attached to an isometric lever (Type C. 109, C. F. Palmer, Lond.) by means of a thread. The lever was fixed to a watchspring, the size of

which was varied according to the tension which was developed, to prevent the muscle from shortening by more than about 0.25 to 0.35 mm during contractions. Watchsprings of the following dimensions were suitable for the species indicated:

0.65 mm wide × 0.09 mm thick—rat uterus
1.05 mm wide × 0.08 mm thick—guinea-pig uterus
1.80 mm. wide × 0.14 mm thick—guinea-pig, rabbit and human uterus

A thread attached to the tip of the isometric lever passed upwards over a pulley and was attached to the upward pointing operating rod of a linear potentiometer transducer (type 542/2, J. Langham Thompson). Contraction of the uterus lifted the operating rod, which returned to its original position by gravity when the uterus relaxed. The isometric lever magnified shortening of the uterus about six times, so that the transducer rod, which had a maximum range of movement of about 6 mm, moved about 2 mm during a contraction.

The transducer potentiometer formed two arms of a d.c. excited bridge circuit, the remaining arms of the bridge being adjustable for zero output. The output voltage from the bridge was coupled via two pairs of emitter followers to a resettable electrical integrator motor with

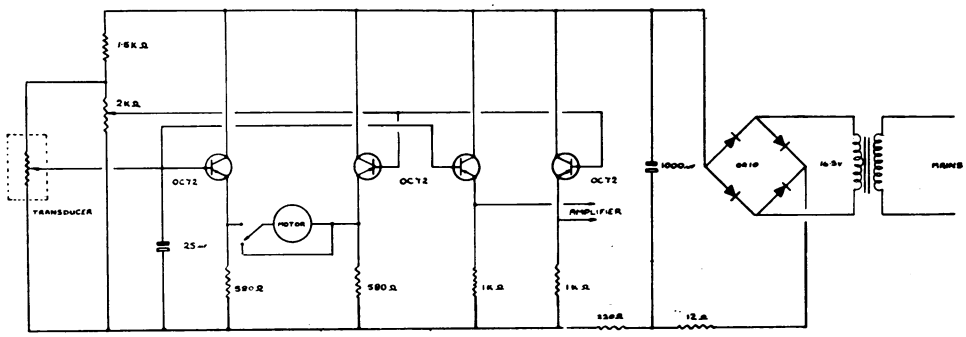

Fig. 2. Circuit diagram of the motor integrator unit.

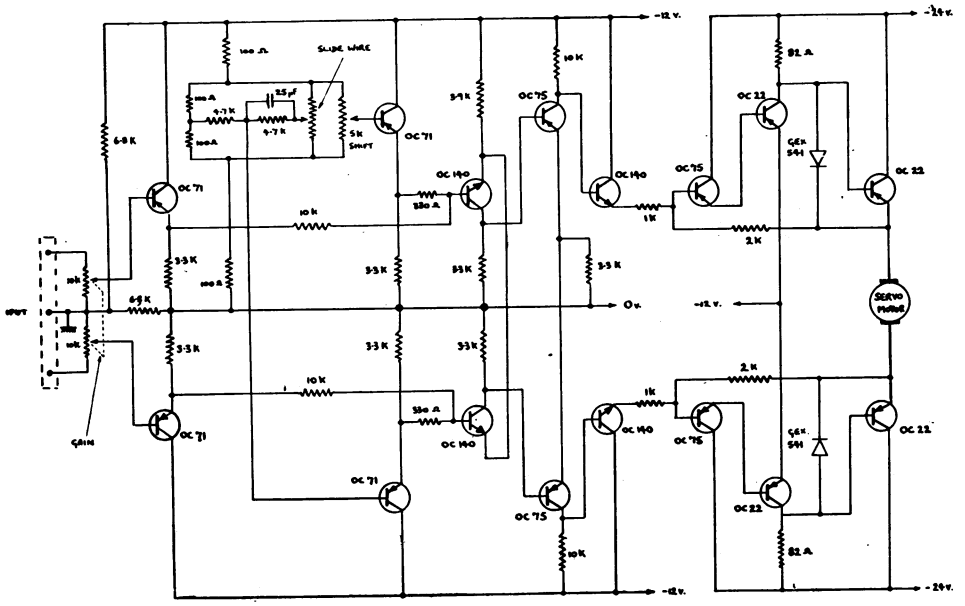

Fig. 3. Circuit diagram of the servo recorder.

a response time of 0.012 sec (type 916—4, Electro Methods) (Fig. 2) and to a servo recorder (Fig. 3).

For convenience, the servo recorder was designed to operate in conjunction with a kymograph and gave a maximum deflection of 15 cm. The writing pen was cord-driven from a permanent magnet-geared d.c. motor (type T d m 37a, Siemens and Halske), the cord also driving a feedback low torque slidewire potentiometer (type ACS—01, Ancillary Developments).

The servo motor amplifier used germanium transistors throughout, and was powered by a Mullard unstabilized subassembly (type YL 6101). Full-scale response time of the servo recorder was approximately 0.2 sec.

One of the drawbacks of the system was the limited resolution (0.0254 mm: 1/1,000 in.) of the potentiometer transducer. This could be overcome by the use of a capacitative or inductive transducer with appropriate circuitry giving infinite resolution.

Hysteresis of the recording system was negligible.

Procedure. A modified Krebs solution was the most satisfactory salt solution for both the spontaneously acting and electrically stimulated uterus. The composition in mM/l. was as follows: NaCl 154, $NaHCO_3$ 5.95, KCl 5.65, $CaCl_2$ 2.54, KH_2PO_4 1.18, $MgSO_4$ 1.18. Dextrose 1 g/l. is added. During the experiments gas (95% O_2 and 5% CO_2) was bubbled through the organ bath.

After setting up the uterus in the organ bath, it was stretched until "resting length" was reached (Csapo, 1954), at which the muscle functioned optimally.

Throughout the experiments Krebs solution was slowly perfused through the organ bath except when drugs were added to the bath.

The spontaneously acting uterus was studied at 37° C. During electrical stimulation of the muscle, spontaneous activity, if it persisted, was abolished by lowering the temperature of the bath and the calcium content of the salt solution. The automatic stimulator gave 50 c/s a.c. of predetermined duration. A stimulus of 15 V for 5 sec was repeated at intervals of one or more min. This particular voltage was found to be optimal for the rabbit uterus, in agreement with Csapo & Goodall (1954), and also for the guinea-pig and rat uterus.

When the uterus was relaxed, the integrator was adjusted to zero and the baseline of the tracing adjusted on the kymograph paper. In many experiments it was found satisfactory to record uterine activity on the integrator over periods of 5 min, though sometimes spontaneous activity was so irregular that longer periods were necessary to obtain consistent results.

Oxytocic drugs were introduced into the organ bath either by adding them to the perfusing Krebs solution, to measure the effect of a given concentration continuously over a long period of time, or directly from a hypodermic syringe.

The apparatus was calibrated by attaching weights to the isometric lever at the point of attachment of the muscle and recording the integrator readings for the same period of time as that used for readings taken during the experiment. A graph was then plotted of weight in g against integrator readings. The integrated tension in g sec corresponding to any reading on the integrator obtained during the experiment was then given by weight in g multiplied by the time in sec.

In most experiments, virgin rats of 150 to 200 g body weight and virgin guinea-pigs of 500 to 700 g body weight were used, and were given 25 and 50 μg oestradiol monobenzoate respectively by subcutaneous injection 24 hr before the experiment.

RESULTS

In Fig. 4, kymograph tracings are shown of the activity of spontaneously acting and electrically stimulated guinea-pig uteri. The integrated tension developed in response to each dose of oxytocin (m-u./ml. in the organ bath) is given below the

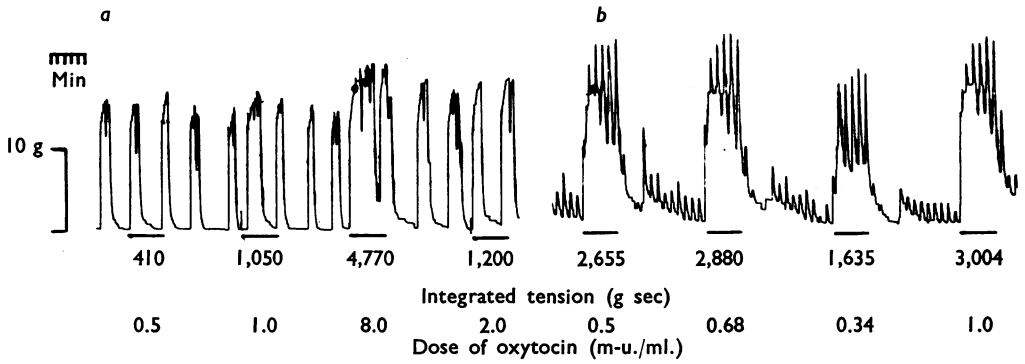

Fig. 4. Kymograph tracings showing the response of the guinea-pig uterus to doses of oxytocin, (*a*) spontaneously acting and (*b*) electrically stimulated. Doses were in the organ bath for 5 min (at horizontal lines), and the integrated tension developed in response to each dose is given beneath them.

corresponding part of the tracing. Doses of oxytocin were left in the organ bath for 5 min. The uterus was washed several times during the 5 min after each dose, and a control reading was taken for a further 5 min before adding the next dose. The integrated tension developed in response to oxytocin was calculated by subtracting the mean value for all control observations from the values recorded in response to individual doses of the drug.

On the spontaneously acting uterus oxytocin, 8 m-u./ml., produced a clear effect, but because of the variable spontaneous activity in between the additions of the drug it would be difficult to assess the response to 0.5, 1.0 and 2.0 m-u./ml. without measuring the integrated tension developed in the muscle. With the larger doses, however, individual parameters such as amplitude and duration of contraction can be separately assessed from the tracing and related to the integrated tension developed.

For the electrically stimulated uterus, the temperature of the bath was lowered to 28° C and the concentration of $CaCl_2$ in the Krebs solution to 0.25 mM/l. In response to electrical stimulation at intervals of 1 min, the muscle gave consistent contractions of small amplitude. When oxytocin was added to the bath the uterus responded to and discriminated between doses which produced only marginal responses in the spontaneously acting muscle. Under these conditions, responses to concentrations of oxytocin as low as 25 μ-u./ml. were sometimes obtained. Similar results were found with the rat uterus, one preparation responding to 12.5 μ-u./ml.

Log dose-response curves for oxytocin obtained from both spontaneously acting and electrically stimulated uteri are shown in Fig. 5. Drug concentrations are expressed in μ-u./ml. in the organ bath. Each point represents one dose in the graph from the electrically stimulated muscle and the mean of three doses in the graph from the spontaneously acting muscle.

In four-point assays of oxytocin, 16 doses were given in a Latin Square using a dose ratio of 1.33. The index of precision (λ) (Gaddum, 1953a) was calculated

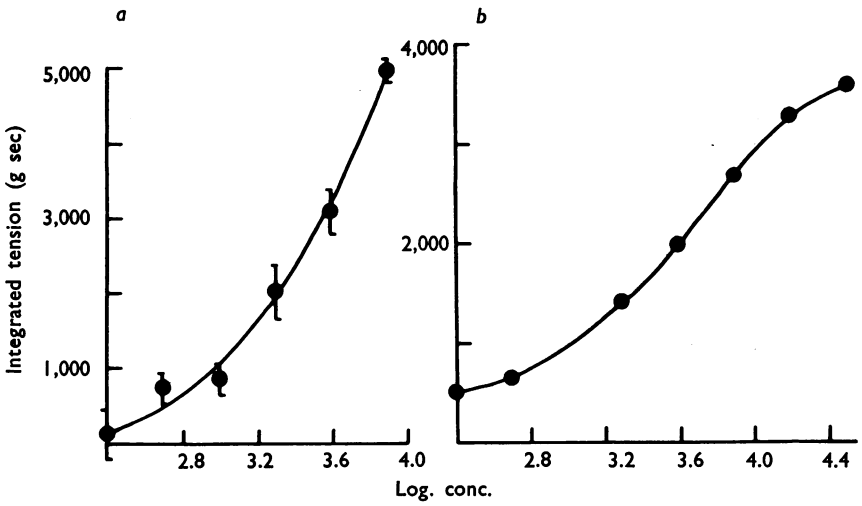

Fig. 5. Log dose-response curves of guinea-pig uterus to oxytocin (μ-u./ml.). (*a*) Spontaneously acting uterus. Each point is the mean of three results. S.E. shown. (*b*) Electrically stimulated uterus. Each point represents one dose.

from the readings obtained for the integrated tension. With the electrically stimulated guinea-pig uterus, the mean value for L $(1/\lambda)$ in two experiments was 12.0 ; with the electrically stimulated rat uterus the mean value in two experiments was 11.3. In one experiment with the spontaneously acting guinea-pig uterus the value for L was 5.1.

DISCUSSION

This technique offers a quantitative method of studying the motility of the spontaneously acting uterus *in vitro* and of measuring the effect upon it of pharmacologically active agents.

By comparing the response of the spontaneously acting and electrically stimulated uteri to drugs, it is possible to differentiate between selective actions which drugs may have upon the mechanisms of activation and contractility.

The potency of drugs can be assayed on the electrically stimulated uterus, and the method can be modified so as to employ the technique of superfusion (Gaddum, 1953b), which might increase the sensitivity of the uterus, for instance, to small doses of oxytocin (Fitzpatrick, 1961). The indices of precision obtained in four-point assays on the electrically stimulated uterus compare favourably with that calculated from data given by Holton (1948), where L $(1/\lambda)$ was found to be 10.2 using a dose ratio of 1.6.

Though commercial chart recorders are available, they are not readily applicable to pharmacological work. The servo recorder described here is constructed specifically for use with a kymograph and in combination with the motor integrator. It can be made for about one-fifth of the price of a commercial recorder.

Although other varieties of smooth muscle have not been studied with this apparatus, the method is applicable to the study of any slowly contracting tissue.

The authors are grateful to Professor R. S. Stacey for advice and criticism during the preparation of this paper, and to Mr D. G. Bray for technical assistance.

REFERENCES

COUTINHO, E. M. & CSAPO, A. (1959). The effect of oxytocics on the "Ca-deficient" uterus. *J. gen. Physiol.*, **43**, 13–27.

CSAPO, A. (1954). Dependence of isometric tension and isotonic shortening of uterine muscle on temperature and on strength of stimulation. *Amer. J. Physiol.*, **177**, 348–354.

CSAPO, A. & GOODALL, M. (1954). Excitability, length tension relation and kinetics of uterine muscle contraction in relation to hormonal status. *J. Physiol. (Lond.)*, **126**, 384–395.

FITZPATRICK, R. J. (1961). The estimation of small amounts of oxytocin in blood. In *Oxytocin*, pp. 358–377, ed. CALDEYRO-BARCIA, R. & HELLER, H. London: Pergamon Press.

GADDUM, J. H. (1953a). Bioassays and mathematics. *Pharmacol. Rev.*, **5**, 87–134.

GADDUM, J. H. (1953b). The technique of superfusion. *Brit. J. Pharmacol.*, **8**, 321–326.

GARCIA DE JALON, P., BAYO BAYO, J. & GARCIA DE JALON, M. (1945). Sensible y nuevo método de valoración de adrenalina en útero aislado de rata. *Farmacoter. act.*, **2**, 313–318.

HOLTON, P. (1948). A modification of the method of Dale and Laidlaw for standardization of posterior pituitary extract. *Brit. J. Pharmacol.*, **3**, 328–334.

SANDBERG, F., INGELMAN-SUNDBERG, A., LINDGREN, L. & RYDÉN, G. (1958). *In vitro* studies of the motility of the human uterus. *J. Obstet. Gynaec. Brit. Cwlth.*, **65**, 965–972.

SCHOFIELD, B. M. (1954). The influence of oestrogen and progesterone on the isometric tension of the uterus in the intact rabbit. *Endocrinology*, **55**, 142–147.