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QUANTITATIVE ASSAYS OF OXYTOCIC DRUGS ON THE HUMAN POSTPARTUM UTERUS

BY

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Quantitative assays of oxytocic drugs were performed on the postpartum human uterus by means of external tocography. Since, owing to the rapid involution of the uterus, only two doses could be given to each patient, the assays were designed as incomplete randomized blocks of 2. A dose/response curve for ergometrine was established, and a comparative assay of ergometrine and methyl ergometrine carried out, the design and analysis of which is described in detail.

Assays of oxytocic drugs on the animal uterus or on isolated strips of the human uterus frequently give a misleading indication of their potency in clinical use, and it is thus desirable to test these drugs on the intact human uterus. There are basically two methods of tocography that can be used to assay drugs on the human uterus, namely the internal and the external methods. The internal methods are in some ways more reliable since the pressures inside the uterus are directly measured, but they have the disadvantages that they are potentially dangerous and cannot easily be used on a sufficient scale to give results which are statistically valid. The external methods utilize an instrument measuring through the abdominal wall the hardening of the uterus during contraction. The advantage of the external method is that it does not in any way endanger the patient, and in fact causes no or practically no discomfort, nor does the method itself modify uterine activity.

Although external tocography has been used to study the effect of drugs during pregnancy and parturition this method has not apparently been used for the quantitative assay of oxytocic drugs after delivery. Because of the simplicity and safety of external recording and despite the apparent difficulties of obtaining satisfactory results from the involuting uterus in many difficult patients, we set out to use this method to establish the dose/response relationship for small doses of ergometrine, and subsequently used the method to compare quantitatively the effects of ergometrine and methyl ergometrine.

An attempt was made to design the experiments on a similar basis to those used for biological assay of drugs in animals, but we were limited by the fact that only two doses could be given to each patient owing to the rapid involution of the uterus and the interval necessary between doses. The assays were therefore designed as incomplete blocks of two doses. In this type of assay it is possible nevertheless to extract an activity ratio which is based only on dose contrasts within blocks, but the analysis is rather more complicated than that of an ordinary four-point assay. A comparison of ergometrine and methyl ergometrine was carried out in this way, and the analysis of variance and calculation of fiducial limits of this assay are described in detail. The calculations are adapted from Finney (1952), who discusses more complex designs. This type of design is of general interest for testing drugs on man when only two doses can be administered to each subject.

METHODS

A strain-gauge tocograph was used in these experiments which was devised and made by Dr. C. N. Smyth. The instrument consists of a brass ring, held firmly on to the abdomen by a thin elastic belt. Mounted radially within the ring is a flat strip of beryllium-copper spring, to which strain-gauges are fixed. The ebonite sensing probe passes through a collar attached to the central end of the spring. The output from the resistance bridge circuit is fed directly to a Fielden Servograph pen-recorder. The pen deflexion was proportional to the load on the spring throughout the working range.

The instrument was applied to the abdomen in the midline over the most prominent part of the surface of the uterus. The probe was adjusted to protrude against the uterus so that the load on the spring was 80 to 100 g. Each test lasted for 1 hr. For the first 20 min. the spontaneous activity, if any, of the uterus was recorded as a control period. The test dose of oxytocic was then given intravenously and its effect recorded for 40 min.

During the control period the uterus was inactive, or gave occasional intermittent contractions. Intravenous injections of small doses of ergometrine or methyl ergometrine produced either larger and more frequent intermittent contractions or sustained contractions as shown in Fig. 1. The tracings were then transcribed from circular to linear co-ordinates and the area enclosed between the base line and the tracing was used as the criterion of effect. The values obtained during the first 20 min. period after injecting the drug were used in the subsequent calculations.

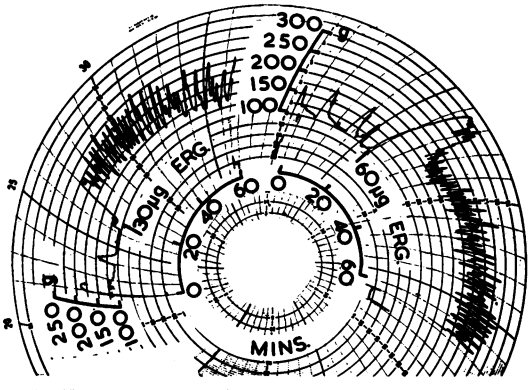

FIG. 1.—Tocograph records following intravenous injections of 30 and 60 μg. ergometrine obtained in the same subject on successive days.

Other calculations based on the whole 40 min. test period did not differ materially in the relative values obtained. Tests were made during the first four days of the puerperium, and patients of differing parity were included. Subjects with obese abdominal walls were not suitable, and, when the fundus of the uterus could not be clearly outlined by palpation, recording was not satisfactory. The nature and purpose of the tests was explained to each patient, and their consent obtained. The drugs used were ergometrine maleate and methyl ergometrine tartrate, and their activities are expressed in terms of these salts.

RESULTS

Dose/Response Curve of Ergometrine. — It seemed likely that, if several measurements could be made in the same patient, variability would be reduced particularly if these measurements could be made on the same day, since the variability due to involution of the post-partum uterus would also be thus eliminated. In a first series of experiments we therefore studied the effects of two doses of ergometrine administered to the same patient on the same day. The results of this trial are given in Table I, which shows that in each patient the first dose produced a proportionately greater effect than the second dose. We concluded that the method of giving more than one dose on the same day was unsound, since the effect of the second dose was depressed by the earlier dose.

TABLE I

EFFECTS OF TWO DOSES OF ERGOMETRINE TO THE SAME PATIENT ON THE SAME DAY

The effect is given in terms of the area between the base line and the response curve.

		Patient				Sums of Effects
		A	B	C	D	
First dose	15 μg.	13	14	12	14	53
	30 ,,					
Second dose	15 ,,	7	7	15	7	36
	30 ,,					

In the next series two doses of ergometrine were given to the same patient on consecutive days. The design was as follows: Three dose levels of ergometrine were used in all (15, 30 and 60 μg.) which had been shown in earlier trials to produce a graded response of the uterus. Each subject received two doses, arranged in such a way that every combination of doses in each order was given twice, so that 12 subjects were employed.

The results of this series are shown in Fig. 2. The pairs of doses in each subject are linked and it is seen that in each individual case the greater dose produced the greater effect. The broken line is the best fitting straight line, and shows the overall increase of response with dosage.

Certain conclusions can be drawn from these results which are relevant to the use of the method for bio-assay: (1) The basic condition of a quantitative assay, dependence of effect on dose, is fulfilled. (2) The relation between log. dose and effect is approximately linear. (3) Responses obtained in the same patient are more consistent than those obtained on different patients. These various points were borne out by an analysis of variance, which showed a highly significant slope; no significant deviation from linearity; and significantly greater variation between than within subjects.

Since the two doses were given on consecutive days it was of interest to find out whether the response of the uterus changed in a systematic

fashion during the experimental period. Our experiments are insufficient in number to establish this point with any confidence, especially since most were carried out on the second and third days and only a few were done on the first or fourth days. Nevertheless, Fig. 3 shows that there is no consistent trend in response with passage of time. This applies particularly to the response obtained on the second and third day.

Comparison of Ergometrine and Methyl Ergometrine.—This assay was also arranged in such a way that each patient received two doses of drugs. Since a 2+2 assay was planned there were six possible combinations of the four doses. Furthermore, each of these combinations could be given in the reversed order. This gives 12 possible dose combinations, or 12 patients each receiving two doses. The dose combinations were allocated to patients at random.

The results (Table II) were computed in the first instance in an approximate manner as a

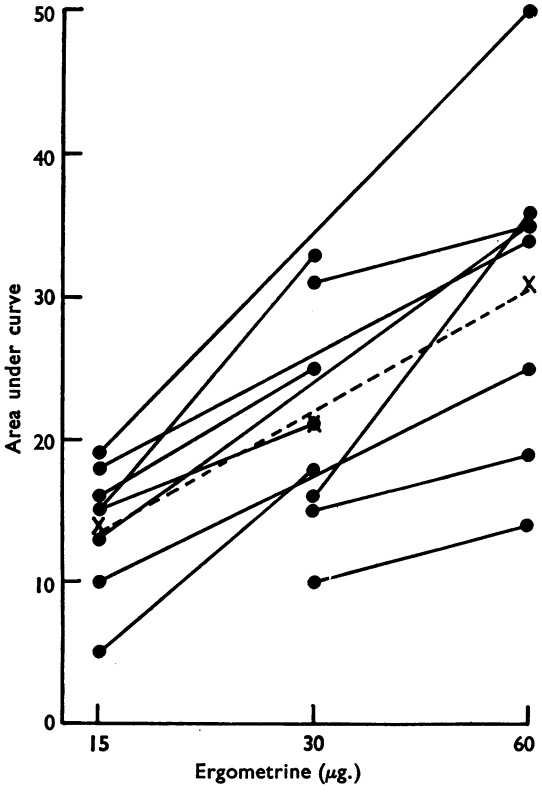

FIG. 2.—Dose/response curves of ergometrine. Points joined by a solid line refer to effects obtained in the same patient on successive days. The broken line is the best-fitting straight line.

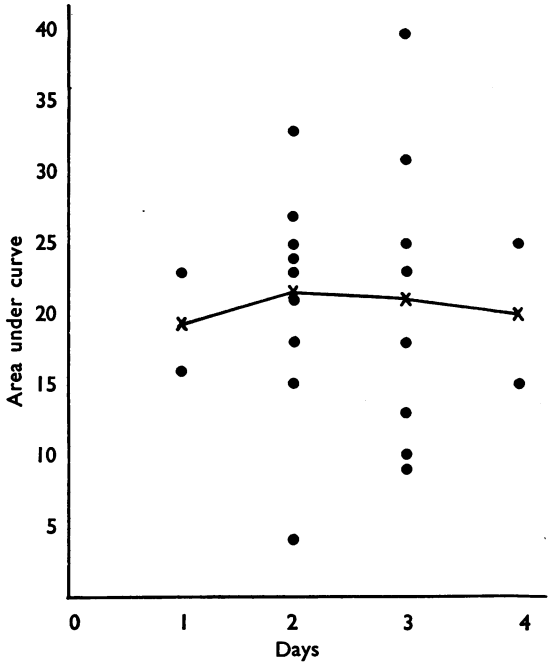

FIG. 3.—Responses to ergometrine in relation to days after delivery. (An arithmetic correction, equal to the difference in the means, was applied to equalize the three dose levels.)

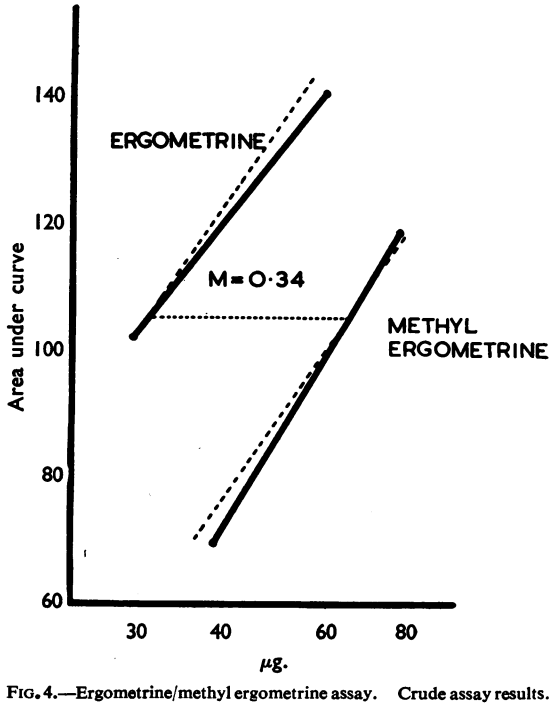

FIG. 4.—Ergometrine/methyl ergometrine assay. Crude assay results.

TABLE II

COMPARATIVE ASSAY OF ERGOMETRINE AND METHYL ERGOMETRINE

Effects measured as in Table I.

Doses	1	2	3	4	5	6	7	8	9	10	11	12
Ergometrine: 30 μg.=E_1	19	16		12			29		14	19		
60 ,, =E_2		21			23	20	40				15	25
Methyl ergometrine: 40 μg.=M_1	8		10	16				8			12	16
80 ,, =M_2			24		16	22		13	19	27		

" straight " 2+2 assay of 24 randomly distributed responses. The mean responses are shown in Fig. 4. The resulting activity ratio was 2.2 in favour of ergometrine and the fiducial limits were 1.3 and 11.2. (The asymmetry and wide fiducial limits are due to a large g-factor.)

The results were next computed taking into account the fact that they comprise 12 blocks of two responses with different variances within and between blocks.

The analysis of variance is divided into two sections, variation within blocks and variation between blocks. Since none of the patients received all four doses, the contrasts between doses are *partially confounded* with contrasts between patients. The object of the analysis of variance is to divide the total sum of squares in such a way that dose contrasts obtained on the same patients are separated from dose contrasts obtained on different patients.

The responses given in Table II were then used to prepare two further sets of values of the sums and the differences of the two responses for each patient. These sums and differences are multiplied by *orthogonal coefficients* as shown in Table III, to provide sums of squares for *preparations*, *regression* and *parallelism*. (The orthogonal coefficients in Table III are obtained by addition of the standard orthogonal coefficients for a four-point assay: $L_p = +1+1-1-1$; $L_i = -1+1-1+1$; $L' = -1+1+1-1$; for example, the L_p coefficient for $E_1 + E_2 = +1+1 = 2$, the L_i coefficient for $E_1 + E_2 = -1+1 = 0$, etc.).

TABLE III

ORTHOGONAL COEFFICIENTS FOR CONTRASTS BETWEEN AND WITHIN PATIENTS

E_1, E_2, M_1, and M_2 as in Table II.

Doses	Totals	Preparation (L_p)	Regression (L_i)	Parallelism (L')
E_1+E_2	106	+2	0	0
E_1+M_1	55	0	−2	0
E_1+M_2	79	0	0	−2
M_1+M_2	55	−2	0	0
M_1+E_2	68	0	0	+2
E_2+M_2	81	0	+2	0
Sum of products		102	52	−22
Divisor		32	32	32
E_1-E_2	−16	0	−2	−2
E_1-M_1	7	+2	0	−2
E_1-M_2	−13	+2	−2	0
M_1-M_2	−19	0	−2	+2
M_1-E_2	−12	−2	−2	0
E_2-M_2	5	+2	0	+2
Sum of products		22	120	−10
		64	64	64

Calculation of sums of squares proceeds as follows:

a. Total $Sy^2 = 9438$

b. Correction factor $\frac{(Sy)^2}{N} = 8214$

$a-b = 9438-8214 = 1224$

c. Sums of two doses for each patient

$[(x_1+x_2)^2+(x_3+x_4)^2 \ldots +(x_{23}+x_{24})^2]/2 = 9070$

$c-b = 856$

d. Differences of two doses for each patient

$(x_1-x_2)^2+(x_3-x_4)^2 \ldots +(x_{23}-x_{24})^2/2 = 368$

e. Between patients (from Table III)

Preparation $102^2/32 = 324$

Regression $52^2/32 = 85$

Parallelism $-22^2/32 = 15$

f. Within patients (from Table III)

Preparation $22^2/64 = 8$

Regression $120^2/64 = 225$

Parallelism $-10^2/64 = 2$

The resulting analysis of variance is shown in Table IV.

The upper part of Table IV deals with dose contrasts in different patients. It shows no significant regression and therefore provides no information about the assay. The error variance in this section is significantly greater than in the middle section, which supports the separation of the variance into two parts. The lower part of Table IV deals with dose contrasts in individual patients. It shows a significant F value for regres-

sion ($P<0.01$) and no deviation from parallelism and can therefore be utilized to provide a new activity ratio which depends entirely on measurements in individual patients and new fiducial limits.

TABLE IV
ANALYSIS OF VARIANCE

	df	Sum of Squares	Mean Square
Between patients	11	856	78
Preparation ..	1	324	324
Regression ..	1	85	85
Parallelism ..	1	15	15
Error (1) ..	8	432	54
Within patients ..	12	368	31
Preparation ..	1	8	8
Regression ..	1	225	225
Parallelism ..	1	2	2
Error (2) ..	9	133	14·8

$F = 54/14{\cdot}8 = 3{\cdot}65 \quad P < 0{\cdot}05$
$F = 225/14{\cdot}8 = 15{\cdot}2 \quad P < 0{\cdot}01$

The logarithmic activity ratio is given by:

$$M = \bar{x}_M - \bar{x}_E + \frac{\bar{Y}_E - \bar{Y}_M}{b} = \bar{x} - \bar{x}_E + \frac{Lp}{Li}\,d = 0.12 + \frac{22}{120}\,0.3 = 0.175$$

The calculation of fiducial limits for $\frac{\bar{Y}_E - \bar{Y}_M}{b}$ may be simplified by using $\frac{d}{4} = 0.075$ as the dose metameter with regression coefficient b′. The fiducial limits are then given by

$$\left[\frac{\bar{Y}_E - \bar{Y}_M}{b'} \pm \frac{ts}{b'}\left\{(1-g)\left(\frac{1}{N_E}+\frac{1}{N_M}\right) + \left(\frac{\bar{Y}_E - \bar{Y}_M}{b'}\right)^2 / S_{XX}\right\}^{\frac{1}{2}}\right] \div (1-g)$$

where $\frac{\bar{Y}_E - \bar{Y}_M}{b'} = \frac{Lp}{Li}\,\frac{d}{0.075} = \frac{22}{120}\,\frac{0.3}{0.075} = 0.733$

$t = 2.26$ (for 9 df at the 5% level)

$s = \sqrt{14.8} = 3.85$

$b' = \frac{L_i}{S_{XX}} = \frac{120}{64}$

$g = \frac{s^2 t^2}{b'^2 S_{XX}} = \frac{(14.8)(2.26)^2(64)}{120^2} = 0.336$

$N_E = N_M = 8$

The limits are evaluated as

$$\left[0.73 \pm \frac{(2.26)(3.85)(64)}{120}\left\{(1-0.336)\left(\frac{1}{8}+\frac{1}{8}\right) + 0.733^2/64\right\}^{\frac{1}{2}}\right] \div (1-0.336)$$

$= 4.023;\ -1{,}824$

These quantities must be multiplied by 0.075 in order to convert them to decimal logarithms.

$$4.023 \times 0.075 = 0.3017$$
$$-1.824 \times 0.075 = -0.1368$$

The lower and upper limits are therefore

$$M_U = \bar{x}_M - \bar{x}_E + 0.3017 = 0.12 + 0.3017 = 0.4217$$

$$M_L = \bar{x}_M - \bar{x}_E = 0.1368 = 0.12 - 0.1368 = 1.9832$$

Taking antilogarithms the activity ratio is 1.5 in favour of ergometrine with (5%) fiducial limits: 0.96 and 2.7. The adjusted activities as derived from within block comparisons of ergometrine and methyl ergometrine are shown graphically in Fig. 5.

DISCUSSION

These results show that it is possible to assay oxytocic drugs quantitatively on the postpartum human uterus. The external recording method involves very little discomfort for the patients, and no danger, since very small doses of drugs will stimulate the uterus when they are administered intravenously. The tocograph responses show considerable variability, but we have found that most of the variation is associated with differences between patients, presumably due to differences in the size of the uterus, its position, and the thickness of the abdominal wall. Responses obtained from the same patient were reasonably consistent, even when they were obtained on successive days. The index of precision for within-patient comparisons for the ergometrine/methyl ergometrine assay was $\lambda = 0.15$, which is within the range of precision obtainable in bio-assays in animals. It is somewhat surprising that the uterus should give consistent responses in spite of involution, but in our limited series of experiments it seemed that at least between the second and third days postpartum there was no consistent diminution in response.

Since assays on the same patient are so much more efficient than on different patients, it is desirable to give more than one dose to each patient. Ideally, groups of three or four doses in each patient would be desirable, since this would give a complete assay block for each patient and provide a more efficient method of analysis, but we have found it impracticable to give three doses to each patient, and have therefore used the device of incomplete blocks with two doses only. This is less efficient than the

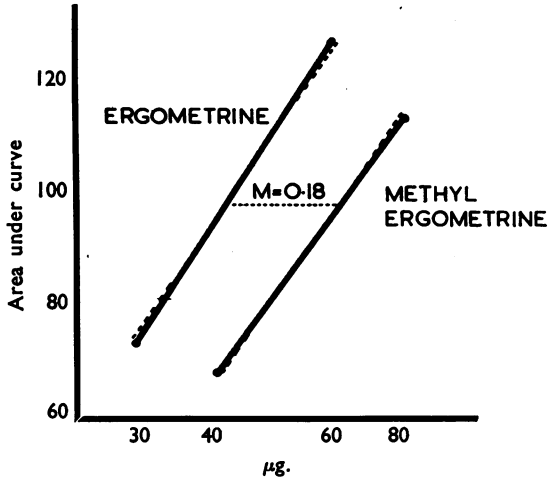

FIG. 5.—Ergometrine/methyl ergometrine assay. Adjusted values derived from comparisons in individual patients.

three doses scheme, and the analysis is more complicated, but nevertheless it is possible to obtain results with reasonable fiducial limits without using a very large number of patients.

Our finding that ergometrine is about 1.5 times as active as methyl ergometrine contrasts with the results of most other workers, who agree in claiming that methyl ergometrine is either as active as ergometrine (Kirchof, Ricely, Wilson, and David, 1944) or slightly more active than it (Gill, 1947). However, none of these comparisons were quantitative or based on concentration/action curves, and their statistical significance therefore cannot be assessed.

Although we found that both ergometrine and methyl ergometrine, when administered in small intravenous doses, gave a satisfactory dose/response relation, it does not necessarily follow that such a relation would be found with other oxytocic drugs. The effects of ergometrine and methyl ergometrine are qualitatively similar, and consist in a prolonged contraction of the uterus which can be accurately measured. Drugs which produce intermittent contraction of the uterus, such as oxytocin, are more difficult to quantitate, as suggested by the findings of Bainbridge, Nixon, Schild and Smyth (1956) with synthetic oxytocin.

We have found that the method of testing oxytocic drugs on the postpartum uterus is of value even in cases where a quantitative assessment of activity is not desired or not possible. Thus we have had occasion to test (unpublished observations) several synthetic oxytocic compounds which were sent to us by manufacturers and which had previously been tested on the animal uterus. In each case it was found that the activity of the oxytocic as compared with ergometrine was very much less on the postpartum human uterus than on the animal uterus. It would seem that for the tesing of new oxytocic drugs an assay on the intact human uterus is indispensable.

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