THE BIOLOGICAL ASSAY OF OXYTOCIN IN THE PRESENCE OF ERGOMETRINE

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The oxytocin content of a solution of oxytocin and ergometrine can be assayed with great accuracy by the chicken blood pressure method provided the rooster preparation is highly sensitive to oxytocin. The hormone can also be assayed with reasonable accuracy by measuring the response of the milk-ejection pressure in the mammary gland of the lactating rabbit. However, the rat uterus *in vitro* method yields erroneously high results due to the additional uterotonic effect of the ergometrine.

THE place of oxytocin as well as that of ergometrine is firmly established in obstetrical practice. Recently, ampoules containing 5 I.U. of synthetic oxytocin and 0·5 mg. of Ergometrine Maleate B.P. became available under the name "Syntometrine", and clinical investigations (Embrey, 1961) showed this combination to have merits which are of considerable interest in the management of the third stage of labour and in the prophylaxis and therapy of maternal blood loss.

As a statutory requirement, the potency of pharmaceutical preparations containing oxytocin must be controlled by biological methods of assay and declared in International Units (I.U.) with reference to the Third International Standard for Oxytocic, Vasopressor and Antidiuretic Substances. The British Pharmacopoeia (1958) prescribes two assay procedures, one based on the uterotonic action of oxytocin, the other on its avian depressor effect. The United States Pharmacopeia (1960) gives only the avian blood pressure lowering test. Since ergometrine influences both uterine contractility and arterial blood pressure, it seemed likely that its presence in a solution of oxytocin would interfere with these two methods of assay.

To clarify this question, experiments were performed to test the accuracy of the rat uterus and avian blood pressure methods for the biological assay of oxytocin in the presence of ergometrine. In addition, the milk-ejection pressure test was evaluated, for, although this method is not included in the pharmacopoeias, it is a convenient and specific procedure for the assay of oxytocin.

METHODS

In vitro preparations of rat uteri were used following the procedure described by Holton (1948) and applying the four-point-assay scheme of Schild (1942).

The blood pressure of white Leghorn roosters was measured by the method of Coon (1939) using the experimental design of Thompson (1944).

The milk-ejection pressure of anaesthetised lactating rabbits was measured as described previously (Berde and Cerletti, 1960), the experimental arrangement being an adaptation of that employed by Cross and

Harris (1951/52) and by Van Dyke, Adamsons and Engel (1955). Here also the four-point-assay scheme was used.

As Syntometrine ampoules contain synthetic oxytocin, "Syntocinon", this was used as the reference standard in many of the assays. In others, the Third International Standard for Oxytocic, Vasopressor and Antidiuretic Substances was taken as the reference standard.

TABLE I

THE APPARENT OXYTOCIN CONTENT OF SYNTOMETRINE IN DIFFERENT TESTS (THE ACTUAL SYNTOCINON CONTENT OF SYNTOMETRINE IS TAKEN AS 100 PER CENT)

Test	Value (per cent)	Note
Rat uterus *in vitro*	119 ± 5	11
	100 ± 15	5
	138 ± 30	5 } 4-point-assays
	142 ± 27	8
	171 ± 38	12
	151 ± 28	10
Chicken blood pressure—high sensitivity	100·5 ± 2·2	12
	100·9 ± 2·8	12
	102·6 ± 3·6	12 } doses of unknown
	99·4 ± 3	10
	101 ± 4·8	10
low sensitivity	86 ± 3	10
	71 ± 8	6 } doses of unknown
	90 ± 4	6
	76 ± 11	5
Rabbit mammary gland *in situ*	107 ± 11	6
	98 ± 10	4
	102 ± 9	10 } 4-point-assays
	92 ± 7	5
	104 ± 9	5

RESULTS

The results are summarised in Table I which gives the means and the standard errors of the assays, the actual Syntocinon content of Syntometrine being taken as 100 per cent.

The oxytocin contents as determined by the rat uterus method are obviously far too high.

Chicken blood pressure assays yield excellent results provided the rooster preparation is highly sensitive to oxytocin, that is to say provided 20 mU. oxytocin i.v. elicits a blood pressure fall of 30 to 50 mm. Hg. If, however, the sensitivity is low, thus, if 40 to 100 mU. oxytocin are required to provoke a fall of 30 to 50 mm. Hg, the measured content is lower than the actual Syntocinon content of the Syntometrine.

The oxytocin contents assayed by the rabbit mammary gland method are in agreement with the actual contents of the test solutions.

DISCUSSION

Bearing in mind the well-known uterotonic effect of ergometrine, it is hardly surprising that the rat uterus *in vitro* should prove unsuitable for the biological assay of an oxytocin solution containing ergometrine. Indeed, it has been reported (Pennefather, 1961) that the isolated rat uterus can be used for the estimation of ergometrine. In our experience

this method of assay is not particularly suitable for ergometrine, since the sensitivity of the rat uterus to this oxytocic tends to fluctuate and the dose-response relationship is not always satisfactory. Be that as it may in the assay of Syntometrine, ergometrine is present in a concentration of 10–40 μg./litre, and in this concentration is liable to elicit contractions of the isolated rat uterus or to reinforce contractions due to oxytocin, thereby yielding erroneously high values for the oxytocin content. The inconsistent influence of ergometrine is reflected by the high standard errors, which greatly exceed the standard errors for assays on solutions containing oxytocin only. On *in situ* uterine preparations of, for example, the rabbit, the summation of the oxytocic effects of the two drugs is evident.

The excellent results obtained with highly sensitive chicken blood pressure preparations show that amounts of ergometrine as small as, for example, 2 μg., do not counteract the vasodilatation provoked by oxytocin. In higher doses, however, the pressor effect of ergometrine attenuates the fall of pressure due to oxytocin. In fact, 10 to 20 μg. ergometrine—without oxytocin—may actually elevate the blood pressure of roosters by 20 to 40 mm. of Hg.

A satisfactory rise of milk-ejection pressure within the mammary gland of the lactating rabbit is elicited by only a few milliunits of oxytocin, so that the oxytocin content of Syntometrine can be assayed with the usual accuracy of this method. The small amount of ergometrine accompanying the effective dose of oxytocin is without any apparent effect.

The studies reported in this paper show that the chicken blood pressure test is the most accurate method for the biological assay of oxytocin in a solution containing oxytocin and ergometrine, provided the rooster preparation is highly sensitive to oxytocin. If this method is not practicable the assay on the rabbit mammary gland is an acceptable, although less accurate, alternative. The isolated rat uterus gives erroneously high values and should not be used for this purpose.

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