A Sensitive and Specific Bioassay for Oxytocin[1] (34643)

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(Introduced by R. I. Dorfman)

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In 1960, Méndez-Bauer *et al.* (1) reported the action of oxytocin on the isolated strip of lactating rabbit mammary gland suggesting its use for the bioassay of oxytocin. The threshold dose found was 500 μU/ml.

The method was further studied by Moore and Zarrow (2) who found a high specificity but a very low precision and confirmed the low sensitivity. Smith (3), Sjöholm and Rydén (4), and Rydén and Sjöholm (5) employed the same method using strips of rat mammary gland. The sensitivity and the precision of this preparation were much higher than that of the rabbit. It was found (5) that the rat mammary gland was highly sensitive to several pharmacological agents other than oxytocin. In our studies on the participation of oxytocin in human reproduction (6) we frequently used this bioassay to measure the very low concentrations of the hormone isolated from the blood. In view of the differences in sensitivity and specificity of mammary tissues corresponding to different species we considered it of interest to try the isolated strip of the lactating mouse mammary gland for the bioassay of oxytocin. We report here some features of the reliability and practicability of this bioassay.

Materials and Methods. Lactating mice between the 8th and 15th days after parturition were used. The litters were taken away from the mouse a few hours before the bioassay. The animal was killed by a blow on the head and the mammary gland was immediately dissected. A strip of approximately 20 × 2 × 3 mm was cut off and kept under Tyrode's solution.

One end of the strip was attached to a hook at the bottom of a microbath of 2.0-ml capacity. The microbath was immersed in a thermostatic bath at 37 ± 0.5° and filled with Tyrode's solution at the same temperature by a catheter which served to change fluids.

The other end of the strip was attached by a cotton thread to a Statham strain gauge 0.15 oz connected to a recording system (Sanborn Polyviso or Twin-viso).

The tension applied to the strip was adjusted by a micrometric screw. With these arrangements isometric records of mammary contractions were obtained (Fig. 1).

Experimental Methods. A. The influence of tension. In order to determine optimal working tensions, the influence of this parameter was studied by tension–response curves under

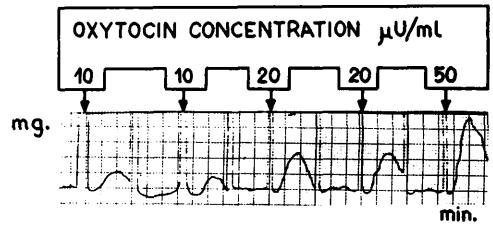

FIG. 1. Isometric recording of the contractions of an isolated strip of lactating mouse mammary gland; concentration of oxytocin was from 10 to 50 μU/ml.

[1] This research was supported by Grants M-67135 and M-6938 from the Population Council of the City of New York. We are grateful to Dr. de Freitas of the Dirección de la Lucha contra la Fiebra Aftosa for supplying us with mice.

[2] Deceased December 2, 1968.

[3] Recipient of fellowships, awarded by the Population Council, New York, Augusta & Petrus Hedlunds Stiftelse, Stockholm and Carl-Bertel Nathhorsts Stiftelse, Stockholm.

two constant doses, 50 and 100 μU/ml of oxytocin (Syntocinon, Sandoz). The initial resting tension, 100 mg, was increased by 50 mg at a time up to 500 mg. At any given tension the doses were repeated four times and the arithmetic means was plotted as a response. The best discrimination between these two doses of oxytocin and the highest responses were obtained with a tension of around 200 mg (Fig. 2). Although bigger differences between the responses could be obtained with higher tensions these were then so irregular that the reliability became considerably lower. Thus a tension of 200 mg was usually used but sometimes the tension had to be adjusted to lower values in order to get a stable base line.

B. The reliability. The reliability of the assay is presented in terms of the following criteria (accuracy, precision, sensitivity, and specificity), proposed by Borth (7). The accuracy was tested by recovery experiments. An arithmetic mean of 97% of added oxytocin was obtained.

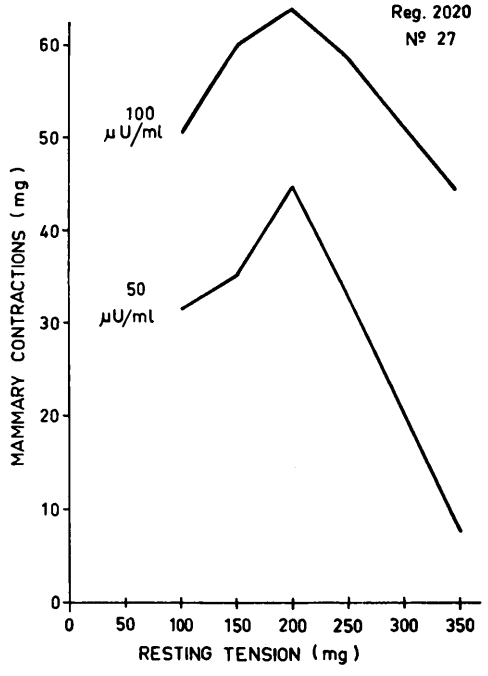

FIG. 2. Isolated strip of lactating mouse mammary gland; tension-response curves. Effect of increased resting tension upon mammary contractions was elicited by oxytocin at two constant doses.

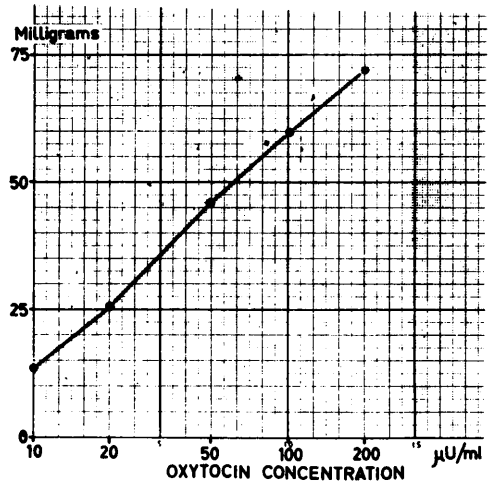

FIG. 3. Isolated strip of lactating mouse mammary gland; dose–response curve under a constant tension of 200 mg.

The precision, in series of duplicate determinations at a level of 100-200 μU/ml, was calculated according to the formula $s = (\Sigma d^2/2N)^{1/2}$ where s equals the standard deviation, d is the difference between pairs of duplicate values and N is the number of duplicates. The s value was 2.8 and the coefficient of variation (C) was 2.6%.

In other series, at a level of 100–200 μU/ml, symmetrical 4-point assays were made with 4 groups = 16 determinations and with statistical treatment according to the method of orthogonal contrasts [Gaddum (8)]. The arithmetic mean λ value was 0.046 or the L value, 24. These values indicate a high degree of precision. The fiducial limits at a probability level of $p = 0.05$ were 110–80% of the found value. Thus the method could distinguish between doses differing around 10% at the mentioned level.

The discrimination and the sensitivity were studied by dose–response curves under constant tension. The responses were plotted against the logarithm of the concentration of oxytocin. A steep slope was found in the range of 20–200 μU/ml and thus a good discrimination was encountered within this range. The threshold dose was usually 20 μU/ml and frequently was 10 μU/ml as shown in Figs. 1 and 3 (see also Table I).

TABLE I. Comparison of the Action of Different Agents on the Isolated Strip of Mammary Gland of the Lactating Rat and Mouse.

	Rat [Ryden and Sjoholm (5)]		Mouse (present work)	
Drug tested	Lowest dose giving a response (μg/ml)	Effect on the oxytocin response	Lowest dose giving a response (μg/ml)	Effect on the oxytocin response
Acetylcholine	10^{-6}–10^{-5}	Decreasing	2.10^{-2}	None
Adrenaline	10^{-5}–10^{-4}	None or decreasing	10^{2}[a]	Decreasing
Noradrenaline	10^{-5}	None	10^{2}[a]	Decreasing
Histamine	10^{-5}–10^{-2}	None	10[a]	None
Serotonin	10^{-2}–1	None	10^{3}[a]	None
Bradykinin	—	—	1	None
Oxytocin	10^{-6}	—	10^{-5}	—
Vasopressin	—	—	5.10^{-5}	—

[a] Adrenaline, noradrenaline, histamine, and serotonin gave no responses at these doses.

The specificity was tested by the action of different drugs on the preparation. The results are shown in Table I and compared with the results reported by Rydén and Sjöholm (5) for the rat. It appears that the mouse tissue in comparison with the rat tissue is very insensitive to the agents tested.

Discussion. It is generally agreed that the efficiency of a given method depends on two main factors—its reliability and its practicability [see Loraine and Bell (9)]. Regarding reliability, the mouse mammary gland has a higher precision than that of the rat mammary gland, which is $\lambda = 0.11$–0.14 (5), and a precision as good as that of the isolated rat uterus, which is $\lambda = 0.022$–0.14 (10). It is as sensitive to oxytocin as the superfused rat uterus (11) and the rat mammary gland (5). Its specificity is high and it is practically insensitive to acetylcholine, adrenaline, noradrenaline, histamine, and serotonin to which the rat mammary gland is sensitive. It is also insensitive to bradykinin. It is simple to perform and, having the recording system, it is inexpensive.

It may thus be stated that the isolated strip of the lactating mouse mammary gland is a satisfactory bioassay for oxytocin in terms of reliability and practicability. These conclusions were confirmed by a hundred bioassays carried out in this laboratory.

Summary. A method for quantitative assay of oxytocin using strips from the mammary gland of lactating mice has been studied. The method is suitable for determinations of small amounts of oxytocin. Doses as small as 10 μU/ml can be estimated. The precision of the method is high and it is highly specific.

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Received Oct. 8, 1969. P.S.E.B.M., 1970, Vol. 133.