A NOTE ON THE *IN VIVO* AND *IN VITRO* DOSE RESPONSE CURVES FOR THE RAT UTERUS

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Received January 16, 1953

THE use of orthodox Latin Square designs in obtaining dose-response curves for acetylcholine on the isolated rat uterus was described in an earlier communication.[1] This has been superseded by use of a Latin Square in which no repetition occurs in the order of treatment.

The experimental method has been extended to recording curves for the rat uterus *in situ* with the blood and nerve supply intact. A mid-line incision in the abdominal wall of virgin albino rats under pentobarbitone anæsthesia permitted the use of the abdominal cavity in place of the isolated organ bath, the animal resting on a warmed operating table. Contractions were recorded from the intact uterus by passing the recording threads through the wall of each horn, and running over suitable small pulleys to two orthodox frontal-type writing levers giving magnification of × 3. The cavity was filled with previously warmed de Jalon[2] solution and was drained by suction and refilled through specially mounted tubes whose open ends were protected by fine surgical gauze. Acetylcholine chloride solutions were made up in saline solution so that addition of 1 ml. gave graded doses of uniform logarithmic increment over the desired range.

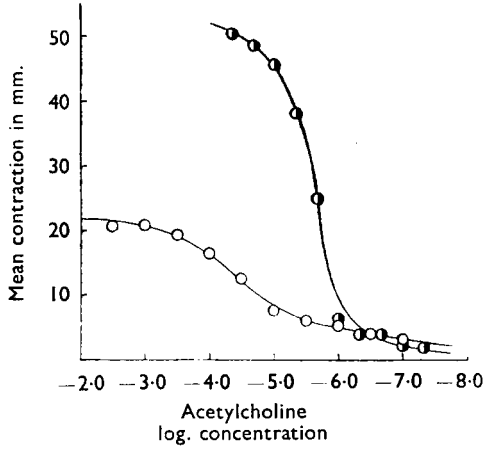

FIG. 1. Mean *in vivo* and *in vitro* dose reponse curves for acetylcholine. Recorded on four horns in each case, using a 10 × 10 Latin square with un-repeated precedents. Œstrus.

4 experiments were performed on the uteri of rats in each of the four main stages of the oestrous cycle, and the average contraction to each dose was calculated and plotted against the log. concentration (w/v) of acetylcholine base in de Jalon solution. Figure 1 shows mean curves during œstrus *in vivo* and *in vitro*, using different concentration ranges.

The main point of difference is apparent in the concentrations necessary to produce equal percentages of the maximal contraction in the two types of preparation. By extrapolation of the *in vitro* curve to the horizontal it is seen that peak contractions are obtained at concentrations of the order of 1 in 10,000, considerably more dilute than corresponding concentrations

required *in vivo*, Confirmation was sought in experiments *in vivo* and *in vitro* on the same uterus. Using identical orders of dosage and the same 8 drug concentrations, comparative dose-response curves of the type in Figure 2 were obtained. These show again the steeper *in vitro* curve, flattening off at relatively lower concentrations than the *in vivo* curve and illustrating the greater concentration required *in vivo* for 50 per cent. of the maximal contraction.

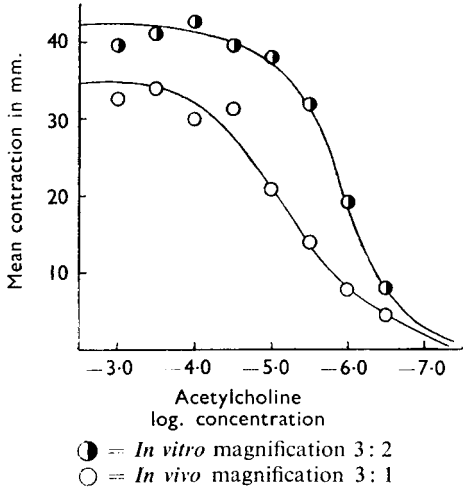

FIG. 2. *In vivo* and *in vitro* dose response curves recorded on the same uterine horn using an 8 × 8 Latin square with unrepeated precedents. Pro-œstrus.

In 3 isolated cases during *in vivo* experiments marked changes were noted in the character of contractions with approaching death. Although direct comparison between contraction magnitudes in the different preparations is not strictly valid, the larger size of isolated organ contractions is shown clearly in Figure 1. Figure 3 is reproduced from a kymograph tracing of contractions *in situ* to a constant dose of acetylcholine, made for purposes of variance estimation. It shows a consistent

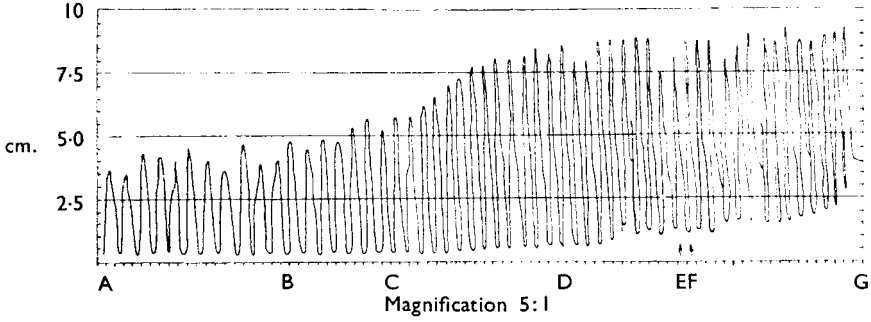

FIG. 3. Uterine contractions to 1 in 100,000 acetylcholine base (w/v). A-B normal contractions (66/hour); B-C slight increase in size (70/hour); C-D an increase in speed and amplitude (approx. 90/hour); D-G progressively poorer relaxation. E, F points of respiratory and cardiac failure respectively. Time trace, 30-second intervals.

increase in contraction size and frequency over the 22 minute period immediately before respiratory and cardiac failure. It is suggested that this phenomenon may have been due to the removal of normal sympathetic inhibition and the use of dihydrogenated ergot alkaloids is proposed in future experiments on this topic.

All results therefore suggest a quantitative difference in uterine response to acetylcholine between the isolated and the intact organ. In a later communication it is hoped to discuss the influence of the œstrous cycle on the response of both preparations to histamine and to acetylcholine.

A grant-in-aid from The Royal Society, for the purchase of animals, is gratefully acknowledged.

References

1. Fletcher, *Nature, Lond.*, 1950, **166**, 117.
2. de Jalon, Bayo Bayo and de Jalon, *Farmacoterap. Actual.*, 1945, **2**, 313.