

tricin. Analytical data on the methylated and acetylated compounds, and mixed melting points, gave further confirmation.

It cannot be assumed that tricrin is the only flavone occurring in lucerne, and we have noted certain lucerne flavone preparations possessing greater activity on smooth-muscle movements than the purified isolated tricrin.

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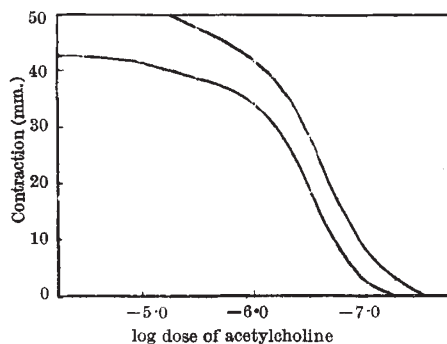
<sup>1</sup> Ferguson, W. S., Ashworth, de B., and Terry, R. A., *Nature*, **163**, 606 (1949).

<sup>2</sup> Anderson, J. A., *Can. J. Res.*, **9**, 80 (1933).

### Statistical Planning in Experiments on the Isolated Rat Uterus

THE use of statistical methods for obtaining estimates of error in biological assays was demonstrated by Schild<sup>1</sup> in a paper on the assay of histamine using guinea pig gut as test object. In 1943, Beauvillain<sup>2</sup> used the rat uterus for assays of oxytocic activity in pituitary preparations, and recently Holton<sup>3</sup> has applied Schild's methods to assays of posterior pituitary extract using the same preparation. Holton has shown that assays on the isolated rat uterus may be made more rapidly than on the isolated guinea pig uterus as prescribed in the *British Pharmacopœia*<sup>4</sup>, and the accuracy of her assays was greater.

In order to study the use of the rat uterus for assay purposes, tests were made using acetylcholine solutions as standards for reference. It was found that the cycle of contraction and relaxation was rapid in comparison with that for the guinea pig. The use of statistical planning was then invoked in order to determine the relationship between the dose of acetylcholine and the contraction produced. Using the balanced experimental design known as the Latin Square, it was found convenient to obtain up to ten contractions to each of the same number of doses, the experiment lasting between three and five hours. If the mean contraction to each dose is then plotted against the logarithm of the concentration of acetylcholine, a smooth dose-response curve is obtained.



Experiment 35: dose response curves for acetylcholine in the case of the rat uterus, using the  $8 \times 8$  Latin Square; recorded on the two horns of the same preparation

The advantage of the use of this design is in the minimization of the effects of variations in sensitivity of the preparation. The accompanying graph shows the type of curve obtained.

The experimental technique has been extended to the recording of curves showing the inhibitory action of histamine and some antihistamines and other substances on the responses of the rat uterus to acetylcholine. Details of these experiments will be published in due course. It is a pleasure to acknowledge the assistance of Mr. K. N. Chandler, of the Mathematics Department of this University, in their design.

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<sup>1</sup> Schild, H. O., *J. Physiol.*, **101**, 115 (1942).

<sup>2</sup> Beauvillain, A., *C.R. Soc. Biol., Paris*, **137**, 284 (1943).

<sup>3</sup> Holton, P., *Brit. J. Pharmacol.*, **3**, 328 (1948).

<sup>4</sup> "British Pharmacopœia", 813 (London: Constable, 1948).

### The Soil-Plate Method for Isolation of Fungi from Soil

RESEARCH on the ecological distribution of fungi in soil, in progress in this laboratory, has been facilitated by the use of a simple plating technique, whereby soil is distributed throughout a thin layer of nutrient medium. A soil plate is prepared by transferring a small amount of the soil to be examined into a sterilized Petri dish. 8–10 ml. of cooled medium is added and the soil particles dispersed throughout the agar. With sandy soils an adequate dispersal may be obtained by shaking and rotating the plate before the agar solidifies. If, however, the soil is very dry, or contains a high proportion of clay, it is preferable to mix the particles with a drop of sterile water in the plate, before the medium is added. The amount of soil used in the preparation of a soil plate varies with the soil investigated, and is determined by trial. With many natural surface soils about 0.005–0.015 gm. of soil has been found to give a convenient number of colonies on each plate. Czapek-Dox + 0.5 per cent yeast extract agar, acidified with phosphoric acid to pH 4.0, has been found a satisfactory medium for the growth and sporulation of many soil fungi and has been extensively used as an isolation medium. A microspatula made by flattening the end of a nichrome needle has been found useful for transferring soil and crushing soil aggregates.

Trials have shown that soil plates are easier to prepare and have several advantages over isolation plates prepared by either the direct method<sup>1</sup> or from soil dilutions. The direct method has been found unsatisfactory because a film of water soon surrounds soil particles placed on agar and favours excessive growth of bacteria even on acid media. Dilution methods, although widely used, are very laborious and are also selective with respect to species and groups of soil fungi isolated. Comparisons have been made of the numbers of species of fungi isolated from soil samples by soil plate and by dilution methods. The accompanying table records the numbers of species obtained from two different soils. Ten plates were prepared by each method; in the dilution method, isolation plates were prepared both from the soil suspension and from the residue of heavy soil particles left at the bottom of the container.