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An improvement on Vane's stomach strip preparation for the assay of 5-hydroxytryptamine

SIR,—Vane (1957) described a practical and sensitive preparation for the assay of 5-hydroxytryptamine (5-HT) using a strip of fundus from the rat stomach. Since then, this preparation has been widely used by others. Two difficulties were encountered in practical application: the slowness of the muscle to relax after responding to 5-HT and the fluctuation in the resting length of the muscle. These were overcome by stretching the muscle for 15 sec after each contraction. A working cycle required at least 4 min with this procedure.

Because of a need to assay over 30 samples of intestinal perfusate for 5-HT at one time, we have modified Vane's preparation to obviate the need for stretching the muscle after each contraction and also to shorten the time cycle.

The modifications we have made are:

(1) The manner of cutting the stomach strip was slightly different, five instead of six incisions being made, three from the fundus end of the opened-out plate of tissue alternating with two incisions made from the pyloric end of the plate.

(2) An auxotonic frontal writing pendulum lever was used. The small counterweight can be varied to give the required tension to the strip. The baseline position of the lever was set at about 10° below the horizontal and had a load of 2·5 g at this position.

(3) On setting up the preparation, it was left to stretch in the organ bath for 2 hr with the physiological solution flowing through at a rate of about 30 drops/min.

(4) A magnesium-free Krebs solution was used: (g/litre) NaCl, 6·92; KCl, 0·353; $CaCl_2$, 0·282; KH_2PO_4, 0·161; $NaHCO_3$, 2·1; glucose, 2·0. The solution was oxygenated with a 3% carbon dioxide and 97% oxygen mixture.

(5) A temperature of 39·5° for the organ bath was used. At this temperature the muscle responded and relaxed more rapidly than at 37°.

Under these conditions the sensitivity of response of the muscle to 5-HT was adequate, a 1 ng dose (in 6·5 ml bath volume) normally causing a recorded

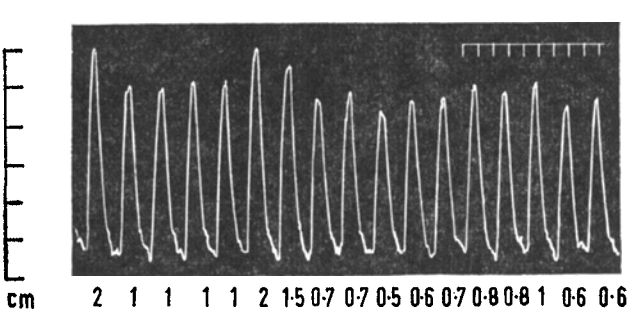

FIG. 1. Record of response of the stomach strip to various doses of 5-HT in ng added to the organ bath (volume 6·5 ml). Time in min. Scale in cm.

contraction of 3 cm. The muscle responded within 10 to 15 sec of adding the 5-HT to the bath and reached maximum contraction in 45 sec; relaxation to the baseline occurred 1 min after 5-HT was washed out; another dose of 5-HT could be added after 2 min. The preparation attained maximum sensitivity after 10 to 12 additions of 5-HT and its response remained consistent for at least 3 hr. Methysergide (Sandoz) in a concentration of 10^{-7} g/ml completely abolished the response to 10^{-9} g/ml of 5-HT.

Doses and concentrations are in terms of base.

A tracing showing graded response by the rat stomach strip preparation with the above modifications to various doses of 5-HT is shown in Fig. 1. We have not been able to determine precisely the cause for this improvement. However, we would like to share this experience with others who may wish to make use of this preparation for the rapid assay of 5-HT.

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Autoxidation of linoleic acid

SIR,—Mehta (1962) has earlier reported on a possible mechanism for the "autoxidation of linoleic rich oils in emulsion". In that report the emulsions studied contained poppy seed oil, safflower oil, and methyl linoleate ester, and were prepared using acacia and tragacanth as emulsifying agents. The mechanism indicated that in the early stages of autoxidation oxygen appeared to add to the double bond to form cyclic peroxides, which were then converted to conjugated dienoic hydroperoxides. The amount of conjugated trienes was insignificant in the oils and ester from which the emulsions were prepared and in all the systems after preparation. The trienes did not develop to any significant extent even after 42 days of autoxidation. The samples were stored at 25° ± 2 in ground glass stoppered bottles. Conjugated dienes and trienes were estimated before and after isomerisation by the method of Hilditch (1951).

We have now examined pure linoleic acid*. The surfactant, Brij 35†, 5 g, was used to obtain a solubilised and an emulsion system, containing linoleic acid 1·07 g and 1·33 g respectively, with distilled water to 25 ml. Samples were stored as before.

In both systems, the amount of conjugated dienes reached a maximum value after about 10 days and then the dienes were further oxidised. E (1%, 1 cm) at 268 mμ for the unisomerised sample (corresponding to the conjugated trienes) was 6·16 for the linoleic acid. The occurrence of trienes corresponded to the disappearance of the dienes. After 20 days of autoxidation, E (1%, 1 cm) at 268 mμ was 17·15 and 14·92 for the emulsion and the solubilised system respectively. The formation of significant amounts of trienes was thus indicated. The 30 day values indicated that the trienes were further autoxidised.

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