preparation is stimulated at low frequencies (0·1–1 Hz). Thus, in the presence of morphine, the evoked output per volley is low and almost constant at frequencies between 0·1 and 10 Hz, whereas without morphine the output per volley is usually much higher at low frequencies of stimulation and falls with increasing rates of excitation. When trains of 2–16 pulses, with intervals of 20–500 ms between pulses, are repeated at intervals of 10 s between trains, the output of acetylcholine caused by the later pulses in a group is lower than that due to the earlier pulses.

While there is so far no unequivocal evidence that morphine reduces the acetylcholine release evoked by the earlier pulses more than that induced by the later pulses, electrophysiological experiments are in favour of such an interpretation. When a single rectangular current pulse is applied to the myenteric plexus-longitudinal muscle preparation, a nerve action potential is obtained which, after a junctional delay of about 200 ms, is followed by a complex of spikes due to activity in the muscle cells (Kosterlitz & Lydon, 1969). Morphine (0·06–0·4 μM) depresses the muscle action potential without affecting the nerve action potential. The depressant action of morphine is less with trains of 16 pulses than with shorter trains or single pulses; this observation is in agreement with the assumption that the acetylcholine release evoked by the later pulses in a train is not affected by the depressant action of morphine.

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A further method of eliminating interfering compounds in the gas chromatographic determination of urinary methylimidazoleacetic acids.

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Estimation of the histamine metabolite 1-methylimidazole-4-acetic acid (1-MeIm4-AA) and its isomer 1-methylimidazole-5-acetic acid (1-MeIm5-AA) in urine by the method of Tham (1966) has presented difficulties in this laboratory due to the presence of interfering peaks on the gas chromatograms. A method of eliminating these has been described (Kelvin, 1968). We now describe an alternative procedure involving elution of urinary methylimidazoleacetic acids from Dowex 1 with a 0·1 M acetate buffer instead of the 0·5 M acetic acid previously used. This simple modification substantially improves the gas chromatograms (Fig. 1).

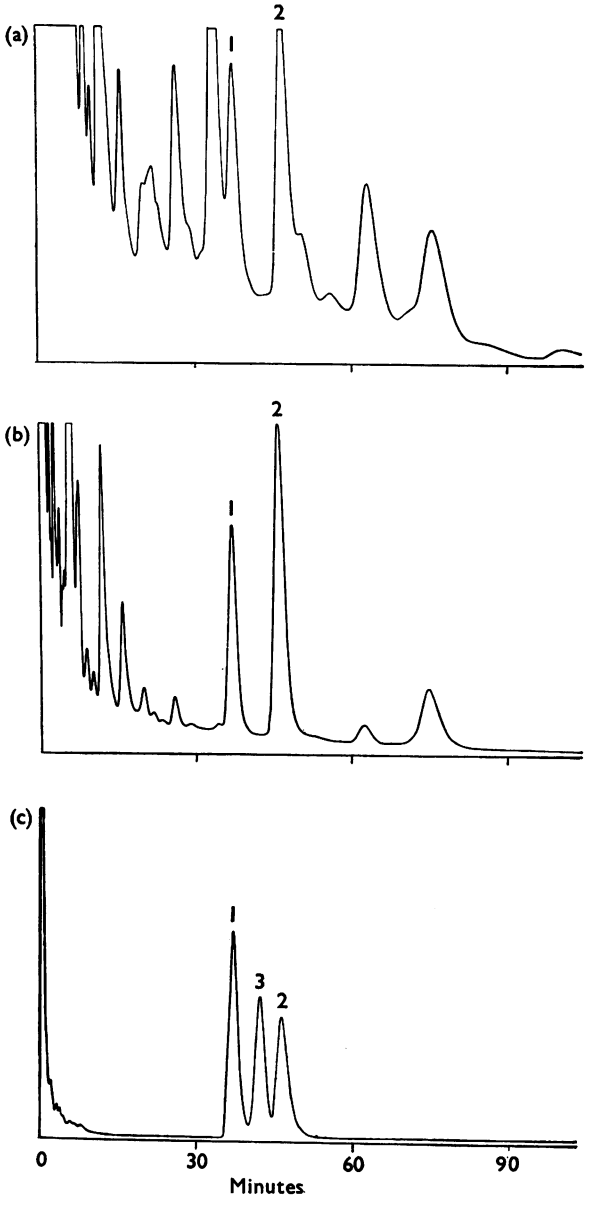

FIG. 1. (a): Gas chromatogram of a urine extract prepared as described in the text except that the methylimidazoleacetic acids were eluted from Dowex 1 with 0·5 M acetic acid. (b): Gas chromatogram of an extract of the same urine, prepared as described in the text. (c): Gas chromatogram of authentic 1-MeIm4-AA, methyl ester (peak 1), 1-MeIm5-AA, methylester (peak 2) and anthracene internal standard (peak 3) (not added to urine extracts (a) and (b)).

Urine specimens are run on Dowex 1–X8 ion exchange columns (Kelvin, 1968). The MeImAAs are eluted with 0·1 M acetate buffer (pH 4·0) at a flow rate of 25 ml/hr. One hr fractions are collected. The pH of the fractions falls sharply from about 6 to about 4. The fraction preceding this point and the subsequent two fractions are combined, 1 ml of concentrated hydrochloric acid is added and the solution is evaporated to dryness, esterified, neutralized, filtered, and the filtrate evaporated to 1·0 ml as described previously. This solution is mixed with 2 ml of pH 8·0 buffer (3 M K_3PO_4/1·5 M citric acid, 3/2) and shaken for 10 min with 5 ml of redistilled A.R. chloroform. After allowing the phases to separate, 4·6 ml of the lower (chloroform) layer is transferred to a tube containing the gas chromatographic internal standard (50 μg of anthracene disssolved in 0·5 ml of chloroform) and evaporated to 0·1 ml at 40°C under reduced pressure. An aliquot (10 μl) of this extract is analysed by gas chromatography as described previously.

Recovery of 1-MeIm4-AA and 1-MeIm5-AA added to urine is $87{\cdot}6 \pm 1{\cdot}4\%$ (mean $\pm$ S.E. of mean, $N=42$) and $87{\cdot}6 \pm 2{\cdot}1\%$ ($N=14$) respectively.

Using this technique the 24 hr excretion of 1-MeIm4-AA and 1-MeIm5-AA in eleven healthy adults (non-smokers) under standardized dietary conditions (Granerus, 1968) was found to be $2{\cdot}43 \pm 0{\cdot}5$ mg (range 1·51–3·21 mg) and $2{\cdot}53 \pm 1{\cdot}06$ mg (range 1·29–4·83 mg) respectively (mean $\pm$ S.D., $N=11$). The values for 1-MeIm4-AA are remarkably similar to those obtained with a thin-layer chromatographic method of assay by Granerus (1968), who found $2{\cdot}31 \pm 0{\cdot}30$ mg (range 1·7–2·8 mg) in eleven non-smokers.

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The mechanism of a drug effect in man studied by a multivariant technique.

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Drug actions are usually analysed by excluding from experiments all variations which are not of prime interest. This method is indispensable to show how systems can respond. It cannot show whether responses are equivalent to those which arise spontaneously in nature, nor measure their contribution amongst other natural variations. The method is weakest with self-regulating systems, since the search for a one-to-one response usually involves the disruption of feedback links.

When a function is determined by several self-regulating systems, the complex may set in unstable equilibria, from which it tends to be disturbed unpredictably by diverse stimuli (Rashevsky, 1960). Ordinary experiments then yield conflicting results on repetition, or when attempts are made to show reversibility of an effect.

A technique is presented in which concomitant variations are encouraged, not excluded, and self-regulation permitted to occur. Numerous relevant variables are