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.

REFERENCES

GRANERUS, G. (1968). Urinary excretion of histamine, methylhistamine and methylimidazole acetic acids in man under standardized dietary conditions. *Scand. J. clin. Lab. Invest.*, **22**, suppl. 104, 59–68.

KELVIN, A. S. (1968). Determination of methylimidazoleacetic acids in human urine by gas chromatography. *J. Pharm. Pharmac.*, **20**, 659–661.

THAM, R. (1966). Gas chromatographic analysis of histamine metabolites in urine. Quantitative determination of ring methylated imidazole-acetic acids in healthy man. *J. Chromat.*, **23**, 207–216.

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

measured synchronously, and their small changes subjected to covariant analyses (Scheffé, 1953) using a computer. The logical steps involved in defining causal relationships are described. The results are expressed not as reproducible, or reversible, relationships between variables, but as reproducible probabilities of finding such relationships.

The method has potential value in the study of drug effects on intact natural systems.

REFERENCES

RASHEVSKY, N. (1960). *Mathematical Biophysics*, p. 69. 3rd revised edition, vol. 2. New York: Dover.

SCHEFFÉ, H. (1953). A method for judging all contrasts in the analysis of variance. *Biometrika*, **40**, 87–104.

The multiple emulsion formulation for the slow release of drugs.

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Multiple emulsions are water-in-oil emulsions which are redispersed in a second aqueous phase. Such emulsions have the advantage of being much less viscous than the primary water-in-oil emulsion and can be easily injected through a fine bore needle. The use of such emulsions as an alternative to the Freund type antigen-carrying adjuvant (water-in-oil emulsion) was suggested by Herbert (1965). Such a formulation for the slow release of drugs has been developed in our department since 1965.

The release of drugs from multiple emulsions is dependent on at least two types of mechanism, the break-up of larger particles and the osmotic gradient between the internal and the continuous aqueous phases. It can be influenced by altering three parameters, the osmotic gradient between the two aqueous phases, the internal phase volume and the concentration of the detergent necessary to form the primary water-in-oil emulsion. The release rate can be assessed by *in vitro* and *in vivo* methods.

The preparation of multiple emulsions will be demonstrated, as well as their naked eye and microscopic appearance. Examples of slow release of drugs from such emulsions will be shown using an *in vitro* technique as well as results of biological assay. The effect of manipulating the three parameters on release rate will be illustrated.

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REFERENCE

HERBERT, W. J. (1965). Multiple emulsions, a new form of mineral-oil antigen adjuvant. *Lancet*, **2**, 771.

A method of investigating ureteral activity in the rat.

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Although the existence of a nerve supply to the ureter has been demonstrated (Wharton, 1932; Gruber, 1933; Lapides, 1948) the nature and function of this nerve supply is still in doubt. In an electron microscopic examination of the upper ureter of