A note on the analysis of disodium cromoglycate (Intal) in human urine

The technique for the assay of disodium cromoglycate in urine has been described (Moss, Jones & others, 1971). It has been of importance in this laboratory in clinical studies of the relation between airways obstruction in respiratory disease, and disodium cromoglycate uptake after aerosol administration (Benson, Curry & others, 1973). Urine is mixed with 30% formic acid, and the mixture is passed through columns of Amberlite CG 4B Type II resin. Most urinary constituents, but not cromoglycate, come through the column with the dilute formic acid. Cromoglycate is later eluted with 98% formic acid, and this fraction is evaporated to dryness. The residue is dissolved in an aqueous potassium carbonate solution, and the solution is divided into two equal portions. One portion is heated, converting the cromoglycate into a bis-acetophenone. Both portions are then diazotized and coupled with *p*-nitroaniline to give a coloured solution. The heated solution gives the most intense colour, the unheated tube providing a blank for each sample. The difference in colour relates to the amount of cromoglycate present.

We were unable to reproduce the recovery, precision, and accuracy reported by the original authors, largely because of variations in the properties of different preparations of the resin. We have found that the various problems involved can be overcome by the use of a radioactive internal standard. Tritiated disodium chromoglycate (190 μCi mg^{-1}) was obtained through the courtesy of Fisons, Limited, Loughborough, U.K. Our modification of the method involved addition of 0·05 μg of this material to each urine sample (samples contained 1–10 μg ml^{-1}), and determination of the appropriate recovery correction for each sample from the yield of radioactivity in the final solution used in the colorimetric measurements. A small calculation correction was needed to allow for the 0·05 μg.

The value of this modification is shown by the data in Table 1. A calibration line was prepared from a series of 15 standards in the range 1–12 μg ml^{-1}. The best straight line, together with a series of statistical assessments, was calculated by the method of least squares. The modification resulted in a line which was more satisfactory by all of the statistical assessments. The modification also led to a steeper calibration line, with an intercept nearer to zero.

Apart from their relevance to cromoglycate analysis, the data in Table 1 are of interest as a demonstration of the internal standard principle.

Table 1. *Statistical evaluation of disodium cromoglycate analysis data fitting the equation* $y = a + bx$.

	Original method	Including radioactive internal standard
Correlation coefficient (r)	0·675	0·992
Slope of regression line (b)	0·022	0·081
Standard deviation of b (Sb)	0·0075	0·0033
Intercept at y axis (a)	0·130	0·025
Standard deviation of a (Sa)	0·048	0·021
Standard deviation about regression line (S)	0·078	0·034
Coefficient of variation (V)	0·310	0·071

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The transport number of noradrenaline as a function of pH

A recent paper by Bevan, Bradshaw & others (1973) has expressed the view, supported by a theoretical calculation, that the transport number of noradrenaline in a solution at pH 5·0 is lower than that at pH 3·1, because of the addition of sodium and hydroxyl ions to the noradrenaline solution during the process of pH adjustment from 3·1 to 5·0. It appears that the authors have overlooked the consideration that because pH 5·0 is still in the acid range, there will be virtually no excess or free hydroxyl ions. Instead, all the added hydroxyl ions (from NaOH) will combine with the removed (buffered) hydrogen ions to form water. A number of sodium ions will be added to the noradrenaline solution equivalent to the number of hydrogen ions removed. This means that some hydrogen ions are replaced by much less mobile sodium ions when the noradrenaline solution is raised from pH 3·1 to 5·0, which would tend to increase the fraction of current carried by noradrenaline ions. In this pH range there is little change in percent of noradrenaline in the cationic form (Frederickson, Jordan & Phillis, 1972). Thus the transport number of noradrenaline at pH 5·0 should be the same or greater than the transport number of noradrenaline at pH 3·0.

The literature is of little use in deciding this issue because of the very wide range and overlap of values of transport numbers for noradrenaline ejection from micropipettes determined empirically (see below).

Source	pH	Concentration of noradrenaline	Transport number
Bradley & Candy (1970)	5·5	1% (≃0·05M)	0·09 (average)
	not stated	10% (≃0·5M)	0·19 (average)
Krnjević & others (1963)	3–4	1·7M	0·34, 0·37, 0–0·07, 0–0·02*
Hoffer & others (1971)	not stated	0·5M	0·05–0·30 (range)

*Values for four different pipettes

In our own experience, using solutions of (−)-noradrenaline bitartrate (Sigma, 0·2M) at pH values of 4·5, 5·0, 5·5, 6·0, 7·4, there is little difference between the distribution of effects (i.e. percentage of cortical cells depressed) or depressive potency of noradrenaline.

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