A Modification of Current Micro-methods for the Estimation of Diodone (3:5-di-iodopyridone-*N*-acetic acid)

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The investigation of renal function in health or disease demands a reliable method for the estimation of diodone in blood and urine. The methods of Alpert (1941), Flox, Pitesky & Alving (1942), Corcoran & Page (1943) all involve the use of bromine to split iodine from the diodone molecule and to convert this into iodate. Having had a fairly wide experience in the use of diodone in the estimation of renal blood flow and tubular mass in normal men and patients, as well as in animals, we have re-examined the various methods, and now present a procedure which, by a suitable combination of the best features of the other methods, combines accuracy with speed. By this method it is possible to perform the analysis of the blood and urine samples from a clearance test in about 30 min., whereas Alpert's method takes the greater part of a day. In the procedure here presented the diodone iodine is converted by a bromide-bromate mixture into iodate; the excess Br_2 removed by sodium formate and the iodate estimated colorimetrically by acidification and the addition of excess KI.

METHOD

Reagents

Bromide-bromate mixture. 10 g. KBrO_3 (A.R.) and 80 g. of anhydrous NaBr (A.R.) are dissolved in 100 ml. of distilled water, 40 ml. of 85% H_3PO_4 are added and the mixture shaken and allowed to stand for 24 hr. before use. The solution is stored in a glass-stoppered bottle and kept in the refrigerator when not in use.

Sodium formate solution 10%. 10 g. of the A.R. solid are made up to 100 ml. of solution with 80% ethanol.

Alkaline potassium iodide. 50 g. KI (A.R.) are made up to 100 ml. of solution with N-NaOH .

Phosphoric acid 85%. A.R. grade acid of specific gravity not less than 1.7.

Procedure

2 ml. of a tungstic acid filtrate of plasma, or urine diluted to the plasma concentration of diodone, are placed in a $5 \times \frac{5}{8}$ in. hard-glass test-tube and 1 drop of the bromide-bromate mixture added. The tube is shaken until the solution is an even yellow, and then placed in a boiling water-bath for 3 min. It is removed, and, in order to speed up the procedure, cooled in iced water. 2 ml. of 10% Na-formate are added from a blow-out pipette in such a manner as

to wash down the sides of the tube. The tube is shaken vigorously for a few seconds and then allowed to stand at laboratory temperature for 15 min. with occasional shaking. 2 drops (0.1 ml.) of 85% H_3PO_4 are added and the tube shaken until the *schlieren* figures are no longer visible in the solution. One drop (0.05 ml.) of alkaline KI is then added and the solution mixed. The colour is allowed to develop for 10 min. at laboratory temperature, at the end of which time it is estimated in a photo-electric colorimeter, using a filter of blue Chance glass (O.B.2). A calibration curve is prepared from aqueous solutions of diodone over the range of 0–10 mg. of diodone/100 ml.

It is essential to leave the tubes for 15 min. after the addition of the formate in order to ensure that all the residual Br_2 has been discharged. If any noticeable colour is developed in a blank estimation, each reagent should be checked, and, if necessary, made up afresh.

RESULTS

The ideal method should allow a quantitative recovery of iodine from diodone. We performed the estimation on standard aqueous solutions of recrystallized 3:5-di-iodopyridone-*N*-acetic acid freed of diethanolamine and diethylamine, and in Table 1 we show the recovery by various methods, using as

Table 1. *Amount of diodone (3:5-di-iodopyridone-*N*-acetic acid) recovered from aqueous solutions by different methods*

Amount of diodone present (mg./100 ml.)	Diodone recovered (mg./100 ml.)				
	Composite method	Corcoran & Page	Flox, Pitesky & Alving	Colorimetric modification of Alpert's method	Composite method using oxalate instead of formate
0	0	0.30	0.30	0.50	0
0.1	0.10	0.60	0.60	0.60	0.13
1.0	1.00	1.40	1.10	1.20	1.10
2.0	2.00	2.40	2.50	2.00	2.00
4.0	4.00	4.80	4.70	4.80	4.80
5.0	5.05	5.70	—	5.70	5.25
6.0	5.97	6.50	6.36	—	6.30
7.0	6.94	—	7.40	7.60	7.20
8.0	8.08	8.20	—	—	8.40
10.0	10.00	10.50	11.50	10.00	11.00

standard the colour developed by solutions of iodate containing the equivalent amount of iodine. Fig. 1 shows the recovery of the acid added to samples of normal human urine.

Fig. 1. Recovery of diodone (3:5-di-iodopyridone-N-acetic acid) added to normal human urine.

A point to be established is the best method of removing the bromine. Boiling it off, although apparently the most satisfactory method, does not, as our examination of Alpert's method shows, result in complete removal. Moreover, it is too tedious for routine use; the analyses of the urines and blood from one patient take about a day. Mass methods of boiling in a brine-bath lead to still more unsatisfactory results.

We have tried a number of reagents for this purpose and find that sodium formate is the best (Table 1). Oxalate is the only other substance that approaches it in efficiency. It should be noted that one or two of the other methods, if used on both blood and urine diluted to the same range, would give figures for clearances which would be reasonably accurate, since the blank value appears to be fairly constant.

Plasma is always liable to present difficulties. Occasionally a perfectly clear filtrate will, after addition of bromine and subsequent clearing, become quite cloudy. This cloudiness, due presumably to traces of protein, makes a colorimetric estimation

impossible, and a titrimetric as well, since the bromine can never be completely removed under these conditions. Indeed the titrimetric application of Alpert's procedure has invariably given more inaccurate results than the colorimetric estimation when the filtrates are anything but perfectly clear. Titration of standard aqueous solutions treated by the Alpert method appears to give results slightly inferior to those obtained by colorimetry. The cloud is more usual in pathological material, but it sometimes occurs in normal human and animal plasma. We have therefore presented (Table 2) recoveries from various filtrates so that the effect of using a different protein precipitant can be assessed. It will be seen that the use of a detergent, suggested by Corcoran & Page (1943), increases the error, doubtless because its presence appears to give a much finer precipitate.

Table 2. Amount of diodone (3:5-di-iodopyridone-N-acetic acid) recovered from normal human plasma deproteinized by various methods

Amount of diodone added (mg./100 ml.)	Diodone recovered (mg./100 ml.)			
	Deproteinizing agent			
	Tungstic acid	Trichloro-acetic acid	Zinc hydroxide	Zinc hydroxide + detergent
0	0	0	0.30	0.50
1.0	1.00	1.00	1.10	1.10
2.0	2.04	2.00	2.20	2.20
4.0	4.00	4.00	4.48	5.00
5.0	4.92	4.90	5.50	6.50
6.0	5.82	6.30	—	—
8.0	8.16	7.60	9.62	9.60
10.0	10.25	9.50	12.00	11.50

Since trichloroacetic acid filtrates have not in our experience turned cloudy, and since, as Table 2 shows, they give presentable recoveries, they may be used when difficulty is encountered with other filtrates.

DISCUSSION

It appears almost certain that the amount of bromine present, whether as saturated bromine water (as used in the method of Corcoran & Page, 1943), or as a bromine mixture (Flox, Pitesky & Alving, 1942), is sufficient for the oxidation of the quantities of diodone iodine present, and, as the figures bear out, the real difficulty is in getting rid of the traces of residual bromine. In Alpert's method, where bromine is added directly, a fairly large residual blank is left, and perhaps accounts for the point he noted that the smallest amounts of diodone gave high readings. A bromide-bromate mixture is more satisfactory than a solution of bromine as it ensures that the amount of Br_2 added is precisely the same each time, which is unlikely with a bromine solution, even though it is in contact with liquid bromine.

Sendroy & Alving (1942) point out that increasing the amount of KI added to develop the colour increased the light absorption of the solution. Adding ethanol has the same effect. However, we find that this is not the case in the reaction used here; increasing the amount of KI or the concentration of acid or ethanol has no effect on light absorption, but the presence of ethanol increases the stability of the iodine colour, both in respect to time and to light. For this reason we have used an ethanolic solution of sodium formate as our clearing agent.

The ideal colour filter for photoelectric estimation of the iodine colour has been shown by Sendroy & Alving (1942) to be Corning glass Violet Ultra no. 586. This glass is not available in this country, and has the disadvantage of having so low a transmission as to require a very much more powerful

source of light than is usual with photoelectric instruments. This latter condition also applies to the violet-blue Chance glass O.B.1 and the Wratten C filter, although, if the illumination of the instrument is adequate, one of these filters is to be preferred to the O.B.2 glass. We have, however, routinely used the Chance O.B.2 filter and have found it quite satisfactory and sufficiently sensitive over the range required.

SUMMARY

Results of a study of the methods in current use for the estimation of diodone are presented together with details of a method incorporating the best features of previous methods.

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