A NOTE ON THE DETERMINATION OF IODINE IN BIOLOGICAL MATERIAL

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(Received for publication, April 13, 1936)

In a previous communication (1) we described a modification of the Leipert technique (2) for the microdetermination of iodine by means of which we were able to determine amounts of iodine up to 50 micrograms with fair rapidity and with an error not exceeding 10 per cent. With continued use of the method we found it frequently impossible to recover satisfactorily amounts of iodine with which we formerly had obtained good results. At first it was thought that the relatively large excess of phosphorous acid which we employed for reduction of the excess dichromate and of the oxidized iodine might cause incomplete recoveries by reducing some of the iodic acid to hydriodic acid instead of liberating the iodine quantitatively. Attempts were made to control the amount of excess phosphorous acid, but this procedure did not improve the results. It was then found that iodine added as iodide to a reduced digestion mixture containing excess phosphorous acid could be distilled more rapidly than could iodine present as iodate. The difficulty, therefore, lay not in the formation of hydriodic acid, but in the slow reduction of iodic acid by the phosphorous acid. A number of reducing agents were substituted for phosphorous acid but for one reason or another proved undesirable. Antimonious and ferrous salts formed a difficultly soluble precipitate which adhered to the digestion flask. Cuprous oxide and stannous oxide liberated sulfur dioxide from the sulfuric acid and thus prevented the recovery of iodine. Nascent hydrogen was unsatisfactory, and oxalic acid, although giving good iodine recoveries, was deemed undesirable because of the danger attending liberation of carbon monoxide during the distillation. Because of recent pub-
lished work (3) with Leipert's original method, we again tried to use arsenious acid as the reducing agent, this time carefully controlling the temperature during the distillation, but we again found that under the conditions necessary for complete recovery of iodine, a variable but appreciable "blank" was always to be found in the distillate. This finding has been confirmed by other workers.¹

We then undertook a study of a variety of conditions under which it seemed that it might be possible to increase both the speed of reduction of iodic acid by phosphorous acid and the rate of distillation of the resulting iodine. It was finally found that the addition of more water to the digestion mixture before distillation hastened the reduction of iodic acid, and that the liberated iodine could be distilled rapidly from such a boiling mixture by means of a current of air.

Two other changes in procedure have been made, both of which shorten the time required for a single determination. It has been found that acetic acid and other volatile substances liberated in the digestion may be removed in most cases by a single heating of the digest to 195–200° after oxidation is complete. At this temperature there is no loss of iodine from blood or urine with or without the addition of such compounds as diiodotyrosine and sodium monoiodo methyl sulfonate. It also has been found that the addition of sodium bisulfite to the distillate may be omitted without altering the final results. In a series of duplicate determinations on blood, the omission of bisulfite from one of each pair resulted in no significant variation.

The procedure which we recommend is as follows: The material to be analyzed is digested with potassium dichromate and sulfuric acid in the presence of a small amount of cerous sulfate in the manner described in our previous paper (1). There will be no loss of iodine if the digestion is carried out as rapidly as physical conditions will permit. The digestion mixture is then heated over a free flame to a temperature of 195°. If the odor of acetic acid, bromine, or chromyl chloride is still detectable after this treatment, it is necessary partially to cool the digest, to add 10 to 20 cc. of water, and again heat until the temperature reaches 195°. The digest is now cooled and water added, the amount varying with

¹ Personal communications from Dr. Bernard Brodie; New York University, and Dr. Emil J. Baumann, Montefiore Hospital, New York.
the amount of reagents which have been used in the digestion. If
50 cc. or less of concentrated sulfuric acid were used, 50 cc. of water
should be added; with more than 50 cc. of sulfuric acid, a volume of
water equal to the acid used is required. The digest is now
treated with phosphorous acid. We use an 84 per cent solution,
by weight, of this reagent, of which about 5 cc. are needed to
reduce the average blood digest. Failure to recover any iodine at
the end of the procedure usually indicates that an insufficient
amount of phosphorous acid was employed; in such a case, an
additional 1 or 2 cc. of phosphorous acid and 30 to 40 cc. of water
should be added and the mixture redistilled. It has been found
that a large excess of phosphorous acid has no effect upon iodine
recovery.

After the digestion flask and the receiver are attached to the
apparatus, the iodine-containing digest is heated and a slow cur-
cent of air, just sufficient to mix the digest, is drawn through the
apparatus. The air is first washed by bubbling through a 4 inch
column of dilute sodium hydroxide. When the digest begins to
boil, the suction is increased so that the air current produces a
rapid, but not violent, bubbling through the receiver (50 to 75
liters per hour). The boiling and aeration are continued for 15
minutes if less than 20 micrograms of iodine are present, or for 30
minutes with amounts of iodine from 20 micrograms to 1 mg.
With quantities of iodine exceeding 50 micrograms, a second alka-
line receiver attached in series with the first is required. At the
end of the distillation, from 30 to 40 cc. of liquid should have con-
densed in the first receiver and the temperature of the boiling digest
should not exceed 150°. The temperature of the digest is ascer-
tained by means of a short thermometer of 110–170° range at-
tached to the central aeration tube by means of a small rubber
band. If the temperature has reached 150° before the distillation
is finished, the flame under the flask should be lowered so that a
temperature of 145–150° is maintained during the remainder of the
aeration period. The distillate is made acid without previous
treatment with sulfite, brominated, evaporated, and treated as
described in our previous paper. For amounts of iodine of 10
micrograms or less, the solution should be evaporated to a final
volume of 2 cc.; with amounts of iodine as large as 1 mg., the vol-
ume may be as much as 25 cc.
There are some further points in connection with the procedure which merit brief discussion. It has been found by Harvey (4), and confirmed by the present writers, that the amount of iodate reacting with KI and starch in a volume of 1 cc. must be equivalent to 0.06 microgram of iodine in order that the color be visible. This fact must be taken into consideration in the calculation of results, both in standardizing thiosulfate solutions and in the determination of the iodine content of unknown solutions. To obtain an absolute factor for the standard thiosulfate solution, the equation used is:

$$1 \text{ cc. Na}_2\text{S}_2\text{O}_3 = \frac{\text{micrograms } I_2 \text{ as } K\text{IO}_3}{\text{cc. thiosulfate used}} - 0.06 V$$

where $V$ equals the volume of the solution in cc., after titration. In the determination of unknowns, the following calculation is made:

$$\text{Micrograms } I_2 = (\text{factor } \times \text{ cc. thiosulfate}) + 0.06 V$$

The volume correction may be omitted entirely if the thiosulfate is standardized against an amount of iodate containing within 10 per cent of the amount of iodine to be determined in the unknown material. In such a case the standardization and subsequent titrations must be carried out in the same volume of liquid. It is expedient to standardize thiosulfate solutions in the presence of approximately that amount of sodium or potassium sulfate which will be present in the final titration of unknowns, since it has been found that the presence of salts influences to some extent the sensitivity of the titration.

When KI is added in the final titration, care must be taken to keep the amount added within a fairly narrow range, as it appears that significant variations in the titration figure may be produced by using uncontrolled amounts of this reagent. The following figures show the effect of varying amounts of KI on the titration of 1 cc. of 0.0001 N KIO₃.

<table>
<thead>
<tr>
<th>KI added (mg)</th>
<th>Thiosulfate used (cc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.088</td>
</tr>
<tr>
<td>10</td>
<td>0.094</td>
</tr>
<tr>
<td>20</td>
<td>0.099</td>
</tr>
<tr>
<td>50</td>
<td>0.105</td>
</tr>
</tbody>
</table>
The KI may be added either in solution or in the dry form; we prefer the latter because the instability of KI solutions necessitates their daily preparation, with resulting inconvenience and needless waste. The finely granular KI may be measured by means of a small aluminum spoon, constructed to hold approximately 20 mg., with sufficient accuracy to insure consistent titration values.

Employing the method as described, we have determined amounts of iodine ranging from 0.2 to 1000 micrograms in over 100 samples of material with an error not exceeding, and usually less than, 10 per cent.

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