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The Iodometric Micro-determination of Arsenic in Biological Material

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The method of Allcroft & Green [1935; see also Kiese, 1937] for the titrimetric estimation of arsenic in tissue digests yielded satisfactory results with 0·05–1 mg. As. As is separated as AsH_3, which is passed into $AgNO_3$ solution, the arsenite formed being titrated with dilute I_2. It has been found possible to adapt this technique for 5–50 μg. As by reducing the volume of the solution to be titrated till the I_2 required for a distinct colour change is a small fraction of that required to oxidize the arsenite.

Tubes have been constructed in which 1·5 ml. N/50 $AgNO_3$ are sufficient for efficient absorption of AsH_3 at the rapid rate of passage of H_2 necessary for complete separation of As as AsH_3. Titration with N/200 I_2 is carried out in the absorption tube. Under the conditions of the experiment, a distinct colour change is produced by 0·003 ml. I_2, although the amount may vary slightly with the observer and with the lighting. The microburette described by Conway [1939], with a ground-glass joint in place of the rubber stopper in the standard model, is used in the titration. If the reservoir is shielded from light, there is little change in the I_2 over intervals of weeks.

The procedure described has been found reliable in thousands of routine estimations carried out by several workers in these departments and elsewhere. No difficulty has been encountered in adapting it to many types of material.

OUTLINE OF METHOD

Organic matter is destroyed by digestion with a mixture of HNO_3, $HClO_4$ and H_2SO_4. The digest is treated with $(NH_4)_2C_2O_4$ to remove interfering N compounds. When the biological material is rich in As and the sample taken for analysis is small ('Digestion—small scale'), AsH_3 is evolved directly from the digest. With large samples of material ('Digestion—large scale'), As is distilled from the digest as $AsCl_3$, and AsH_3 is evolved from the acid distillate. The procedure thenceforth is the same in both cases. After adjusting the acid concentration and adding $CuSO_4$ and $SnCl_2$ to catalyze H_2 evolution, Zn is introduced into the As-containing solution and the gas liberated is passed through 10N NaOH and N/50 $AgNO_3$. The dimensions of the $AgNO_3$ tube are shown in Fig. 1. To avoid risk of breaking the tip of the Conway burette, the gas inlet (thick-walled capillary tubing of 1 mm. internal diam.)

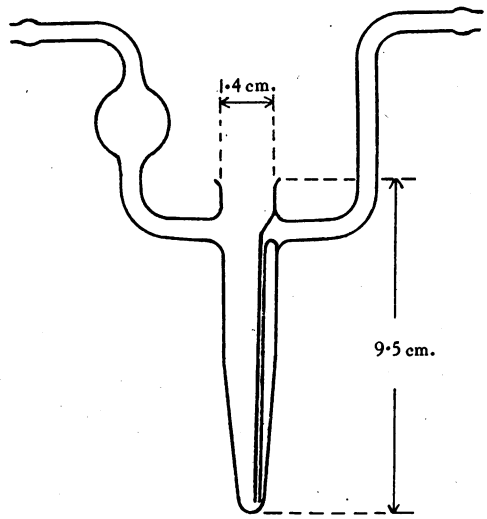

Fig. 1. The absorption tube.

should be situated close to the wall, as shown. Before titrating the arsenite in the absorption tube, solid KI is added to remove excess Ag^+ which would otherwise prevent the appearance of the I_2-starch colour. This is followed by $NaHCO_3$ to remove HI produced in the titration, and starch indicator. The tip of the Conway burette is immersed in the solution, which is stirred during the titration by passing air through the gas inlet of the absorption tube.

Since a large number of digestions can be carried out simultaneously, the time involved per sample at this stage of the determination is negligible. By working in serial fashion with six sets of apparatus, estimations can be put through the stage of AsH_3 separation and arsenite titration at about 10 to the hour. When preliminary separation of As as $AsCl_3$ is necessary, the distillations can be done at the rate of one every 10 min. by using duplicate apparatus.

EXPERIMENTAL

Reagents

All reagents should be of analytical or As-test quality

HNO_3, conc.	$SnCl_2$, 40% in conc. HCl
$HClO_4$, 60% (w/w)	$CuSO_4$, $5H_2O$, 5%
H_2SO_4, conc.	Zn shot, freed from dust and extra large shot
HNO_3, fuming	H_2SO_4, 20% (w/v)
$(NH_4)_2C_2O_4$, sat. solution	KI, cryst.
$FeSO_4$, $7H_2O$, cryst.	$NaHCO_3$, powdered
KBr, cryst.	Starch solution, fresh
NaCl, cryst.	I_2, N/200
$AgNO_3$, N/50	Standard arsenite solution, prepared from As_2O_3
NaOH, 10N	
Glycerine	

Procedure

Digestion—small scale. Up to 5 ml. blood, 10 ml. urine or 2 g. tissue are measured into a 100 ml. Kjeldahl flask, and conc. HNO_3 (5 ml.), $HClO_4$ (3 ml.), conc. H_2SO_4 (10 ml.) and two or three clean glass beads are added. The flask is warmed, with shaking, over a naked flame till brown fumes are given off, and heating is continued on a digestion stand over a very small flame (to avoid bumping) till they are no longer evolved. The contents are then gently boiled till H_2SO_4 fumes appear, and the clear liquid becomes colourless. Heating is continued for 10 min. If the mixture chars at any time, the flask is cooled slightly and a few drops of fuming HNO_3 added.

When digestion is complete, the flask is allowed to cool and $(NH_4)_2C_2O_4$ solution (5 ml.) is added. The liquid is brought to the boil and heating continued till white fumes appear. Distilled water (5 ml.) is added to the cooled liquid, which is again brought to the boil, and heating continued till 5 min. after the first appearance of white fumes.

Control experiments, with reagents alone, are carried out in exactly the same manner. It is important that heating of the digest at each stage should be continued for at least the period specified.

Digestion—large scale. Tissue. Up to 20 g. of minced, moist tissue are wrapped in very thin paper and placed in a 300 ml. Kjeldahl flask (with ground joint). After the addition of conc. HNO_3 (30 ml.) and two or three clean glass beads, the flask is set aside overnight.

Blood. Up to 30 ml. blood are treated as described above.

Urine. To not more than 150 ml. urine one-fifth its volume of fuming HNO_3 and two or three glass beads are added, the liquid evaporated to about 10 ml., and the digestion then carried out.

$HClO_4$ (15 ml.) and conc. H_2SO_4 (20 ml.) are added to the flask, which is warmed cautiously with shaking over a naked flame till reaction sets in, and is then placed on a digestion stand. After the first violent reaction subsides, the burner is lit and the contents brought to the boil. When digestion is complete, 20 ml. of $(NH_4)_2C_2O_4$ solution and of water are added. It may be necessary to make several additions of fuming HNO_3 before digestion is complete.

$AsCl_3$ *distillation.* This is necessary only after large-scale digestion. A delivery tube is connected, with conc. H_2SO_4 as lubricant for the ground-glass joint, to a 150 ml. Fresenius flask containing distilled water (40 ml.) immersed in ice-water [see Bang, 1925].

Distilled water (50 ml.) is added to the Kjeldahl flask and the contents are cooled thoroughly. Through a wide-bore funnel, $FeSO_4$, $7H_2O$ (8 g.), KBr (2 g.) and NaCl (25 g.) are added and the flask immediately connected to the previously lubricated joint of the delivery tube. A 200 ml. volumetric flask is placed over the exit of the Fresenius flask. The liquid in the Kjeldahl flask is boiled vigorously for exactly 10 min., the ice around the receiver being renewed as necessary. With the liquid still boiling, the delivery tube is disconnected from the Kjeldahl flask. The contents of the Fresenius flask are then transferred to the 200 ml. volumetric flask, and, when cool, diluted to the mark.

Separation of As *as* AsH_3. Two absorption tubes (Fig. 1), the second of which acts as guard tube, are charged with N/50 $AgNO_3$ (1·5 ml.). The tubes are stoppered and connected to a small wash-tube containing 10N NaOH (renewed after 12 estimations), glycerine being used as a lubricant for rubber tubing. A right-angle tube, bearing a stopper to fit the AsH_3 generation flask, is attached to the wash-tube.

After a small-scale digestion, the contents of the Kjeldahl flask are transferred to a 150 ml. conical flask, with 40 ml. distilled water as washing fluid. Alternatively, a measured fraction of the diluted digest is made up to 50 ml. with 20% H_2SO_4. 40% $SnCl_2$ (10 drops) and 5% $CuSO_4$ (3 drops) are added. The liquid is warmed if necessary, Zn shot (15 g.) quickly introduced, and the flask at once connected to the previously prepared absorption apparatus.

After a large-scale digestion, if the portion taken is less than 100 ml., it is made up to this volume in a 150 ml. conical flask with 20% H_2SO_4. When the total distillate is used, it is transferred to a 250 ml. conical flask. 40% $SnCl_2$ (10 drops/100 ml. total vol.) and 5% $CuSO_4$ (3 drops/100 ml. total vol.) are added and the liquid is warmed. Zn (25 g.) is introduced and the flask immediately connected to the absorption apparatus.

Titration of arsenite. When H_2 has been passed through the absorption apparatus for 30 min. the tubes are disconnected. The rubber stopper of the absorption tube is washed down with a few drops of distilled water, and the gas inlet is connected to the compressed air. KI is added till the precipitated AgI redissolves. After the addition of a spatula-point of $NaHCO_3$ and a drop of starch solution the tube is slipped over the jet of the Conway burette. It is held in place by sliding the titration platform under it, and with air passing through the liquid at a gentle rate I_2 is run in till a red-brown colour appears and persists for 30 sec. Before the end of the titration, the gas exit is washed down by increasing the flow of air till a little of the liquid is blown into it. A daylight lamp is of advantage in observing the end-point. If the liquid in the guard tube darkens, it is titrated in the same way.

At the beginning of each day, the glass tubing in the burette is thoroughly washed out with fresh I_2 from the reservoir. To standardize the I_2, two different quantities of arsenite solution are measured into absorption tubes, the volume made up to about 2 ml. with N/50 $AgNO_3$, and the titrations carried out as described above. The amount of As (μg.) is plotted against ml. I_2, and the analytical results are read off from the straight line joining the two points. This line cuts the I_2 axis at the volume required to cause a colour change in 2 ml. N/50 $AgNO_3$ treated as above, in absence of arsenite.

RESULTS

In 20 experiments in which 20 μg. As were added as Na_2HAsO_3 to 100 ml. 20 % H_2SO_4 and separated as AsH_3, the mean recovery was 19·6 μg., and the standard deviation of a single observation from the mean was 0·73 μg. In the subsequent pages of this paper, 'standard deviation' always means the standard deviation of a single observation from the mean. In 26 determinations of 20 μg. As added to 3 ml. blood for small-scale digestion, average recovery was 18·9 μg. (standard deviation 2·3 μg.). These two series of estimations were carried out by a technical assistant who understood that he was dealing with amounts of As differing slightly from each other.

In 19 analyses in which three portions of the same digest, averaging 12·1 μg. As, were taken for the final stage (separation of AsH_3 and titration of arsenite) of the estimations, the standard deviation was 0·50 μg. As. A research worker investigating the procedure found the average recovery in 24 determinations of 20 μg. As added to 1 ml. blood to be 18·8 μg. (standard deviation 2·5 μg.). The results in Table 1 for As added to 20 g. of minced mouse illustrate the recovery after large-scale digestion and preliminary separation of As as $AsCl_3$.

Table 1. *Recovery of arsenic added to minced mouse prior to large-scale digestion*

As added (μg.)	As recovered (μg.)	Recovery (%)	As added (μg.)	As recovered (μg.)	Recovery (%)
8·0	7·4	93	48·0	46·3	97
10·0	9·3	93	50·0	45·8	92
12·0	12·4	103	56·0	55·6	99
16·0	17·8	111	60·0	54·7	91
20·0	17·5	88	64·0	60·1	94
30·0	32·2	107	72·0	63·4	88
32·0	30·6	96	72·0	69·4	96
40·0	42·4	106	80·0	77·2	97
40·0	38·1	95	80·0	77·1	96
40·0	34·4	86	100·0	96·6	97
48·0	47·6	99			

The accuracy is largely determined by the care taken in carrying out the control experiments with reagents alone. The lower limit for accurate estimations depends upon how much As is present in the reagents, the lighting during titrations and the sensitivity of the individual to the end-point. Below 10 μg. As, the titration error (about 0·3 μg.) assumes increasing importance as the amount of As decreases. The percentage As not retained in the first $AgNO_3$ tube varies with the rate at which H_2 is developed, but as a rule little AsH_3 reaches the guard tube with less than 50 μg. As or passes through it with less than 100 μg. present. With special care it is possible to work in the range of 2–10 μg. As with the accuracy found for larger amounts of the element.

DISCUSSION

Allcroft & Green [1935] found HNO_3 and $HClO_4$ to be the most satisfactory reagents for digestion in their As determinations. Their figures, however, showed a constant loss of As, particularly marked with 5 and 10 μg. As_2O_3, which should probably be attributed to the digestion rather than to titration or separation of AsH_3. In trials carried out here, poor recoveries were invariably obtained when H_2SO_4 was omitted from the digestion mixture. With a mixture of all three acids [Kahane, 1932; Hinsberg & Kiese, 1937; Cassil, 1937], loss of As during the combustion has not been noted, even though no special precautions were taken when fairly large amounts of Cl^- were present [see How, 1938; Klein, 1920].

The Sub-Committee of the Society of Public Analysts on the Determination of As [1930], Winterfeld, Doerle & Rauch [1935] and Kiese [1937], all found it necessary to carry out a double separation of As after digestion of large samples of biological material. NaCl is employed as a source of gaseous HCl in the preliminary separation of As as $AsCl_3$. Bang [1925] recommended KCl. T. B. B. Crawford & I. D. E. Storey (private communication) found a 20 % loss of As when HCl [Cox, 1925; Ramberg, 1921; Winterfeld *et al.* 1935; Kiese, 1937] was used. With small amounts of blood or urine, Dr Ena Brown (Mrs G. M. Henderson) has dispensed with the $AsCl_3$ distillation without increasing the error. The separation of As as AsH_3 directly from acid digests was recommended by Allcroft & Green [1935].

The most satisfactory reaction with Zn, in presence of Cu^{++} and Sn^{++}, for the separation of As as AsH_3, is obtained with 4N H_2SO_4. A similar rapid, but not violent, evolution of H_2 from the $AsCl_3$ distillate is obtained after it has been diluted to 200 ml., giving a concentration of HCl of approximately 2N. The use of NaOH to remove H_2S from the stream of AsH_3 and H_2 was recommended by Hinsberg & Kiese [1937]. According to Kiese [1937], N/50 $AgNO_3$ is sufficiently dilute to avoid the formation of any arsenate in the reaction with AsH_3. Our results indicate that both the separation of As as AsH_3 and the conversion into arsenite proceed quantitatively. Contrary to the suggestion of Conway [1939], we have found air stirring to be entirely suitable for iodometric titrations, in spite of the volatility of I_2.

Methods based on the matching of stains have been considered unsatisfactory by many authors, e.g. Winterfeld *et al.* [1935], Cassil [1939]. Thus Barnes & Murray [1930] found a standard deviation of 4·8 μg. in 150 estimations of 27·3 μg. As_2O_3, and Neller [1929] a 15 % standard deviation in the recovery of As (22–94 μg. As_2O_3) by a similar method.

In these studies, the length of stain produced by AsH_3 on $HgBr_2$ paper was measured and the corresponding figure for As read from a graph obtained with standard solutions. It should be noted that variation in length of stain for a given amount of As enters into the preparation of the graph as well as into analysis of an unknown sample, thus introducing the possibility of further error in the final result. The standard deviation of a single determination of 20 μg. As by the micro-titrimetric procedure was 3·7 %. While it is as sensitive as the usual Gutzeit procedure, the titrimetric technique is much more objective and does not call for the same careful control of physical conditions in the evolution of AsH_3.

Cassil & Wichmann [1939] have reported a procedure for small quantities of As very similar in principle to that described above. As was separated as AsH_3 and passed into 1 ml. 1·6 % $HgCl_2$. I_2 was added and the excess back-titrated with arsenite, in contrast to our direct titration. The final volume of solution titrated was about 8 ml. After the absorption of AsH_3 in $HgCl_2$, one atom of arsenic was found to correspond to eight atoms of iodine. The sensitivity of the estimation must, therefore, have been identical with that observed in titrating, in a volume of 2 ml., the product of the reaction between AsH_3 and $AgNO_3$, which requires two atoms of iodine for each atom of arsenic. It seems that the sensitivity in using $HgCl_2$ could be further increased by decreasing the volumes of the reagents added before the back-titration. Cassil & Wichmann's accuracy was somewhat greater than that claimed for the present procedure.

SUMMARY

1. With a special tube for the absorption of AsH_3 in $AgNO_3$ solution and the Conway micro-burette for the titration of the arsenite formed, the iodometric estimation of As in digests of biological material was modified to cover the range 5–50 μg.

2. The recovery of 20 μg. As was 98 %, the standard deviation of a single observation from the mean being 3·7 %.

3. Methods for the digestion of As-containing material were examined, and the use of a mixture of HNO_3, $HClO_4$ and H_2SO_4 adopted.

4. Preliminary separation of As as $AsCl_3$ from the digest was found to be necessary only when large samples of material were taken for analysis.

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The Components of the Antigenic Complex of *Salmonella Typhimurium*

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Recent work on the antigenic complex of *Eberthella typhosa* (*Bact. typhosum*) has shown that the substance may be obtained in a state approaching chemical homogeneity by trichloroacetic acid or diethylene glycol extraction of the bacterial cells, and by tryptic digestion of the dried organisms. The complete antigenic complex may be broken down by mild acetic acid hydrolysis with the libera-