XCVI. RAPID TITRIMETRIC ESTIMATION OF ARSENIC IN BIOLOGICAL MATERIAL.

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IN the course of work upon the fate of arsenic in avian metabolism the need arose for a rapid method of estimating quantities of arsenic from 1 mg. down to 0.005 mg. As_2O_3 per 10 g. sample of biological material, with a probable error not exceeding $\pm 5\%$ for quantities around 0.005 mg. In a balance experiment on a laboratory animal in which it is desired to account quantitatively for administered arsenic as eliminated in faeces and urine or retained in various tissues, such a degree of accuracy meets most demands for physiological information; but wider errors are objectionable and in our opinion most of the older statements regarding prolonged retention of considerable quantities of arsenic in the body arose from faulty recovery of excreted arsenic and the unjustifiable assumption that the unrecovered arsenic was still in the tissues.

The range of accuracy arrived at is also satisfactory for public health administration concerning arsenic in foods where 1.4 parts per million is the permissible figure suggested by the Royal Commission of 1903.

A survey of existing procedures revealed laborious methods sufficiently accurate and rapid methods open to higher error, but despite the voluminous literature on arsenic determination it seemed probable that a more serviceable routine could be devised. The present communication claims to supply one which is effective with a few γ of material but has an "average working time" of only 20 mins. per determination in a routine series of half a dozen analyses. Since it involves a wet combustion, a separation as arsine and a final microtitration, a single determination cannot very well be completed in less than 90 mins., but since a rack of six blood analyses can be finished in 2 hours the procedure becomes as simple as the Kjeldahl process for total nitrogen. The economy of time is effected by combining a very rapid perchloric-nitric acid wet combustion with a rapid subsequent separation of arsine in a fast stream of hydrogen, absorption in dilute silver nitrate and direct micro-titration of the liberated arsenious acid with iodine in presence of sufficient potassium iodide to retain excess silver in clear solution and so render possible a delicate starch end-point. The iodine is arranged to express results directly on the burette and obviate arithmetic (1 ml. $N/495 = 0.1$ mg. As_2O_3 or 1 ml. $N/375 = 0.1$ mg. As).

Discussion of existing methods.

Since arsenic determination for biological purposes usually involves destruction of large amounts of organic matter and subsequent estimation of real quantities below 0.1 mg. ordinary gravimetric and volumetric methods are excluded and colorimetric or nephelometric methods and micro-titration or mirror-matching technique come into consideration. For literature on such methods the reader is referred to Heller [1934] who, in collecting references on micro-methods since 1928, cites 54 publications dealing with arsenic; to Kleinmann and Pangritz [1927] who give numerous references to previous technique; to

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for supply one ... per determination

read supply one which is effective for quantities of arsenic down to 5 γ but reduces the average working time to about 20 minutes

Minot [1926] who does the same and to Cox [1925] who concerns himself chiefly with English public analyst requirements and with checking the methods of Bang and Ramberg.

The precise technique of combustion is important since small losses are liable to occur both in wet and dry methods, and it is usual to effect a preliminary separation of arsenic from the combustion residue either as arsine or as arsenic trichloride before proceeding to actual estimation. Where the quantity of arsenic can be arranged to be relatively large (say 5 mg.) as in estimations on organic arsenicals the very simple method of Ewins [1916] is serviceable. Here the organic matter is destroyed with sulphuric acid as in an ordinary Kjeldahl combustion, with concomitant reduction of arsenic to arsenious acid, the excess sulphuric acid neutralised with soda lye and finally with sodium bicarbonate and the arsenic titrated directly with iodine. But where the amount of arsenic is small loss of traces of it by volatilisation and the possible presence in the combustion residue of substances which liberate iodine invalidate the procedure. Nitric-sulphuric acid combustion, in which the bulk of the organic matter is destroyed at lower temperature, is the generally recognised procedure and the technique of Ramberg [see Cox, 1925] is preferable to that described in the Report of the Conjoint Committee on the detection and approximate estimation of minute quantities of arsenic [1902]. The American Association of Official Agricultural Chemists [1930] also adopts combustion with nitric and sulphuric acids. Scott [1925] gives the unsatisfactory hydrochloric acid and potassium chlorate method. Hitherto no one seems to have recorded trials of perchloric acid with nitric or sulphuric acid. Amongst the little used dry methods, combustion with magnesium nitrate, as described below, would seem to be satisfactory.

In most cases where the nitric-sulphuric acid wet combustion is used it is now customary to remove residual traces of nitric acid by dilution, addition of oxalate and recombustion—a procedure which adds to the working time but is necessary when ensuing separation of arsenic is to be as trichloride.

Minot [1926] then proceeds to final determination without separation of the arsenic, reducing to trivalent form by saturating the diluted combustion residue with SO_2 , expelling this by evaporation and finally titrating direct with $N/500$ iodine. Blanks, however, amount to 0.01 mg. As_2O_3 and vary $\pm 20\%$ (0.08 ml.—0.12 ml.), and the time saved in separating as AsH_3 or AsCl_3 is lost by reduction with SO_2 and final evaporation.

Cox [1925], the English Sub-committee on the determination of arsenic in food colouring materials [1930], von Fellenberg [1930] and most recent workers agree that arsenic must be separated from the combustion residue before final determination. Cox considers that separation by distillation of the trichloride [Bang, 1925] followed by micro-titration with standard potassium bromate as used by Ramberg is satisfactory if the amount of arsenic to be determined is not less than 0.01 mg.; below this he recommends the Marsh-Berzelius method following the Ramberg technique for destruction of organic matter. Nevertheless the personal trials of Cox with the bromate titration, using pure carbohydrate with known quantities of arsenic, gave recoveries of 0.06 mg. in place of 0.05 mg. (20 % too high), 0.03 mg. in place of 0.02 mg. (50 % too high) and 0.02 mg. in place of 0.01 mg. (100 % too high). The results from 0.05 mg. downwards are thus much too high and this is a more serious fault than being too low; in the latter case empirical correction can be justified if duplicates are good and small losses consistent.

Kleinmann and Pangritz [1927] regard colorimetric methods of the Gutzeit type as inherently unreliable and consider that amongst the titrimetric methods

the technique of Billiter [1923], who dissolves the Marsh mirror in iodine and back-titrates with thiosulphate, is the most accurate. They themselves describe a nephelometric method sensitive to 0.5γ H_3AsO_4 in pure solutions and accurate to 5 % even with quantities below 0.01 mg. The principle of the method is precipitation with cocaine molybdate and nephelometric determination of turbidity; previous combustion being carried out by the nitric-sulphuric method of Ramberg and essential separation of arsenic being effected by the trichloride distillation of Bang [1925]. Unfortunately their recovery figures refer mainly to direct nephelometric determination on pure solutions and insufficient data are supplied on the operation of the whole process with tissues to which known amounts of arsenic have been added in order to test the summation of errors involved in combustion, distillation, precipitation and nephelometric matching.

Amongst newer colorimetric methods may be mentioned those of Maechling and Flinn [1930] and of Youngburg and Farber [1932]. The former use nitric-sulphuric combustion followed by distillation of arsenic trichloride, convert into quinquivalent form by evaporating with nitric acid and bromine, heat with reduced molybdate and compare in a colorimeter with an arsenate standard. The latter precipitate as arsenic sulphide, oxidise this and determine colorimetrically with molybdate and stannous chloride. But no recovery tables for arsenic added to tissues are given in either of these papers.

Von Fellenberg [1930] also precipitates as sulphide (with H_2S after distilling as AsCl_3 from the combustion residue) but titrates the isolated sulphide with permanganate in alkaline solution. Good results are shown even with a few γ but unfortunately in recovering added arsenic (limited data) the smallest magnitudes are returned too high and the higher magnitudes too low so that there is the suggestion of arbitrary compensatory errors just at the point (0.01 mg.) where recoveries by other methods begin to be unduly low.

On the question of separation of arsenic as AsH_3 by zinc and sulphuric acid most authors agree that recovery is good with pure solutions of arsenic provided that a properly activated pure zinc is used, but some consider that it may be rendered imperfect by substances in the combustion residue and therefore recommend a preliminary separation as trichloride even when subsequent arsine separation follows incidentally on the use of Gutzeit papers. This double separation occurs in the method described in the First Report of the Sub-committee on determination of arsenic in food colouring materials [1930], although Cox [1925] recommends the Marsh-Berzelius procedure direct on the Ramberg combustion residue.

Despite the variety of terminal procedures at disposal the most popular finishing estimation is still the colorimetric comparison of Gutzeit papers. But Neller [1929] in his study of the accuracy of the Gutzeit procedure considers the inherent error too high and the English Sub-committee, while regarding the stain produced by 0.005 mg. As_2O_3 as "a very suitable intensity to evaluate", concedes that "a stain actually due to 0.0045 mg. may be estimated at 0.004 or 0.005 according to individual judgment," and that this 20 % difference may appear equally in stating results of 1 part or 20 parts per million, irrespective of the real magnitude of the quantity in the analysed aliquot. Although the procedure is sound enough for most ordinary needs it might well be considered that the tedious combustion, dilution and reconcentration, distillation as trichloride and second separation as arsine, are labour lost if they end with an inherent error of ± 10 % irrespective of whether 0.20 mg. or 0.01 mg. As_2O_3 per 10 g. sample is dealt with. The significance of double separation of arsenic is worthy of further investigation but the usefulness of abbreviating combustion

needs no defence, and the obviation of individual judgment is still more desirable. Since a starch-iodine end-point is as delicate as an arsenic-mercury stain we believe that microtechnique is capable of carrying titration as far as Gutzeit papers. If recovery of arsenic is then incomplete but duplicates agree well, and an empirical upward correction is indicated, varying with the real magnitude of the arsenic at disposal, the correction is at least deliberately made and not unconsciously incorporated.

The proposed method.

The combustion procedure is essentially that described by Bolliger [1932] for general use in destroying organic matter by means of perchloric and nitric acids. The use of sulphuric acid in place of nitric (1 ml. perchloric acid, 1 ml. sulphuric acid per 0.2 g. of dry matter) associated with higher combustion temperature, as used by McCance (personal communication) for subsequent colorimetric determination of total phosphorus in foods, is equally serviceable and even speedier when the quantity of organic matter is sufficiently small for the analyst to view possible conflagration in the combustion vessel with equanimity, but the lower boiling-point of the perchloric-nitric mixture is an advantage when large combustion volumes have to be considered in relation to traces of arsenic liable to loss by volatilisation. The need for secondary dilution, addition of oxalate and recombustion to get rid of residual nitric acid, disappears since separation as AsCl_3 does not follow. The technique of the arsenic titration is a refinement of that used by Green [1918] in which the familiar reaction

is used; precipitation of silver iodide is held up by addition of excess potassium iodide and the arsenic is directly titrated with iodine. It is sufficient to add the observations (a) that perchloric acid does not interfere with the liberation of arsine by zinc and sulphuric acid, (b) that absorption of traces of arsine from large volumes of hydrogen by silver nitrate as dilute as $N/100$ is complete even when the gas is blown through a few cm. depth of liquid at the rate of several litres per minute, and (c) that the starch end-point is still sensitive to less than 1γ when soluble iodides are present in large excess so that micro-titration can take the place of the conventional Gutzeit papers and obviate the personal equation altogether. In general 10 g. of wet tissue form a suitable aliquot and since 1 ml. $N/495$ iodine = 0.1 mg. As_2O_3 the microburette reading gives mg. per 100 g. without further calculation.

Reagents. A.R. quality and As-free materials should be used throughout.

Perchloric acid 60 %.

Nitric acid, conc.

Stannous chloride solution. Dissolve 40 g. $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ in HCl and make up to 100 ml. with the same strength acid [Assoc. Off. Agric. Chem. 1930].

Copper sulphate 5 %.

Lead acetate 1 %.

Silver nitrate, approximately $N/50$.

Starch solution 1 % (fresh).

$N/495$ iodine, freshly diluted from stock $N/10$ (2 ml. $N/10$ made up to 99 ml.).

Sodium bicarbonate.

Potassium iodide.

Zinc shot (tested for interfering impurities).

Sulphuric acid.

Standard arsenic solution. 1 ml. = 0.1 mg. As_2O_3 for intermittent checking of process.

Moir and Jamison [1925] have described an electrolytic generator for use with the titration method of Green [1918] but zinc and acid generators are more usual and more conveniently prepared in large numbers.

Perchloric acid frequently contains traces of arsenic (up to 0.75 mg. per litre) and although it is possible to obtain it As-free (B.D.H. supply usually is so) a sample should be procured before purchasing in bulk and a test made to ensure that it will not introduce too high a blank. As-free zinc shot is easy to obtain but should be tested in the actual process to ensure that it permits good recovery of arsenic from the standard solution. Samples have been encountered which contain no arsenic but which give low results, apparently by retaining traces of arsenic in the generator. In making this test a suitable strength of sulphuric acid to use in the generator is 1:5, with catalysts as usual. It should be needless to point out that glassware often contains arsenic and that for combustion flasks exposed to high temperature and strong acid mere preliminary "pickling" is not enough. We have found monax glass quite suitable.

First stage. 10 g. of finely divided wet tissue or 2-5 g. dry matter are introduced into a 300 ml. Kjeldahl flask and covered with 20 ml. of perchloric acid. The quantity of perchloric acid (which should be noted if not quite As-free) naturally depends upon the material but to avoid the risk of explosion it is essential to err on the side of liberal excess. In the case of dilute liquids such as urine preliminary concentration by boiling precedes the addition of perchloric acid. If dry matter is used care should be taken to ensure thorough wetting with the acid before heating, and for adhesive powders such as flour it is best to wet to a paste with water first. Speculative adjustment of sample aliquots on the basis of anticipated real arsenic values naturally facilitates any final corrections which may have to be made. In the case of material rich in arsenic 1 ml. or 1 g., or less, may be taken and the scale of operation reduced to a test-tube 1 × 7 in. which, if desired, can also serve as subsequent Marsh generator for a reduced scale absorption apparatus.

Two glass beads are added to facilitate even boiling and the mixture is brought to ebullition and appearance of perchloric fumes over a bare flame. The flask is then transferred to a cork ring in the combustion cupboard and concentrated nitric acid added from a dropping pipette. Vigorous reaction sets in almost at once but is easily controlled by adjusting the rate of addition of nitric acid. When no further reaction occurs the yellow-brown mixture is heated on a Kjeldahl or other digestion rack until white fumes of perchloric acid appear. Continuous observation is necessary to control recrudescence of reaction. When the volume is reduced to about half oily drops appear on the sides of the flask and a vigorous reaction usually occurs. If so it is necessary to turn out the flame. After the reaction subsides the digest either turns practically colourless or else dark brown. If the latter then more nitric acid must be added, in which case it is advisable to remove the flask to its cork ring and allow to cool somewhat before adding the requisite further nitric acid and restoring to the digestion stand for completion of combustion. Usually 2 or 3 ml. are sufficient as indicated by the dark brown colour changing to a yellow-brown. Complete combustion is indicated by a clear, pale, yellowish green residue associated with white perchloric fumes. The residue is usually colourless on cooling, but if not can generally be rendered so by final addition of a few drops of perhydrol. Tissues such as muscle, blood and urine give a colourless cold digest; a faeces digest may be slightly yellow.

Although the digestion process requires constant attention it is not at all laborious. The time taken for the whole combustion varies from about 10 mins. for 10 ml. of blood or urine, to about 30 mins. for 10 g. of muscle or liver tissue

and over an hour for 2 g. of dried faeces, but since 6 to 12 combustions can be supervised simultaneously the actual duration of a single digestion is unimportant.

Second stage. The digest is diluted and washed into a generating flask to a total volume of about 100 ml.; 10 drops of stannous chloride solution and 2 drops of copper sulphate are added to speed up evolution of hydrogen. About 15 ml. of concentrated sulphuric acid are added and the contents heated over a flame for a few seconds if the mixture is not warm enough to ensure immediate subsequent vigorous evolution of hydrogen; about 25 g. zinc shot are then quickly added and the flask immediately connected up to the previously prepared absorption tubes (A, B, C and D). Of these, the first tube A contains 1 % lead acetate to remove any traces of H_2S formed by reduction of the hot dilute sulphuric acid. The remaining three tubes B, C, D contain $N/50$ or $N/100$ AgNO_3 , not stronger. Two are sufficient (*i.e.* B and C) in ordinary practice so that the last tube D becomes a guard tube in case unsuspectedly large amounts of arsenic are found (over 1 mg.). For quantities of 0.2 mg. over two-thirds is caught in the first tube; for 0.1 mg. or less the last tube D is unnecessary and C serves as guard. Three or four ml. of $N/50$ AgNO_3 in the first two tubes cover any range up to 1 mg. As_2O_3 . For quantities below 0.01 mg. $N/100$ AgNO_3 (approximately) is preferable.

Evolution of hydrogen from the hot acid is very rapid and the arsenic is swept over completely in 20 mins.—the faster the better so long as the liquid is not blown out of the tubes. Too slow evolution of hydrogen seems to lead to bad recoveries of arsenic.

Tubes B and C are then detached and the contents of the latter added to the former if any trace of silver deposition is noted in it. It is rarely necessary to add the contents of the guard tube. About 0.1 g. of sodium bicarbonate and about 2 g. of potassium iodide crystals, or sufficient to redissolve the silver iodide first precipitated, are added. After final addition of about 0.5 ml. of fresh 1 % starch solution $N/495$ iodine is run in to violet end-point. On a titration volume of about 5 ml. one drop from an ordinary 1 ml. micro-burette with waxed tip, corresponding to 0.01 ml. = 0.001 mg. or 1γ As_2O_3 , gives a sharp end-point but this is not the limit of sensitiveness since change of violet tint can be detected with less. By reducing the titration volume to 1 ml. and using an appropriate adaptation of the Rehberg type of burette an end-point of 0.1 γ could be reached. This would come into consideration if it were desired to reduce the whole scale of technique, combust small aliquots in test-tubes and absorb the AsH_3 in, say, 1 ml. $N/100$ AgNO_3 ; but such refinement is rarely required for physiological work.

With the 1 ml. burette used in this work for quantities up to 0.1 mg. As_2O_3 each division corresponded to $1/100$ ml. = 1γ As_2O_3 in the sample, and the starch end-point was taken to one division. For larger quantities a 5 ml. or 10 ml. burette was used.

Illustrative results. The method has been used extensively in physiological work over the past year with sufficiently good results but the following data may be taken to illustrate the results in routine practice, working at a speed which allowed 24 determinations to be completed in an uninterrupted 8-hour day.

Table I shows the recoveries of arsenic added to the generator flask as standard solution, *i.e.* omitting the combustion process and considering only the perfection of separation as arsine, absorption in silver nitrate and subsequent titration with iodine.

Table I.

As ₂ O ₃ added (mg.)	...	1.0	0.5	0.2	0.1	0.05	0.02	0.01	0.005
ml. <i>N</i> /495 iodine	(1)	9.67	4.87	2.03	0.985	0.505	0.205	0.110	0.055
solution required	(2)	9.82	4.87	1.92	1.015	0.495	0.195	0.100	0.060
	(3)	9.80	4.80	1.92	0.985	0.500	0.205	0.105	0.055
Blank, ml. iodine		0.02	0.02	0.02	0.015	0.015	0.015	0.015	0.015
Average, <i>minus</i> blank		9.74	4.83	1.94	0.980	0.485	0.187	0.090	0.042
mg. As ₂ O ₃ recovered		0.974	0.483	0.194	0.098	0.0485	0.0187	0.0090	0.0042
% of theoretical value		97	97	97	98	97	94	90	84

A few determinations were also carried out in which the two drops of copper sulphate were omitted and only the stannous chloride activator used, to ascertain whether the trace of copper helped or hindered recovery. No noteworthy difference was recorded so that the function of the copper is apparently confined to speeding up the generation of hydrogen. Another series was executed to ascertain the best concentration of silver nitrate to use. Approximately *N*/50 was found to be optimum for quantities between 0.01 mg. and 1 mg. and *N*/100 for quantities below 0.01 mg. *N*/10 was definitely too strong in the sense that recoveries were about 10 % lower than with *N*/50 but the reason for this was not specially investigated.

From Table I it will be noticed that the recoveries are very good down to 0.05 mg., much better than could be counted upon in Gutzeit colour matching, and that triplicates are satisfactory.

There is, however, a definite small loss which diminishes as the real quantity of arsenic diminishes but which is exaggerated when results are expressed on a percentage basis. Triplicates, however, agree within the limitations of the end-point of the burette and the titration volume, and the blank is open to the same titration error, so that nothing better could be expected. This point is brought out again in Table II which represents a series of recoveries on the whole process adding known quantities of arsenic to 10 g. of liver tissue in the combustion flask, and comparing with recoveries on pure solutions.

Table II.

As ₂ O ₃ added in mg.	Recovered			
	From pure solutions		After digestion + 10 g. liver	
	mg.	%	mg.	%
1	0.965 0.980 0.978	97	—	—
0.5	0.485 0.485 0.478	97	0.480 0.485 0.490	97
0.1	0.097 0.100 0.097	98	0.095 0.091 0.093	93
0.05	0.049 0.048 0.0485	97	0.048 0.045 0.048	94
0.01	0.0095 0.0085 0.0090	90	0.008 0.0085 0.008	82
0.005	0.0040 0.0045 0.0040	84	0.003 0.0035 0.003	64

It will be noted that down to 0.05 mg. As_2O_3 the perchloric-nitric combustion process has not involved any significant loss. At 0.05 mg. the difference between 97 % and 94 % recovery is negligible in view of the fact that 0.048 mg. recoveries appear in both sets of triplicates. There is a real loss varying between 1 γ and 2 γ As_2O_3 ; this variation in recovery is of less consequence for quantities over 0.05 mg. but is more serious when results are expressed on a percentage basis with quantities so low as 0.005 mg. The average of 0.0042 mg. recovered from the pure solution and of 0.0032 mg. recovered from the liver tissue means a loss of 16 % in one case and 36 % in the other, but one would hesitate to attribute the difference wholly to the combustion. The agreement between triplicates, however, is within the limits of titration of the volume concerned so that in a balance experiment in which it was necessary to account on the basis of 100 % recovery, an upward correction of 0.002 mg. to the uniformly low results and admission of a variation of ± 0.0005 mg. (± 10 %) in drawing conclusions, would seem to be justifiable.

A further series of trials is offered in Table III, this time on different substances to which "1.4 parts per million", the critical limit for the public health analyst, were added.

Table III. *Recoveries in parts per million and as percentage of arsenic added to various materials. 0.014 mg. As_2O_3 per 10 g. or 10 ml.*

Urine		Beer		Blood		Milk		Flour	
Parts per million	%	Parts per million	%	Parts per million	%	Parts per million	%	Parts per million	%
1.30	93	1.20	86	1.10	79	1.30	93	1.20	86
1.25	89	1.15	82	1.20	86	1.30	93	1.15	82
1.15	82	1.15	82	1.15	82	1.25	89	1.25	89
1.20	86	1.20	86	1.15	82	1.30	93	1.25	89

It will be observed that recoveries vary from 1.15 to 1.30 parts per million, or 82 to 93 % of the added arsenic. Most analysts would agree that this is good and report the figures direct, but since all data are low a higher degree of accuracy could be obtained by correcting upwards by 12 % and stating results within a probable error of ± 7 %.

Comparison of combustion methods. Since the method of digestion utilised here does not seem to have been discussed for arsenic before, it is considered advisable to add comparative data on different methods of combustion. Table IV gives the recoveries of known quantities of arsenic added to 10 g. minced liver tissue in each case. The methods were:

(1) Ordinary Kjeldahl combustion with 3 g. potassium sulphate and a fragment of copper as catalyst.

(2) Combustion with nitric and sulphuric acids as described in Assoc. Off. Agric. Chem. [1930, p. 307].

(3) Hydrochloric acid and potassium chlorate method as given by Scott, [1925, p. 51].

(4) Perchloric-nitric combustion as described above.

(5) Dry ashing with magnesium nitrate, a method sometimes used by Green in dealing with large quantities of rumenal contents or grass. This consists in thoroughly mixing the material with 20 % magnesium nitrate solution (about 40 ml. for 10 g. liver tissue), taking to dryness on the water-bath and ashing at moderate temperature in an electric muffle. The light white ash is then moistened with water, dissolved by warming with 1:5 sulphuric acid and transferred to the generator flask for completion of the determination as usual.

Table IV. *Method of digestion.*

As ₂ O ₃ added mg.	Sulphuric acid	Nitric acid + sulphuric acid	Hydrochloric acid + potassium chlorate	Perchloric acid + nitric acid	Ashing with magnesium nitrate
0.5	77	81	49	97	90
	59	84	41	96	88
	52	77	50	98	95
Average recovery	63	81	47	97	91
0.1	61	71	53	95	95
	72	70	—	91	83
	67	85	—	93	94
Average recovery	67	75	—	93	91
0.05	70	72	48	96	92
	72	72	36	90	84
	66	74	—	96	88
Average recovery	69	73	42	94	88
0.01	90	70	40	80	85
	50	70	50	85	70
	60	80	—	80	80
Average recovery	67	73	45	82	78
0.005	Nil	50	Nil	60	50
	40	70	Nil	70	40
	20	50	Lost	60	50
Average recovery	20	57	Nil	63	47

The combustion with hydrochloric acid and potassium chlorate was so troublesome and unsatisfactory that it was not considered worth completing the series. The high temperature sulphuric acid combustion obviously leads to material percentage losses even with quantities as high as 0.5 mg. Dry combustion with magnesium nitrate appears to be as satisfactory as nitric-sulphuric digestion and may occasionally be of service with certain classes of material. In using it abundant excess of magnesium nitrate should be used and care taken to ensure thorough permeation of the sample. The nitric-sulphuric combustion might have given higher recoveries, especially on the 0.5 mg. and 0.1 mg. quantities, if the precautions emphasised by Cox in discussing Ramberg's procedure had been studiously observed, but the Assoc. Off. Agric. Chem. [1930] method was purposely carried out as it would be in ordinary laboratory practice. The run of results with the perchloric-nitric combustion is obviously best of all so that it appears to have in its favour superiority of recovery as well as greater speed of destruction of organic matter. This is attributed to the lower boiling-point of perchloric acid as compared with sulphuric at the last stage of combustion, as well as to the shorter period at high temperature.

SUMMARY.

A rapid quantitative method for determination of arsenic in biological material is described which enables six determinations to be completed in about 2 hours. A mixture of perchloric and nitric acids is used for destruction of organic matter. Arsenic is liberated as arsine in a fast stream of hydrogen, absorbed in a few ml. of *N*/50 silver nitrate and directly titrated with *N*/495 iodine (1 ml. = 0.1 mg. As₂O₃) to starch end-point in presence of sufficient excess of potassium iodide to keep silver in solution. Micro-titration is regarded as superior to matching Gutzeit papers, the inherent error of which may operate indifferently over large and small real quantities of arsenic.

Recoveries of added arsenic in quantities of 0.50 mg. to 0.005 mg. As_2O_3 compare favourably with recoveries by other methods. Duplicates are good and probably validity of results can be improved by an upward correction for consistent small losses.

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