CII. AN ABSORPTION APPARATUS FOR THE MICRO-DETERMINATION OF CERTAIN VOLATILE SUBSTANCES.

V. THE MICRO-DETERMINATION OF BROMIDE, WITH APPLICATION TO BLOOD AND URINE AND OBSERVA- TIONS ON THE NORMAL HUMAN SUBJECT.

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INTRODUCTION.

A METHOD has been described [Conway, 1935] for the micro-determination of chloride depending on the oxidation of chloride to chlorine by an acid permanganate mixture in the outer chamber of an absorbing "Unit" [Conway and Byrne, 1933]. The chlorine formed is estimated iodimetrically, the whole procedure being conducted at room temperature. Quantitative determinations could be carried out with as little as 0.5 γ Cl, which contained in 2 ml. represents 0.025 mg./100 ml. Cl. Still smaller quantities could be determined in apparatus of reduced size.

The principle and general technique of the chloride determination may also be applied to that of other halogens. Both free bromine and iodine are sufficiently volatile to require about the same absorption times as chlorine. In the present communication the micro-bromide technique is described, bromide being determined to an ultimate standard deviation of 0.08 γ Br, which in milli-equiv. is about one-third that found with chloride. The standard deviation of the blank value may be expressed as 0.037 γ Br, which is only about one-seventh of the equivalent value found with chloride. The difference probably arises from minute traces of chloride likely to be present in water and on glassware. These ultimate errors for chloride and bromide determinations appear to be the lowest yet recorded. The early papers on micro-determination of bromide have been reviewed by Bernhardt and Ucko [1925]. Accounts of the more recent developments may be found in the papers of Quastel and Yates [1934], Dixon [1935], Bertram [1933], Stoll and Brenken [1934], Indovina [1935], Leipert [1935] and others. Here reference will be made only to such previous work having immediate bearing on the present method and the results obtained for normal blood and urine.

Such micro-bromide determinations consist essentially of three stages. Firstly, the ashing of the material. Secondly, the oxidation of the bromide to bromine by a suitable oxidant. (These two stages may be combined in wet ashing methods such as that of Leipert and Watzlawek [1934].) Thirdly, the determination of the liberated bromine after extraction, aeration *etc.*

In the present method these three stages are also followed for blood and urine, ashing being obviously unnecessary for purely inorganic mixtures or such as contain only traces of organic matter (*e.g.* sea water). The stages are conducted

in a manner hitherto undescribed and permit the least absolute error of bromide with the least manipulation. Also, it is possible with this method to carry out large numbers of determinations at the same time.

The apparatus used for the liberation and absorption of the bromine is that already described in previous communications. In the outer chamber of the apparatus is contained a dichromate oxidation mixture and in the centre 1 ml. of 20 % KI in which the formed bromine is absorbed, liberating its equivalent of free iodine.

The ashing procedure. The ashing of the blood is carried out after preliminary removal of the proteins with methyl alcohol and subsequent evaporation of the cleared fluid. This reduces the incineration time to a few minutes and was introduced here for the reason that it is now practically certain that all the bromide in normal blood is present in inorganic form. If a complete ashing procedure is desired, the technique of Indovina [1935] seems very suitable. In this method magnesium oxide is employed to prevent bromide losses on heating.

The oxidation procedure. The oxidant found most suitable for bromide determinations is dichromate and sulphuric acid. The dichromate is used in powdered form and the sulphuric acid as 1 ml. of a solution containing 40 vol. % of sulphuric acid to 1 ml. of the ash extract. (Upwards of 48 vol. % may be used after bromide administration.) Acid dichromate of this strength has a scarcely detectable influence on 1 ml. of 1 % NaCl even after 20 hours' action, whereas all the bromide is oxidised and absorbed in 2-2½ hours.

The presence of iodide interferes with this method as shown in the experimental section. With normal blood and urine this is of little or no account as the final error introduced will not exceed 1 %.

After administration of iodide, the iodide present may be removed as iodine by treating the ashed extract in the outer chamber with dichromate in about 0.1N sulphuric acid, the inner chamber being empty. Raising the acidity to the required degree will then liberate the bromine which is absorbed and determined as usual. Instead of letting the formed iodine escape, it may be determined in a similar manner to bromide.

As referred to in a previous communication [Conway, 1935] the use of a suitable acid permanganate mixture would have the advantage of rendering the method independent of the presence of both chloride and iodide. It was found however to have the disadvantage of causing marked losses as bromate, when the concentration of bromide for determination is very small.

The determination of the bromine formed on oxidation. In the present method the bromine diffuses into the central chamber of the "unit", liberating its iodine equivalent from 20 % KI. The iodine is then determined as for chloride [Conway, 1935] by titrating with thiosulphate if more than 80γ Br are present; an absolute colorimetric determination of the iodine colour itself is likewise found very suitable for the range from 80 to 16γ Br. Below this level, starch is added and the colour produced is colorimetrically determined.

In the method used by Leipert and Watzlawek [1934] for the determination of the bromide content of normal blood the formed bromine is oxidised to bromate after absorption into alkali, the bromate being then determined iodimetrically. An advantage of this procedure is that six times the equivalent of the original bromine is finally present as iodine. This variation could also be used with the "unit", with possible advantage for the minutest bromide determinations.

The delicacy of the method selected however is so great that it is doubtful if a substantial increase in accuracy would result, and further operations with

numerous reagents would be required. Also errors in the bromine formation and absorption are likewise multiplied by six.

With the present method, using starch, each γ Br will in fact cause an increase in the extinction coefficient of the mixed fluid from the central chamber of 0.09 (for a wave-length of $464m\mu$ approximately).

PROCEDURE.

Inorganic.

(a) *Above 80 γ Br.* Into the outer chamber of a "unit" is placed a small quantity of pure powdered potassium dichromate—roughly about 0.2 g.—by means of a spoon spatula, and into the central chamber 1 ml. of 20 % KI. Into the outer chamber is now introduced 1 ml. of the bromide solution and the lid smeared with the fixative (see below) and placed in position. The unit is slightly tilted by resting it on the edge of a spare lid, the lid is slightly displaced and 1 ml. of a solution containing 40 % (by vol.) sulphuric acid is run in quickly without blowing—best from a simple tube with a mark at 1 ml. approximately. A solution containing 45 % acid (by vol.) may be used when the Br/Cl ratio exceeds 1/500. After the introduction of the acid the lid is quickly replaced. The fluids in the outer chamber are well mixed by rotation. After 2 hours the free iodine in the central chamber is titrated with 0.05*N* thiosulphate from the special burette [Conway, 1934], the number of divisions on the burette being multiplied by the bromine equivalent or factor which may be determined as for chloride by making some initial determinations with standards or titrating standard iodine in 20 % iodide in the central chamber. In this latter case the thiosulphate required is multiplied by 0.97 to allow for the slight constant loss of iodine from the central chamber during a determination.

(b) *Below 80 γ Br.* The procedure is carried out in the same way as for the previous method, except that the 1 ml. of iodide (which should have been carefully delivered) in the central chamber is examined colorimetrically. This is done either by direct colorimetric investigation without standards or by the addition of 0.2 % starch solution and subsequent determination.

Without standards (to about 16 γ Br). The contents of the central chamber are pipetted into a micro-colorimeter cup and the plunger set at 8.35 mm. or convenient multiple. Using the "grey solution" of Thiel [1933] and Leitz filter No. 3 the depth in mm. of grey solution multiplied by 10 gives the γ Br analysed. 1.4 γ are added to the result as representing a small constant loss as bromate. It is advisable that the depth of the fixed plunger be so chosen that the mm. grey solution required for a match do not much exceed 10 for this direct colorimetry, though this precaution is not necessary when using starch. For this absolute colorimetry the Pulfrich photometer may also be employed or the new Leifo instrument of Leitz, the extinction coefficient being given directly on the instrument and the bromide concentration being subsequently read from a graph or tables.

With starch addition (to about 2 γ Br). This method is most suitable for bromine quantities under 16 γ , but may be used to cover the whole range from 80 γ Br downwards. Where the amount is under 16 γ , 0.5 ml. of a 0.2 % starch solution is added to the central chamber but for the range between 80 and 16 γ , 2.0 ml. of the 0.2 % starch solution must be used. The mixed contents from the central chamber are pipetted into a micro-colorimeter cup and the iodine is estimated by comparing it with standard iodine in 20 % KI similarly treated,

or by absolute colorimetry. Using the grey solution the fixed plunger in the starch iodide solution is put at 29.0 mm. (or convenient multiple or submultiple) and the reading of the grey solution multiplied by 0.2 with subsequent addition of 1.4 gives the γ bromine analysed. Leitz filter No. 3 (464 $m\mu$) is used. (If 2 ml. starch have been added the reading is multiplied by 0.4.) The values obtained by absolute colorimetry may be checked by standard iodine and starch as described in the chloride method. It may be mentioned however that a greater constancy with absolute colorimetry in relation to the starch-iodide colour was obtained than was anticipated, provided that the same starch solution was used throughout, so that the whole procedure below 80 γ Br could be conducted without standards.

Ashing and extraction procedure for blood and urine.

Normal blood (1 ml.). Into a 15 ml. centrifuge-tube is pipetted 1 ml. of blood, 12 ml. of 95 % methyl alcohol are added and the whole is well mixed. The tube is then capped and centrifuged for 5 min. 10 ml. of the clear fluid are pipetted into a small crucible (20–30 ml. capacity) and 0.2 ml. of a standard KBr solution containing 2 γ Br is added. The fluid is dried in the oven at 90–100° and then the crucible is heated in the Bunsen flame until the bottom and sides have been brought for about 1 min. to a dull red heat. It is then cooled and 0.5 ml. of distilled water is pipetted into the crucible, mixed and rotated around the walls. The solution is then pipetted into the outer chamber of a unit. Another 0.5 ml. water is added and the process repeated. The presence of some carbon particles does not interfere. The determination then follows the inorganic procedure (above outlined) for quantities under 16 γ Br. The 2 γ Br are added to bring the amount analysed on to the linear curve. A blank determination is carried out in an exactly similar manner including the small bromide addition. The calculation of bromide analysed is then made as above, the blank value being subtracted and the addition of 1.4 γ Br here omitted.

Blood after bromide administration (0.2 ml.). A similar procedure is adopted. 5 ml. of 95 % methyl alcohol are used and 3 ml. of the clear fluid pipetted off for drying. No bromide need be added. The mode of determining the free iodine in the central chamber will depend on the order of magnitude of Br expected. It may be noted that the ingestion of 2 g. KBr by the human subject will produce about 16–32 γ Br in 0.2 ml. of whole blood.

The recovery of bromide from Folin-Wu filtrates is described below.

Urine. The same procedure for ashing and extraction may be adopted for normal urine as for urine after bromide administration. Since the bromide in urine very closely follows the chloride it is always advisable to carry out a preliminary chloride determination. The bromide concentration has in fact little significance apart from this chloride figure. Where the urine contains the average quantity or more of chloride, 2 ml. are used for ashing with correspondingly larger volumes with reduced chloride concentrations. 2 ml. urine are pipetted into a crucible and dried in the oven. The crucible with dried contents is now held over the Bunsen flame and cautiously incinerated. The contents char at first, then fumes are emitted, the residue finally melting as a rule when the bottom of the crucible turns red. At this stage the crucible is withdrawn from the flame and cooled. The whole procedure does not require more than a few minutes, and the bottom of the crucible is brought only for about 1 min. to a dull red heat. After cooling, 2 ml. of distilled water are added and mixed with the contents. After a short time 1 ml. is pipetted into the outer chamber of a "unit", the standard method being then followed. If it is found difficult to obtain 1 ml. free

from many charred particles, 2 ml. of extract are centrifuged in a small tube, which procedure may be followed as a routine. After bromide administration, the free iodine present in the central chamber can be titrated with the $N/20$ thiosulphate from the special burette.

Notes on method and solutions required.

No special care need be taken to free the analytical reagents used from bromide, since it is found that they contain none. But it is advisable to clean the units, after the usual cleaning, with a little alcohol and ether, subsequently rinsing them with distilled water. For the smallest bromide quantities the units should not be exposed to direct sunlight during the absorption and may be covered with a light cloth; under these conditions the blank values will show no alteration even after 24 hours or more. Another point that may be emphasised is that 0.5 ml. of 0.2% starch is no longer sufficient when the bromide exceeds 20 γ . 2.0 ml. are then a convenient amount and will be sufficient for the whole range.

40% sulphuric acid. This is made up as a volume concentration, 40 ml. of the purest sulphuric acid (sp.gr. 1.84) being made up to 100 ml. with water. A certain latitude (of a few % at most) is permissible in the actual concentration, but the purest sulphuric acid must be used. 45 vol. % is advisable when the Br/Cl ratio exceeds 1/500.

20% potassium iodide. The purest reagent should be employed, and for the minutest bromide determinations it should be freshly prepared. When over 80 γ Br are being determined, a slight coloration is of no consequence as it will be allowed for in the blank.

0.2% starch. This is best prepared as described for chloride determinations.

The fixative. This is also the same as for chloride.

0.02N iodine. This is made up as a stock solution from N or 0.1N iodine and stored in a brown bottle in the dark.

Standard bromide. 1.489 g. of pure powdered and dry KBr are dissolved in 1 litre of water. Then each ml. contains 1000 γ Br.

EXPERIMENTAL.

Determinations on standard bromide solutions.

Table I gives a summary of the results obtained in solutions containing from 800 to 2 γ Br. The accuracy for the higher quantities resembles that already found with chloride, namely 0.6% as a coefficient of variation for 800 γ Br compared with 0.5% for 355 γ Cl. For the lower quantities, 8–2 γ Br, the bromide determinations are more accurate owing to the smaller blank values. The bromide blank values in fact arise merely from the light absorption by the added starch solution and may be occasionally due to traces of free iodine contained in the iodide solution. It will be seen from the table that for the 16 determinations from 8 to 2 γ Br the maximum deviation was 0.21 γ Br. For 8 determinations of 2.0 γ Br the standard deviation was 0.08 γ . Fig. 1 shows the bromide values obtained (iodine equiv. multiplied by 1.03) plotted against the values added to outer chamber. Here the constant loss of 1.4 γ Br has not been added to the calculated results; the validity of this correction is however apparent from the figure. The line has been extrapolated beyond 2.0 γ Br to cut the abscissa at 1.4. Some determinations of 0.8 γ Br indicate that the full curve leads from somewhat under this point

Table I. *Determination of bromide in standard solutions.*

Bromide analysed γ Br	No. of analyses	Mean value of bromide recovery γ Br (including addition of 1.4 γ Br)	Extinction coeff. of fluid from central chamber minus blank	Standard deviation of the individual determination γ Br	Maximum deviation found γ Br	Method of determining the free iodine in central chamber
800.0	6	803.2	—	4.8 (0.6%)	15.0 (1.9%)	Titration
80.0	7	79.9	0.471	1.4 (1.8%)	4.0 (5.0%)	Direct colorimetry
16.0	3	15.7	1.228	—	0.34 (2.1%)	Colorimetry with starch
8.0	18	8.13	0.578	0.12 (1.5%)	0.38 (4.8%)	"
6.0	4	6.01	0.396	—	0.10 (1.7%)	"
4.0	4	3.99	0.223	—	0.21 (5.2%)	"
2.0	8	2.03	0.054	0.08 (4.0%)	0.16 (8.0%)	"
Blanks	11	—	0.017	0.037	0.09	"

The 11 blank determinations were carried out in the one series. A few obviously spoiled results have been omitted.

The method of calculating the results is described in the text.

Fig. 1.

Fig. 1. — The bromide found (determined from the liberated iodine $\times 1.03$). - - - - The result obtained using the same colorimetric method with the theoretical equivalent of free iodine added immediately to 1 ml. of 20% KI. The calculation in the text allows for the factor 1.03 and the constant addition of 1.4 γ Br.

into the origin. The addition of a trace of bromide (2 γ Br per ml.) in the 40% sulphuric used for the determination and the blank will eliminate the addition of 1.4 γ Br to the result. The same result is achieved by adding 2 γ Br (as bromide) directly into the outer chamber of the units, the strength of the acid added being suitably altered for the extra volume.

The effect of chloride and iodide on the bromide determinations.

For biological determinations it is essential that chloride should have no appreciable influence since it will almost always be present in largely preponderating concentrations. With the above determinations in the 2 hours of bromide absorption the chloride oxidation is scarcely measurable when the chloride concentration in the outer chamber is of the order 0.5–1.0% NaCl (i.e. 1–2% in the fluid for examination). These represent the higher range of

concentration likely to be met with in urine analysis: even after 24 hours' action the amount represents less than 2γ Br. It may be noted however that when the chloride is increased to concentrations of the order of 10% the amount of chloride oxidised is increased in much greater proportion. This effect is shown in Table II. If 1 ml. of 10% NaCl was added to the outer chamber with 1 ml. acid,

Table II. *Effect of chloride on bromide determinations (dichromate oxidation).*

Experimental group	Chloride in outer chamber expressed as its bromine equivalent γ	Bromide in outer chamber γ Br	Time of action hours	Total halogen oxidised as Br equivalent (+1.4 γ Br)
1	136,700	—	21	52
2	13,670	—	20	1.6 (maximum value)
3	13,670	—	2.5	— (indeterminable)
4	13,670	4	2.5	4.06

The free iodine in the central chamber was determined colorimetrically as described in the text. The average room temperature was 15°.

an excess value of about 6γ Br at room temperature of 15° was obtained for the $2\frac{1}{2}$ hours' absorption, the chloride present at the same time representing an equiv. of 136,700 γ Br.

The effect of iodide may be seen from Table III.

Table III. *Effect of iodide on bromide determinations (dichromate oxidation).*

Experimental group	Potassium iodide in outer chamber as Br equivalent γ	NaCl in outer chamber as Br equivalent γ	Bromide in outer chamber γ Br	Total halogen oxidised determined as Br equivalent
5	80	13,670	8.0	48.4
6	8	13,670	8.0	13.8
7	—	—	8.0	8.0

The outer chamber of the units contained besides the 1 ml. of 40% sulphuric acid 0.2 g. solid dichromate and 1 ml. of a halogen mixture in each experimental group. In group 7 only standard KBr solution was used.

About half the equivalent of the iodide present appears as free iodine in the central chamber, the remainder being converted into iodate at the concentration of acid used.

Table IV illustrates how iodide may be removed if present in appreciable quantity. In the outer chamber were placed some solid dichromate, 1 ml. of

Table IV. *Removal of iodide before the bromide determination (dichromate oxidation).*

Into the outer chambers of a number of units were placed 0.2 g. (approx.) dichromate, 1 ml. of N/1000 KI and 0.1 ml. of NH_4SO_4 . The units were then exposed to the air with empty central chambers for varying periods, at the end of which 1 ml. of 20% KI was run into the central chamber and the unit covered by a lid with fixative, the iodine absorption being allowed to proceed for 2 hours at room temperature.

Time of exposure min.	Iodine subsequently absorbed in central chamber γ iodine	Time of exposure min.	Iodine subsequently absorbed in central chamber γ iodine
0	118	36	2.6
2	98	42	1.5
8.5	44	72	0.1
14.5	15	72	0.0
23	11	78	0.0

$N/1000$ KI (equivalent to 80γ Br) and 0.1 ml. $N H_2SO_4$. The iodide is converted into free iodine, which entirely disappears in about 1 hour's exposure. Instead of permitting the iodine to escape, it may be determined in a similar manner to the other halogens. With an acidity of $N/10$ H_2SO_4 in the presence of dichromate, no appreciable fraction is converted into iodate.

Recovery of bromide added to shed blood. To 10 ml. of defibrinated blood was added 1 ml. of a standard bromide solution, 1 ml. of water being added to a second 10 ml. blood. A series of 0.2 ml. samples from both mixtures was then analysed. The results are shown in Table V, where the results from the blood

Table V. *Bromide "recoveries" from blood.*

Bromide added per 0.2 ml. blood γ Br	Blood analysed ml.	No. of analyses	Bromide recovered γ Br	Coefficient of variation of individual determination	Maximum deviation %
145	0.2	10	146	3.3	5.2
14.5	0.2	12	14.6	3.8	6.6

The blood for analysis was prepared by adding 1 ml. of standard KBr solution to 10 ml. o. defibrinated sheep's blood. For the blank values water was substituted for the standard KBr.

containing no added bromide were treated as blank values. Where 145γ added Br were contained in each 0.2 ml., 146γ were recovered as the mean of 10 determinations, a maximum error of 5.2% being observed for the individual analysis. When 14.5γ of added Br were present, 14.6 were recovered as the mean of 12 determinations, the maximum error being 6.6% of the mean.

The bromide recovery from blood is therefore quantitative.

Recovery of bromide added to urine. Some 2 ml. samples of normal urine were evaporated and incinerated as above. To other similar samples of the same urine 1 ml. of standard KBr solution was added, the whole evaporated and incinerated as before. The bromide was then determined, the urine containing no added bromide being used as a blank. The free iodine in the central chamber was determined by titration. The results are shown in Table VI. The recovery of added

Table VI. *Bromide "recoveries" from urine.*

Bromide added to crucible per 1 ml. urine γ Br	Vol. of urine incinerated ml.	No. of analyses	Bromide recovered γ Br	Maximum deviation found %
80	2.0	3	78	4.8
200	2.0	9	199	6.1 (second highest = 3.1)
400	2.0	3	392	3.3

2 ml. of distilled water were used to extract the incinerated urine, 1 ml. of the extract being taken for subsequent analysis.

quantities varying from 80 to 400γ Br was investigated. For the 15 results summarised in the table, 99% was recovered as a mean value. Only 1 of the 15 results showed a deviation of more than 4.8% from the mean.

The bromide content of normal blood and urine.

Normal whole blood and urine samples from 8 students were investigated in the manner described. The mean value for blood was 372γ Br/100 ml. individual values varying between 227 and 572. The results are given in Table VII. The corresponding urine samples gave a mean value of 656γ Br/100 ml. with a range of variation of 297–855. The chloride of the urine samples was determined as well as

Table VII. *Bromide in normal blood and urine.*

Subject	Blood bromide		Urine bromide		Urine chloride		Cl/Br (milli- equiv.) in urine
	γ Br per 100 ml.	Milli- equiv. per 100 ml. $\times 10^3$	γ Br per 100 ml.	Milli- equiv. per 100 ml. $\times 10^3$	mg. Cl per 100 ml.	Milli- equiv. per 100 ml.	
Sh.	360	4.50	705	8.81	501	14.1	1600
Cos.	257	3.21	297	3.71	355	10.0	2700
To.	227	2.84	685	8.56	778	22.2	2590
Cu.	341	4.26	550	6.86	611	17.2	2510
Mu.	572	7.15	702	8.78	688	19.4	2210
Ma.	487	6.08	757	9.46	599	16.9	1790
Con.	455	5.68	700	8.75	593	16.7	1910
Cr.	276	3.45	855	10.69	716	20.2	1890
(Fo.)	(1890)	(23.6)	(2450)	(30.6)			

The above figures for normal blood and urine were obtained on blood and urine samples from 9 students. For the last of the group it was found on subsequent inquiry that a course of bromide had been prescribed (amounting altogether to approximately 10 g. KBr) the last dose having been taken 7 weeks before the date of blood determination. The student was unaware that he had taken bromide.

the bromide, using the method described in a previous communication [Conway, 1935]. The ratio of chlorine to bromine, each expressed as milli-equiv. per 100 ml. is 2150 as an average value, individual values varying between 1600 and 2590. The chloride in the blood samples was not determined. If we assume that the mean ratio of bromine in the whole blood to bromine in the plasma is 1:1.12—a figure derived from Leipert's data [1935]—and that the concentration of chloride in plasma is rather constant and 10.35 milli-equiv./100 ml., then we may derive the mean value of Cl/Br for the plasma as 2180. Comparing this value with the corresponding ratio of 2150 for urine it appears that compared with chloride the bromide in urine is almost identical with that in blood. Leipert obtained a comparatively high ratio, namely 1:1.5.

Recovery of bromide from a Folin-Wu filtrate.

In the preparation of a blood filtrate from defibrinated blood prepared according to Folin and Wu [1919] standard bromide has been added to a final concentration of *ca.* 0.001*N*. A similar filtrate was prepared without added bromide. 1 ml. samples were taken in the outer chamber containing solid dichromate and the method carried out as previously described, the filtrate without added bromide serving as a blank. The free iodine in the central chamber was determined by direct colorimetry using the "grey solution" of Thiel and filter No. 3 (Leitz). The results illustrated in Table VIII show a

Table VIII. *Bromide "recoveries" from a tungstate filtrate.*

No. of determination	Added bromide in 1 ml. filtrate	Bromide recovered from 1 ml. filtrate
	γ Br	γ Br
1	78.9	79.0
2	78.9	82.3
3	78.9	79.0

quantitative recovery. In this determination of bromide in a tungstate filtrate it is better to add 1 ml. of a solution containing 45% sulphuric acid (by vol.) instead of the usual 40%. The use of the Folin-Wu filtrate is suitable only after bromide administration corresponding to a few g. or more in the human subject. Somewhat reduced "recoveries" were noticed in some experiments in which the filtrate contained only 8 γ Br in ml.

The effect of varying the conditions in the above bromide method.

Varying the acid concentration. The effect is illustrated in Fig. 2, the rate of absorption from bromine water being also given for comparison (dotted line). It will be seen that changing the acid concentration has a marked effect of a kind similar to that shown for chloride. 20 min. after adding the 40 % sulphuric acid (as described above) 50 % of the bromide has been oxidised and absorbed. When 20 % acid was added only 4–5 % of the bromide was recovered in the same time. In a similar way to the action of the permanganate system on chloride, a marked deficiency appears in the total amount recovered when the concentration of acid exceeds a certain limit (see curve *C*, Fig. 2). This deficiency

Fig. 2.

Fig. 2. Curves *C*, *D*, *E* and *F* represent the absorption rates of the bromine from the outer chamber containing 0.1 ml. of 0.1N KBr, 1 ml. of saturated dichromate and 1 ml. of sulphuric acid of strengths 75, 40, 30 and 20 % (by vol.) respectively. Curve *A* represents the fall in percentage value of free iodine in the outer chamber introduced before a blank determination (allowance being made for blank values). Curve *B* represents the absorption of bromine from 2.1 ml. bromine water in the outer chamber.

Fig. 3.

Fig. 3. Curves *D*, *E*, *F* and *G* represent the bromine absorption from the outer chamber containing 0.1 ml. N KBr, 1 ml. of 40 % (by vol.) of sulphuric acid and 1 ml. of saturated, half-quarter- and one-sixteenth-saturated dichromate respectively. Experiments conducted at room temperature of about 18°.

however is not evident until the concentration of acid in the mixed fluids exceeds 24 %. The concentration of acid should in fact be maintained within 24–19 %, since a higher concentration than 24 % causes a loss in the total amount recovered and less than 19 % gives an unduly slow absorption.

In Fig. 2, curve *A* shows that a slight constant loss of iodine occurs from the central chamber (approximately 1 % per hour of the total amount present).

The effect of changing the dichromate concentration in the outer chamber. The effect is much less than changing the concentration of acid. Similar results were obtained with the permanganate system and chloride; but whereas the rate of chloride oxidation seemed to be proportional to the permanganate concentration, the influence of varying the concentration of dichromate appears to follow a square root relation.

The effect is shown in Fig. 3. It may be noted that saturated potassium dichromate at room temperature (18°) contains 9.8 g. dichromate per 100 ml. solution.

With regard to changes in the other conditions such as fluid volume, temperature *etc.*, the reader is referred to the previous communication on chloride [Conway, 1935].

DISCUSSION.

This method for micro-bromide determination is applicable to general inorganic analysis and to blood and urine determinations *etc.* With blood the normal bromide concentration may be determined in 1 ml. After bromide administration finger blood (0.2 ml.) can be used.

Pincussen and Roman [1929], supported by Zondek and Bier [1931] advanced the theory that normal blood bromide consisted largely of bromide combined in organic linkage and functioning like thyroxine as an important catalyst in the organism. The pituitary was thought to be the site of the formation of this substance. Its passage from the pituitary into the mid-brain was believed to be associated with the advent of normal sleep and the content of bromide in the blood to be much reduced in maniacal depressive states. The views of Pincussen and Zondek were based however on faulty methods as shown by Fleischacker and Scheiderer [1932].

Ucko [1934], who with Bernhardt [Bernhardt and Ucko, 1925; 1926] had obtained very high bromine values for the pituitary, has now stated that using a revised method he could not find more bromide in the pituitary than in other glands and that no valid evidence exists for assuming the presence of an organic bromide catalyst or for the assumption that a gland is associated with its production. From the work of Quastel and Yates [1934], Dixon [1935] and in particular the recent detailed studies of Leipert [1935], and of Leipert and Watzlawek [1935] there are no valid grounds for supposing that the normal blood bromide is other than the inorganic substance. Leipert has shown that protein precipitation, dialysis or ultrafiltration gives no indication of a bromo-protein compound. Moreover the bromine of blood is completely precipitated by silver nitrate, whereas organic bromide compounds of the type of dibromotyrosine are not precipitated by this reagent.

Concerning the origin of the normal blood bromide the greater part of this must come from the bromide associated with the natural chloride of the food although this does not often exceed 20 % of the total ingested. The Br/Cl ratio in commercial chloride has been found here not to exceed 1:7000 for four samples, whereas the Br/Cl ratio of sea water and of ordinary drinking water (*e.g.* Dublin Vartry water) is of the order 1:700 to 1000. Presumably the natural foodstuffs possess a similar ratio to the latter with regional variations. The commercial chloride taken would therefore dilute the "natural" ratio—the general limiting value of which may be presumed to be that of the sea.

The failure of the evidence hitherto presented for an organic bromide compound in the blood by no means removes biological interest from micro-determinations of bromide. Among other considerations the study of the Br/Cl ratio may throw considerable light on the chloride metabolism as well as on the functioning of such organs as the kidney. From the clinical side also an easy and accurate method for carrying out micro-bromide determinations on very small quantities (finger blood after a single therapeutic dose) is of considerable interest.

SUMMARY.

1. A method is given for the micro-determination of bromide, using the special absorbing apparatus already described. With the method, bromide can be determined to an ultimate standard deviation of 0.08% Br.

2. Using standard KBr solutions, the coefficient of variation for a single determination of 800 γ Br was found to be 0.6 % and with 80, 8 and 2 γ Br it was found to be 1.8, 1.5 and 4.0 % respectively.

3. In the method the bromide ion is oxidised to free bromine in the outer chamber and the formed bromine is then absorbed in 1 ml. of 20 % KI in the central chamber, the whole procedure being conducted at room temperature. The free iodine liberated is titrated with 0.05*N* thiosulphate (using the special burette already described) when the bromide exceeds about 80 γ . It is colorimetrically determined below this level, either directly or after starch addition, without standards, using modern methods of absolute colorimetry and in a similar manner to that already described for minute chloride determinations.

The determination may be also carried out with an ordinary colorimeter, after starch addition and using standards.

4. The oxidant selected as most suitable is acid dichromate. For all biological determinations where the chloride strength does not much exceed 1 % as NaCl, the determination is entirely independent of the presence of chloride even when the molar concentration of chloride is 7000 times that of bromide. With acid dichromate the method is not independent of the presence of iodide, but iodide can be easily and completely removed by a preliminary treatment which may be also made the basis of an initial iodide determination.

5. Applications of the method to the determination of bromide in normal blood and urine are described. Such determinations may be carried out on 1 ml. samples. After administration of bromide the determination can be carried out on finger blood (0.2 ml.). Bromide added to blood and urine can be quantitatively recovered.

6. The concentration of bromide in normal blood varied for 8 students between 227 and 572 γ /100 ml. The mean values for blood and urine were 372 and 656 γ /100 ml. respectively, the average chloride concentration of the urine being 605 mg./100 ml. The average Br/Cl ratio in urine was 1:2150, the halogen concentrations being expressed as milli-equiv.

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