CXLIII. INVESTIGATIONS INTO THE PRESENCE AND THE RÔLE OF BROMINE IN THE BODY.

BY H. UCKO.

From the Physiology Department, Guy's Hospital, London.

(Received April 28th, 1936.)

THE elements of the halogen group being generally associated in nature (as in minerals, sea-water, mineral springs) are absorbed together by plants and animals and should be present as normal constituents of the living tissue. The presence of chlorine, iodine and fluorine in the body is known while that of bromine, denied for a long time, is doubted even in the latest editions of some biochemical handbooks.

In the past, some authors claimed to have demonstrated occasionally the presence of bromine in urine and tissues, but only a few workers employed a suitable technique. Damiens [1922] found bromine in the organs of dogs, and Bernhardt and Ucko [1925; 1926] showed that it was constantly present in the blood and organs of man.

Zondek and Bier [1932; 1933] pointed out:

(1) That the normal bromine content of the blood and the cerebrospinal fluid was greatly diminished during the different stages of manic-depressive psychoses.

(2) That the hypophyseal bromine diminished or disappeared during artificial sleep whereas the bromine content of the mesencephalon increased. An extract containing the hypophyseal bromine was said to produce apathy when injected into dogs. The authors presumed that the pituitary secreted a substance containing bromine and that this substance could act upon the cerebral centres, facilitating the production of sleep.

The method of Roman [1929] and Pincussen [1932] employed by Zondek for these investigations has been studied by different authors [Fleischhacker and Scheiderer, 1933; Hahn, 1933, 1; Holtz and Roggenbau, 1933] and has been shown to be subject to gross errors. Bier and Roman [1933] did not succeed in the quantitative determination of bromine solutions given to them by the above-named authors. The method on which Zondek based his conclusions seems therefore to be unsuitable for the estimation of bromine in blood and tissues.

Other methods have recently been described. Leipert and Watzlawek [1934] destroyed the organic matter by sulphate of silver and chromic and sulphuric acids in a special apparatus, collected the halogenous vapours in NaOH and, using the formation of hypochlorite to oxidise bromides to bromates, determined bromine by iodimetric titration. Dixon [1934; 1935] utilised the same reaction, destroying the organic substance by incineration with alkali. As Dixon pointed out, in this type of reaction there will always be formation of chlorate which will liberate iodine. The quantities of iodine liberated in "blank" estimations were equivalent to 0.4 ml. of *N*/500 thiosulphate (=10.7% of bromine). After ignition with alkali, Francis and Harvey [1933] liberated bromine by a chromic-phosphoric acid mixture, collected it directly in a KI solution and titrated with thiosulphate. Yates [1933] used a chromic-sulphuric acid mixture of a certain concentration which is said to oxidise bromides completely without oxidising chlorides.

As a specific reaction for the determination of bromine seemed to be more satisfactory than one based on the general principles of oxidation-reduction, the author studied the colorimetric bromine reactions of Guareschi [1913] and Baubigny [1897]. The former, used in Bernhardt and Ueko's investigations, was found to be less suitable for exact quantitative determinations than Baubigny's reaction. This reaction consists in the formation of tetrabromofluorescein (eosin) by the action of bromine on fluorescein. The method employed is as follows.

Method.

To 10 ml. oxalated blood (0.2 g. potassium oxalate per 100 ml. of blood) or an equivalent weight of triturated organs in a nickel crucible is added 0.25 g. pure NaOH per g. dry weight of the material. The mixture is evaporated to dryness on a water-bath and then heated on a wire-gauze over a small flame. After the swelling of the mass and the formation of tarry vapours have ceased, it is heated again over a flame at a gradually increasing temperature and kept at a dark red heat for 2-3 min. (observed in a dark room). After cooling, the ash is moistened with water to dissolve the pyrophosphates which inhibit the further ignition. It is then dried and heated in the same way and this procedure is repeated till almost white ash remains (in case of blood the ash is brick-red). In this manner the organic matter is completely destroyed at a low temperature in 7-8 hours. The ash is moistened with very small quantities of hot water, thoroughly stirred up with a glass rod over a small flame and the contents filtered into a 10 ml. flask. This process is repeated several times and, provided that these precautions are adopted, 10 ml. of water are sufficient to extract the bromides quantitatively. If a very low bromine concentration is expected, the liquid is filtered into a small Erlenmeyer flask and the volume reduced by heating to 3-5 ml. The filtrate is then neutralised in presence of a small piece of litmus paper with 25 % H_2SO_4 and 33 % NaOH. The solution is vigorously shaken after the addition of each drop to aid the elimination of CO_2 . The total amount of the liquid must be noted.

For the colorimetric reaction, 0.04-0.06 up to 0.26 ml. of a ten times diluted standard solution, containing 0.1489 g. KBr (=0.1 g. Br) per litre, are delivered into a series of test tubes of 5 ml. capacity and absolutely uniform diameter. The contents of the tubes, containing 0.4-0.6 up to 2.6 γ of bromine, are made up with water to 1 ml.

Preliminary investigations have shown that a well-defined colour is given with amounts as small as 0.1 γ Br, provided the reaction is adjusted to p_H 5.3-5.4. Therefore, 0.5 ml. of a buffer solution consisting of a mixture of 1 part of *N* acetic acid and 4 parts of *N* sodium acetate is added to each tube.

One drop of a 0.125 % solution of fluorescein is added to each tube. This solution is prepared with fluorescein twice recrystallised from alcohol, 1.2 g. being dissolved in 25 ml. *N* NaOH and made up with water to 1 litre. It can be kept in the dark for several weeks.

To oxidise the bromides to bromine, one drop of a 0.4 % solution of chloramine T is added. The use of chloramine instead of chlorine water or hypochlorite was suggested by Hahn [1933, 2].

The tubes are shaken at once and the reaction is stopped, after exactly 1 min., by addition of two drops of 20 % ammonia and one drop of sodium bisulphite. On examining the different pink colours on a white ground in the day-light, differences of 0.1 γ of bromine are easily recognised.

The reaction is carried out in the same way with different quantities of the tissue filtrate, the colours compared with those of the standard range and the

arithmetic mean noted. This comparison gives better results than the use of a colorimeter.

About 200 bromine estimations have been carried out by this method. The only objection is the time and care to be devoted to the destruction of the organic matter (which has to be watched carefully). A suitable method of wet ignition would be more satisfactory.

Blank determinations showed that the quantities of reagents and of doubly distilled water employed did not contain appreciable amounts of bromine so that special purification of the reagents was unnecessary. The colorimetric estimation in the blood filtrate itself, without isolation of the bromine compounds, has the further advantage of avoiding any loss of bromine. The addition of phosphates, sulphates, chlorides and carbonates to bromine solutions did not affect the colorimetric reaction.

The presence of chlorides does not affect the reaction. Iodine forms with fluorescein the similarly coloured tetraiodofluorescein. Normally, there is less than 0.1 γ iodine in the quantities used for one estimation. In the case of tissues rich in iodine the latter must be previously separated.

Control of the method.

(1) In about thirty estimations of aqueous bromide solutions containing different quantities of bromides not known to the operator (from 0.4 to 3.5 γ) the error was below 0.1 γ .

(2) After the addition of dibromotyrosine to an organic mixture, otherwise free from bromine, and ignition with alkali, bromine was found with an accuracy of 3-4 %.

(3) The differences in about a hundred duplicate estimations of organic material were less than 5 %.

(4) The following table shows the amounts of bromine found in human blood and urine together with amounts after bromide has been added in quantities not previously known to the operator.

Table I.

Material	Br without addition mg. per 100 ml.	Quantity of Br added mg. per 100 ml.	Total Br		Differences %
			Calculated mg. per 100 ml.	Found mg. per 100 ml.	
Blood D	0.189	0.150	0.339	0.334	-1.5
" F	0.293	0.150	0.443	0.452	+2.0
" B	0.126	0.120	0.246	0.256	+4.0
" A	0.137	0.100	0.237	0.229	-3.4
" Ph	0.171	0.180	0.351	0.336	-4.3
" M	0.195	0.150	0.345	0.318	-7.9
Urine U	0.441	0.600	1.041	1.040	-0.1

According to the calculation of the probable error, the maximum error of the method would be 8-10 %. Practically, it was seldom higher than 5 %.

Results.

(1) In almost all *salts*, especially in halogen derivatives, bromine has been found. One sample of chloride of sodium, claimed to be chemically pure, contained 1.55 mg. of NaBr per 100 g.

(2) *The blood.* About 100 samples of human blood have been analysed. Bromine was regularly found. The figures, obtained from the blood of normal

persons never treated by bromine-containing medicaments, varied between 0.15 and 0.35 mg. per 100 ml. These values are lower than those previously recorded. Similar figures were found in the blood of dogs. The great variations in normal blood bromine recorded by some authors [Guillaumin, 1933; Yates and Hennelly, 1935] were not observed. As the normal chlorine and iodine concentrations in the blood are very constant, a relative invariability in the normal blood bromine seems to correspond well with the conditions of the other halogens. The ratio Br to Cl, *i.e.* Br/Cl in the blood was 0.0005–0.0014.

After bromine intake, the blood bromine rises markedly, while the total halogen content remains normal. As the rate of bromine elimination is very slow, an increased amount of bromine in blood and tissues can be found many months after the intake of Br has ceased. This fact may be one of the causes for the variable figures recorded by different authors. In France,¹ where these investigations have been performed, bromine-containing drugs are very rarely administered, and the author has had the opportunity of examining a large number of cases untreated by such medicaments [Ueko, 1934].

In ten cases the blood bromine level was high (0.6–3.0 mg. per 100 ml.). In two of these cases no evidence of previous bromine or iodine administration was found, and both suffered from Graves' disease; the blood bromine of one of them was 1.8 mg. per 100 ml. In neither case was iodine separated. Two other patients with Graves' disease showed normal figures.

The relative distribution of bromine between corpuscles and plasma was found to be very constant whether it was present in normal or artificially increased amounts. The ratio was about 1:3, lower therefore than that found for chlorine. No difference was found between the bromine content of whole and defibrinated blood, between plasma and serum and between corpuscles and blood-clot: from this it may be concluded that the Br content of plasma proteins is negligible.

To obtain some idea of the chemical state of bromine, the alcohol-soluble and insoluble fractions of bromine have been separated in two samples of normal blood, as has been done in the case of iodine [v. Fellenberg, 1926; Veil and Sturm, 1925].

To 1 part of total blood 4 parts of 95 % alcohol are added, the precipitate is filtered by suction, washed thrice with alcohol, the alcohol is distilled off and the residue and the precipitate separately ignited and analysed. About 1/5 of the total bromine was found to be in the alcohol-insoluble part of the blood, which suggests that this fraction of the blood bromine is present in some organic complex.

(3) *The urine.* No record of systematic investigations into the normal bromine content of the urine has been found. In about seventy 24-hour specimens examined bromine was constantly present, the amounts eliminated varying from 1.0 to 2.5 mg. The bromine content varied very much in samples passed at different periods of the day, but these variations were always related to the amount of chlorine (Br/Cl=0.0003–0.0005) and to the specific gravity. The Br/Cl is therefore much lower in the urine than in blood and tissue (see below). After the intake of large amounts of bromine the bromine output is increased and the ratio rises. The highest ratio found among the cases examined was 0.1–1. An increased urinary bromine was always associated with a high blood bromine level.

In cases where retention of bromine was very marked, the Br/Cl was found to be very similar in urine and blood.

¹ Hôtel-Dieu, Paris (Prof. Carnot).

In one determination of the alcohol-insoluble part of the urinary bromine, only traces were found suggesting that the urinary bromine was entirely present in an alcohol-soluble form.

After intravenous injection of 1 g. NaBr (=780 mg. bromine) the average bromine elimination was:

	mg. Br
1- 4 hours after injection	7-11
4- 8 " "	2-3
8-24 " "	5-10
Total amount in 24 hours	14-24

The elimination of very small doses (0.3-0.5 mg. daily) was studied in a dog. The animal was fed on a standard diet on which the daily urinary output contained 1.2-1.4 mg. Br. After 8 days of this diet small doses of bromine were given daily with a total of 15 mg. in 35 days. The output of urinary bromine increased in the first 2 days to a figure of 1.7-1.9 mg. and remained at that level for the rest of the experimental period. At the end of the experiment the blood bromine had risen from 0.180 to 0.286 mg. per 100 ml. indicating an increase of about 0.63 mg. of bromine in the blood (=1/13 of body weight). In proportion to the intake much more bromine was eliminated during 24 hours in this experiment than after the injection of one large dose.

(4) *The gastric juice.* In ten normal patients 0.5-0.9 mg. per 100 ml. (in one case 1.2 mg. per 100 ml.) of bromine were found in the gastric juice of the "fasting" stomach. It seems to be present "normally" and in a higher percentage than in the blood. The Br/Cl was between 0.0017 and 0.0055.

The effect of histamine upon the bromine content of gastric juice was studied. After withdrawal of the fasting juice 1 ml. of 1% histamine phosphate was injected and gastric samples collected at 10-min. intervals. The bromine, total chlorine and free hydrochloric acid were estimated in each sample and the Br to Cl ratio calculated. Table II gives the results of three typical experiments. It will be seen that histamine has no apparent effect upon the normal bromine content, despite great variation in the chlorine content and free acidity.

Table II.

Time in min. after injection	Bromine mg. per 100 ml.			Chlorine mg. per 100 ml.			Free acid = mg. per 100 ml. Cl			Br/Cl		
	1	2	3	1	2	3	1	2	3	1	2	3
Initial	1.2	0.76	0.57	308	292	346	102	73	73	0.0055	0.0026	0.0017
10	1.19	0.80	0.58	481	407	316	314	230	175	0.0025	0.0020	0.0018
20	1.15			537			387			0.0021		
30	0.87	0.57		505	316		226	0		0.0017	0.0018	
40	0.73			423			153			0.0017		

The effect of intravenous injection of bromide was studied in a similar manner. 1 g. NaBr was injected intravenously and gastric samples were removed

Table III.

Time in min. after NaBr	mg. per 100 ml. Br after NaBr injection
Initial	0.82
10	10.0
20	12.0
30	13.0
40	—
50	13.0
60	

at 10-min. intervals. Table III gives the result of a typical experiment and it will be seen that there is a marked and maintained rise in the bromine content. The effect of histamine upon this raised output was investigated (Table IV) and no appreciable difference was recorded.

Table IV.

Time in min. after NaBr	Time in min. after histamine	mg. per 100 ml. Br after NaBr and histamine injection	Br/Cl
Initial	—	0.87	0.0024
10	—	—	—
20	—	—	—
30	10	13.4	0.0355
40	20	13.7	0.0299
50	30	13.2	0.0287
60	40	14.1	0.0310

If repeated doses of bromide have been taken so that a large part of the gastric chlorine has been replaced by bromine, histamine seems to have an effect on the gastric bromine level so that, under these conditions, bromine appears to have become an integral component of the secretory response. An example of this is given in Table V.

Table V. *Histamine effect after intake of 6 g. NaBr by mouth daily during 5 days (total amount = 28 g. bromine).*

Time in min. after histamine	Bromine mg. per 100 ml.	Total halogens calculated as mg. per 100 ml. Cl	Chlorine mg. per 100 ml.	Free acid = mg. per 100 ml. Cl
Initial	108	284	236	18
10	160	500	430	280
20	143	572	520	419
30	130	550	492	406
40	135	547	487	391
50	160	543	468	351

(5) *The organs.* Table VI shows the bromine figures obtained by the analysis of different organs.

Unfortunately, human pituitaries could not be obtained for analysis. In animals (except in the case of one bull) the hypophyseal figures were not higher than those of other organs. Dixon found the bromine content of human pituitaries to vary from 0.4 to 2.4 mg. per 100 g., calculated for wet weight; in four exceptional cases it was 4.4, 4.0, 9.0 and 26. The last two cases had previously received bromine and in the first cases some possibility of bromine dosage was presumed.

Serbescu and Buttu [1934] claimed recently that the thyroid gland contains 2–3.1 mg. per 100 g. of bromine although the method they employed failed to demonstrate the presence of bromine in the blood and in most human organs (limit of sensitivity of method 30 γ). The authors have apparently not succeeded in removing all iodine from the thyroid, a necessary preliminary for the determination of bromine by colorimetric methods.

A satisfactory procedure for the removal of iodine is to oxidise the iodides in an acid medium by the addition of a few drops of a 10 % solution of sodium nitrite. Iodine is collected by shaking in chloroform. Nitrous acid does not oxidise bromides if present in a low concentration. The process is repeated till

Table VI.

Organ	Species	Bromine mg. per 100 g.	Chlorine mg. per 100 g.	Br/Cl
Pituitary	Ox	0.59	206	0.0029
"	Ox	0.78	—	—
"	Ox	0.59	—	—
"	Cow	0.61	168	0.0037
"	Cow	0.55	178	0.0031
"	Bull	1.47	182	0.0080
Pituitary, commerc. powder		3.3 = 0.66*	1170	0.0028
Suprarenal	Ox	0.39	—	—
"	Ox	0.58	144	0.0040
"	Cow	0.62	144	0.0043
Suprarenal, commerc. powder		4.9 = 1.2*	586	0.0086
Suprarenal	Man	0.66	171	0.0038
"	Man	0.33	180	0.0018
Testicle	Ram	0.57	175	0.0033
Epididymis	Ram	0.80	—	—
Ovary	Calf	0.55	151	0.0037
Ovary (two corp. lut.)	Cow	0.61	—	—
Pancreas	Dog	0.10	—	—
"	Dog	0.13	—	—
Thyroid	Ox	0.89	123	0.0073
"	Cow	0.75	112	0.0067
"	Dog	0.42	115	0.0037
"	Dog	0.94	—	—
"	Man	0.48	—	—
Thyroid, commerc. powder		5.6 = 1.1*	—	—
Spleen	Dog	0.26	—	—
"	Man	0.33	200	0.0017
"	Man	0.24	158	0.0015
Liver	Man	0.17	—	—
Liver (cirrhosis)	Man	0.14	—	—
Kidney	Man	0.27	165	0.0016

* Wet (calculated).

the chloroform gives no coloration with a starch solution. Using these precautions the bromine content of the thyroid (see Table VI) was found to be not markedly higher than that of other organs.

(6) *Bromine in mental diseases.* Ten typical manic-depressives were examined and the amounts of bromine in blood and urine determined during the active phases of their mental disease. In nine of them the figures for blood and urinary bromine lay within normal limits. The tenth patient had been treated previously with bromides and had a blood bromide of 26 mg. per 100 ml. (Br/Cl=0.096) and a urinary excretion of 19 mg. Br in morning sample (Br/Cl=0.08). Two cases, one immediately after an epileptic fit and one in a state of stupor, showed similar normal bromine figures. Though the number of cases is small, it can be concluded that there is no constant diminution of blood bromine in cases of manic-depressive psychosis.

CONCLUSIONS.

As bromine is eliminated extremely slowly from the body and can accumulate in fluids and tissues in very great amounts, "normal" figures can be misleading and high figures, *e.g.* pituitary, may be due to unrecorded previous administration of bromine-containing drugs. Bernhardt and Ucko [1926] pointed out that it was unlikely that the inorganic bromine of the body had any specific functional significance owing to the fact that it accumulated so easily in the body without

producing any obvious effect. If this assumption is correct, bromine could only have a specific rôle as a normal constituent of the body if it were present in a special chemical state. The observation that 1/5 of the blood bromine is found in the alcohol-insoluble part of the blood, but only traces in the alcohol-insoluble part of the urine, suggests the possibility of the existence of some bromine complex which is not allowed to escape *via* the kidney.

The main source of bromine is the food, especially common salt. Plants regularly contain small quantities of bromine [Damiens and Blaignan, 1931; 1932]. The "normal" figures will therefore only refer to the bromine content on a "normal" diet. The maintenance of a constant level of blood chloride despite great variation in chlorine intake points to a delicate regulation of the chlorine balance in the body: a similar type of regulation would appear to control the bromine level. This regulation is sufficiently delicate to prevent any accumulation of bromine as a result of dietetic variations or following very small doses of bromides. It would appear that the smaller the dose of bromine taken, the smaller is the proportion retained. This may explain why the food bromine does not accumulate in the body. After the administration of large doses, bromine seems to be stored in tissues and body fluids, as previous observations and its appearance in the gastric juice suggest.

Normally, a definite relationship exists between the distribution of bromine and chlorine in the body. The ratio Br to Cl is 5-10 times smaller in the urine than in the tissues.

	Br/Cl (average)
Organs	0.0015-0.0080
Gastric juice	0.0017-0.0055
Blood	0.0005-0.0014
Urine	0.0003-0.0006

An explanation of the low bromine content of the urine would be that normally the kidneys do not excrete bromine above a certain limit. It is therefore retained in the body in higher proportions when present in large amounts. Viewed in this light, the replacement of chlorides in the body by bromides, formerly attributed to a "lack of discrimination" on the part of the kidneys regarding halogens, would be due to a restriction of bromide excretion and to a compensatory increased excretion of chlorides to maintain the normal osmotic equilibrium. Thus the total halogen level in the body remains unaltered.

The kidneys, however, are capable of excreting far larger amounts of bromine than they do normally. If a certain degree of bromine storage has been established, we see that even small quantities of urine may contain several mg. of bromine. Under these conditions, the Br to Cl ratio rises till it becomes of the same order as that of the blood, so that bromine is then excreted in the same proportion to chlorine as it exists in the body.

We do not know whether the physiological retention of bromine depends solely on a renal factor or whether a tissue factor is also concerned. Nor do we know whether the high output after bromine storage is due to an increased permeability of the normal renal filter or to a lack of storage capacity in the tissues.

SUMMARY.

1. A method is described and criticised for the determination of bromine in body fluids and tissues.
2. The figures for bromine found in blood, urine, gastric juice and tissues in normal cases and after the intake of bromine are recorded.

3. Ten manic-depressive patients showed normal figures both in blood and urine.

4. The bromine equilibrium in the body and its relationship to the chlorine content are discussed.

REFERENCES.

- Baubigny (1897). *Compt. Rend. Acad. Sci.* **125**, 654.
Bernhardt and Ucko (1925). *Biochem. Z.* **155**, 174.
—— (1926). *Biochem. Z.* **170**, 459.
Bier and Roman (1933). *Klin. Woch.* **12**, 391.
Damiens (1921). *Bull. Sci. Pharmac.* **27**, 609.
—— (1922). *Bull. Sci. Pharmac.* **28**, 37, 85.
—— and Blaignan (1931). *Compt. Rend. Acad. Sci.* **193**, 1460.
—— (1932). *Compt. Rend. Acad. Sci.* **194**, 2077.
Dixon (1934). *Biochem. J.* **28**, 48.
—— (1935). *Biochem. J.* **29**, 86.
von Fellenberg (1926). *Ergebn. Physiol.* **25**, 176.
Fleischhacker and Scheiderer (1933). *Monat. Psych. Neur.* **84**, 348.
Francis and Harvey (1933). *Biochem. J.* **27**, 1545.
Guareschi (1913). *Z. anal. Chem.* **52**, 454, 538, 609.
Guillaumin (1933). *Progrès Médical*, No. 48.
Hahn (1933, 1). *Klin. Woch.* **12**, 390.
—— (1933, 2). *Compt. Rend. Acad. Sci.* **197**, 245.
Holtz and Roggenbau (1933). *Klin. Woch.* **12**, 1410.
Leipert and Watzlawek (1934). *Z. physiol. Chem.* **226**, 108.
Pincussen (1932). *Klin. Woch.* **11**, 1550.
Roman (1929). *Biochem. Z.* **207**, 416.
Serbescu and Buttu (1934). *Bull. Acad. Med.* **111**, 232.
Ucko (1934). *Compt. Rend. Soc. Biol.* **116**, 48.
Veil and Sturm (1925). *Deutsch. Arch. klin. Med.* **147**, 166.
Yates (1933). *Biochem. J.* **27**, 1763.
—— and Hennelly (1935). *J. Mental Sci.* **81**, 173.
Zondek and Bier (1932). *Klin. Woch.* **11**, 633, 759.
—— (1933). *Klin. Woch.* **12**, 55.