THE DISTRIBUTION OF BROMIDE AND CHLORIDE IN THE BODY *

By STANLEY HELLERSTEIN, CAROLYN KAISER, DANIEL DeS. DARROW AND DANIEL C. DARROW

(From the Department of Pediatrics, Kansas University and Mercy Children's Hospital, Kansas City, Mo.)

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Over sixty years ago, Nencki and Schoumow-Simanowsky (1) suggested that bromide displaces chloride in tissues. Twenty years ago, Wallace, Brodie, Brand and Leshin (2–4) and Weir and Hastings (5) showed that, when large doses of bromide are administered, the ratios of chloride to bromide are the same in serum and in the tissues excepting the brain and spinal fluid. We have been unable to find analyses comparing the ratios of chloride with bromide in sera and individual tissues when the small amounts of bromide suitable for clinical studies are given. However, it has been rather generally assumed that the ratio of serum bromide to chloride after administering a known amount of bromide is a measure of total body chloride (4–10). Cheek and West (8) found that total chloride of rats agreed well with total chloride calculated from bromide dilution. If this finding is correct, total body chloride may be followed for a considerable time after a single dose of bromide. This conclusion follows from the evidence that bromide is excreted slowly and practically entirely in the urine when there is no diarrhea. The present study re-examines the distribution of bromide for intervals of 6, 24 and 72 hours. In addition, bromide space was measured at the same intervals in rats and for ten days in humans following a single dose of bromide. A comparison of total chloride by analysis and by bromide dilution is given for normal rats and rats subjected to alteration in body chloride.

CHEMICAL METHODS

Water and fat were determined by weighing fresh tissues before and after drying at 105° C and after extracting with ether. Duplicate aliquots of dried, fat-free ground tissues were used to determine chloride and bromide, respectively, by the Volhard titration (11) and Brodie and Friedman's (12) iodometric titration of bromate formed by hypochlorite. Hunter and Goldspink's modifications (13, 14) were used, together with certain adaptations described in the following Remarks. Serum chloride was determined on two or three 0.2 ml samples; bromide on a single 1 ml sample of plasma and heparinized whole blood. Serum and whole blood bromide was determined in duplicate on the human subjects.

The chief difficulty of the bromide determination involves preparation of a suitable solution. Bromide added to serum was recovered within 2 per cent when the reaction was carried out on the solution obtained by adding 0.5 ml of 2 N KOH and heating for 15 minutes at 575° C. After breaking up the charred material with a little water, a similar second heating was carried out. Similar treatment of 50 to 100 mg of fat-free dried tissues did not consistently yield a colorless solution. The titrations were slightly high on adding bromide to tissues of rats which had received no bromide. However, when 1 g of fat-free dried tissues was extracted for 18 hours with 15 ml of water, the chloride and bromide were recovered quantitatively in aliquots of the water. Heating this water extract as described for serum yielded colorless solutions for bromide determination. The same water extract was used for chloride using aliquots yielding suitable titrations after adding 1 ml of 0.05 N $AgNO_3$ in HNO_3. Chloride is the Volhard titration minus bromide. Ether extracts have been repeatedly tested for chloride and none found. Similar tests for bromide were not made.

Remarks on bromide determination. The directions of Hunter (13) were followed. Sodium bromide was added to serum to make concentrations of 1, 2, 3 and 4 mEq of Br^- per L. The same amounts of bromide were added to 100 mg of fat-free dried muscle. The recovery varied from 96.5 to 98.2 per cent of the contents, and was more complete when bromide was high. Correction for losses during ashing was applied according to the Br^- content of the sample. Replicate analyses showed a standard deviation of 2.5 per cent of the contents.

When Pyrex flasks were used for repeated ashing, slightly high blanks were obtained when the flasks had been treated solely with dichromate. However, the flasks gave the same blanks as new flasks when they were boiled for 15 minutes in 15 per cent Na_2CO_3 and were then treated with dichromate cleaning fluid. The ashing with KOH produces considerable etching which does not seriously interfere with the titration.

An approximately normal solution of sodium hypochlorite was prepared as follows. Forty-six g of NaOH was dissolved slowly in 700 ml of water. About 90 g of calcium hypochlorite was added. A caked precipitate

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formed. This was broken up by intermittent, rather vigorous shaking for several hours. After standing overnight, the solution was filtered and washed with enough water to make 1 L. A large, wide-mouthed glass jar was convenient. A small amount of bromate contaminates the reagents (14); the blank remains surprisingly constant. The blanks have varied from 0.038 to 0.045 ml of 0.05 N thiosulfate in preparations made over a period of 3 years. The solution remains satisfactory for at least 2 months when kept in a refrigerator in a brown bottle.

Remarks on chloride. Duplicates of skin chloride gave a deviation 1.5 ± 0.8 per cent of the value. The deviation of muscle and carcass was somewhat greater, 3.1 ± 1.0. In the case of the carcass, the deviation may in part be explained by less uniform samples.

RESULTS

Observations on rats

Only male rats weighing 250 to 300 g were used. These were a Sprague-Dawley strain, all obtained from the same breeding farm.

Bromide space. A group of rats was divided into groups of six. The animals were placed in separate cages in order to collect urine for 24 hours to provide control bromide excretions. Enough NaBr was injected into the peritoneal cavity to raise serum bromide to about 3 mEq per L in four rats while two received equivalent amounts of NaCl in order to provide control values for serum bromide. Twenty-four hours before terminating the observation, food was removed from the cages and water containing 10 per cent glucose and 50, 20 and 30 mEq per L of Na, K and Cl^-, respectively, was available. Four hours before killing the rats, both food and drink were removed from the cage. The rats were killed by withdrawing as much blood as possible from the abdominal aorta under ether anesthesia. Washings of the cage were included with the urine in order to assure as complete collection as possible. The intervals after injection of bromide were 6, 24 and 72 hours.

Table I shows the bromide space calculated as follows:

$$\frac{Br - Br_u - Br_r}{Br_s \times 1.13} = \text{bromide space}$$

where Br is the bromide injected; Br_u is urine bromide; Br_r is red cell bromide calculated from the proportion of red cells in blood and whole blood bromide, assuming a blood volume of 80 ml per kg; Br_s is serum bromide while the factor combines a correction of serum water (0.93) and a Donnan factor of 0.95. Control serum bromide was subtracted from values of the experimental rats and control urine bromide from urine bromides.

TABLE I

Bromide space of rats

Number	Weight	Interval	Br^- space
	g	*hrs*	*ml/kg*
4	270 ± 12	6	249 ± 9
4	255 ± 5	24	240 ± 6
4	250 ± 21	72	305 ± 10

Table I shows the average and standard deviation. The bromide space does not differ statistically at 6 and 24 hours but is greater at 72 hours. As is shown later, the ratios of chloride to bromide agree in the serum and in certain tissues (liver, muscle and skin) at 6, 24 and 72 hours. Consequently the increase in bromide space at 72 hours is probably erroneous owing to failure to measure bromide losses completely.

Distribution of bromide and chloride in tissues. Rats were killed as previously described at 6, 24 and 72 hours after receiving 0.32 mmole of NaBr intraperitoneally. Liver and samples of serum, muscle and skin were analyzed for chloride and bromide. Table II shows the ratios of chloride to bromide in serum and tissues together with serum bromide. The average and standard deviation of each group is given. Plotting the serum against the tissue ratios shows good direct correlation in each group despite the small variations within a group. Because there was no significant difference in the relation of the ratios of serum and tissues in the groups, the data were treated as one homogeneous sample for which the average and standard deviations, and the correlation coefficients between the serum ratios and the ratios of liver, muscle and skin, are given. The surprisingly high correlations indicate that the tissue ratios may be predicted with confidence for these tissues. The regression equations are:

$$R_l = 1.07\ R_s - 175 \pm 1.4$$
$$R_m = 0.915\ R_s + 5.4 \pm 0.9$$
$$R_{sk} = 0.986\ R_s + 0.5 \pm 0.5$$

where R_l, R_m, R_{sk} and R_s are, respectively, the ratios of chloride to bromide of liver, muscle, skin

TABLE II

Ratios of chloride to bromide in serum and tissues (R_{st})

		Serum Br^-	Ratio of Cl^- to Br^-			
			Serum	Liver	Muscle	Skin
hrs	*no.*	*mEq/L*				
6	4	3.35	31.5 ± 1.8	32.8 ± 1.6	35.0 ± 2.1	31.3 ± 2.7
24	8	2.75	35.6 ± 2.8	35.4 ± 4.3	38.3 ± 5.7	34.3 ± 4.0
72	8	1.88	54.1 ± 7.3	56.6 ± 3.7	54.3 ± 9.3	53.6 ± 8.1
All	20	2.50	42.2 ± 11.1	43.3 ± 13.3	44.0 ± 11.0	41.0 ± 11.2
R_{st}				0.89	0.93	0.98

and serum. The greater correlation between serum and skin probably results from smaller proportionate errors in these determinations. Since the determination of both bromide and chloride involves a relatively constant titration error, the proportionate error is less in skin and serum, in which the halogen content is higher, than in muscle and liver.

Estimation of total body chloride by bromide. Because muscle and skin contain the largest part of total body chloride, it is likely that the ratio of chloride to bromide in total body tissues is about the same as in serum. Accordingly, total body chloride is equal to retained bromide multiplied by the ratio of chloride to bromide in serum. This hypothesis was tested by injecting 0.32 mmole of NaBr and collecting urine for 24 hours. At this time the rats were killed and serum collected. The carcasses were ground in a meat grinder. An aliquot was dried, extracted with ether, and again ground with a mortar and pestle. Table III shows the serum chloride, the ratios of chloride to bromide in serum and tissues, and Cl^- calculated from retained bromide and direct tissue analyses. Tissue chloride is calculated per kilogram of fat-free tissues.

TABLE III

*Comparison of tissue Cl^- by analysis and Br^- dilution**

	Serum Cl^-	Ratio Cl^- : Br^-		Per kg fat-free tissues		
		Serum	Tissue	Cl^-	$Cl^-_{Br^-}$	Water
	mEq/L			*mEq*	*mEq*	*g*
Normal	97	35	35	30	32	738
Normal	95	33	35	29	29	730
Low Cl	85	30	32	24	25	703
Low Cl	76	26	28	21	22	726
NaCl	91	29	31	30	29	715
NaCl	103	34	37	36	37	706
DCA	102	37	39	39	38	734
DCA	104	34	37	35	36	730

* Low Cl^-: peritoneal losses of Na, 9.5 and 14; of Cl 7 and 9 mEq/kg. NaCl: received about 51 ml of physiological saline per kg. DCA: received same saline with 7 mg DCA in oil. $Cl^-_{Br^-}$: body chloride calculated from Br^-.

The rats labeled normal received no treatment except the injection of bromide. The rats labeled low Cl^- were subjected to removal of Cl^- and Na by the injection of 35 ml of 5 per cent glucose into the peritoneal cavity. After four hours the fluid was removed by opening the peritoneal cavity under ether anesthesia, and bromide was given subcutaneously. The loss of Na and Cl^- per kilogram of fat-free tissues is indicated. The rats labeled NaCl received 15 ml of physiological saline 24 hours before injecting bromide while those labeled DCA received 7 mg of desoxycorticosterone as well as the same amount of saline.

The data indicate that injection of bromide permits calculation of total body chloride as retained bromide multiplied by the ratio of chloride to bromide in serum. The chloride removed in the low Cl^- rats is consistent with the values found. The retention of Cl^- was not determined in the rats receiving saline. The data confirm the study of Cheek and West (8).

Observations on humans

The volume of dilution of bromide in adult males. Four adult males ingested sufficient KBr to raise serum bromide concentration to about 3 mEq per L. Urinary bromide losses were measured and serum and whole blood concentrations were followed for periods up to ten days. The subjects, aged 25 to 31 years, were in good health. During the study they carried out their usual activities; all urines were collected. The usual diet was eaten and presumably contained no unusual source of bromide. Samples of blood and urine were obtained before ingestion of bromide in order to provide control urine and serum bromide con-

centrations. These values were subtracted from those of the experimental periods. Bromide concentration was small before ingestion of bromide, generally about 0.1 mEq per L. Bromide space was calculated as was indicated for the similar measurements of bromide space in rats. The observations are comparable, except the second one on S.H. dated 5/10/57 (Figure 1). In this case five tablets containing 40 mEq of Cl^- were ingested thrice daily after the fifth day (Thermotabs; Merck, Sharp and Dohme, containing 0.45 g NaCl and 0.03 g KCl). Data on the rate of urinary excretion of bromide in this study confirm the well recognized augmentation of urinary bromide when urinary chloride increases. The average rate of excretion from the second to fifth day was 0.101 mEq per hour. The rate from the sixth through the ninth day was 0.15 mEq per hour. At the beginning of the second day 66 mEq of bromide was in the body while at the beginning of the sixth day body bromide was 55 mEq.

The data are illustrated in Figure 1. The volume of bromide space increased beyond the analytical errors from the sixth to the twenty-fourth hour in S.H., 10/29/56, and in L.T., but the other values do not certainly change. Assuming that the volume at 24 hours is most nearly accurate, Subject S.H. showed essentially constant bromide space in one study for six days and in another for ten days. However, the other studies show a slight tendency to rise. Table IV shows the value at 24 hours together with the average after three days. It will be noted that the average shows remarkably little variation after the third day. However, inspection of the figure indicates that the later values are greater than the earlier ones. A rise in measurements of bromide

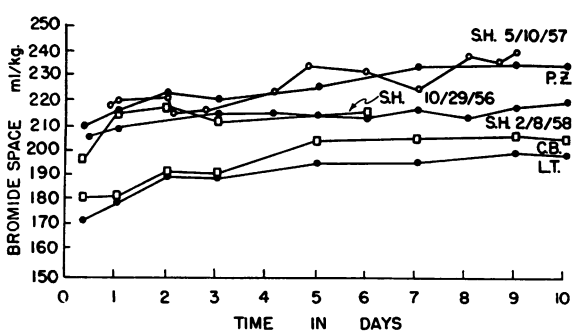

FIG. 1. BROMIDE SPACE IN ADULT MALES. See text for note on S.H., 5/10/57, concerning load of chloride.

TABLE IV

Bromide space in adult males

Subject		Bromide vol. of dilution: At 24 hrs	After 72 hrs: Mean ± SD	After 72 hrs: No. of obs.
	kg	*ml/kg*	*ml/kg*	
L. T.	87.7	179	197 ± 2.2	4
C. B.	91.5	182	206 ± 1.4	4
P. Z.	77.2	216	232 ± 4.2	4
S. H.	87.0 5/10/57	219	231 ± 6.6	9
S. H.	88.7 2/8/58	209	216 ± 2.5	8

space would occur if bromide loss is not completely measured or if bromide is concentrated with respect to chloride in certain tissues over a period of time. Because the rat tissues showed consistent agreement between the ratios of chloride to bromide in serum, liver, skin and muscle for 72 hours, the findings on bromide space are probably due to failure to collect small amounts of urine or to significant losses of bromide in stools or from the skin. The data indicate that measurements of bromide space do not involve appreciable errors over a short period, but significant errors occur over long periods.

DISCUSSION

The previous data comparing the ratios of bromide to chloride in sera and tissues are summarized in Table V. The relationship is indicated by R_s/R_t where R_s is the ratio of chloride to bromide in serum, and R_t is the corresponding ratio in tissues. Unity indicates identity of the ratios while values above or below unity indicate, respectively, relative concentration or dilution of bromide in the tissues. As was recognized by the investigators, the interval after injection was probably too short to assure stable equilibrium in the first five observations. The other data show reasonably good agreement, but the number of determinations is small and occasional discrepancies are shown. A more serious objection is that 20 to 50 per cent of serum chloride is displaced by bromide. Clinical use of bromide should involve concentrations of bromide well below the toxic level. Furthermore, large amounts of bromide may obscure small accumulations of bromide in tissues. The present data support the con-

clusion that bromide occurs in essentially the same ratio in serum and in certain tissues when small as well as large doses of bromide are given.

The concentration of bromide and chloride has been compared in serum and extracellular accumulations of fluid in patients and in experimental animals (4, 5, 9, 15). The agreement is satisfactory. Consequently, the relation of bromide to chloride is essentially the same as in serum in the parts of extracellular fluid which closely resemble an ultrafiltrate. Earlier work recognized that bromide is not found in the brain, in the same relation to chloride, as it is found in serum. Since cerebrospinal fluid may be regarded as representing the extracellular fluid of the brain, the ratios in the brain may be considered to be those of cerebrospinal fluid (3, 15, 16). The ratio of bromide of cerebrospinal fluid to that of serum is 0.75 while the corresponding ratio of chloride is 1.20 (3). Similarly, the ratio of bromide in red cells to that of serum is 0.75 while that of chloride is 0.67 (17–19). Gastric juice is known to contain more bromide relative to chloride than does serum (20). Also, urinary bromide does not precisely vary with the serum ratio (19).

It is true that these recognized discrepancies cannot introduce large errors into the calculation of total body chloride from body bromide and the ratio of chloride to bromide in serum. For example, the sum of the chloride in the central nervous system, red cells and gastric juice is about 3.5 mEq per kg in an adult. Consequently, an error of 30 per cent in these tissues results in an error of only about 3.5 per cent in total body chloride. Since skin and muscle contain about 50 per cent of total body chloride in an adult human, and 60 per cent in the rat, the present data give satisfactory support that total body chloride may be measured by administering bromide.

The errors of the balance techniques are involved in measurements of bromide space or body chloride following a single dose of bromide. Expressing average bromide space in the present human studies as percentage of the 24 hour volume, the following values are obtained: six hours, 95 per cent; two days, 102.5 per cent; three days, 102.6 per cent; four to five days, 105 per cent; six to seven days, 105 per cent; nine to ten days, 107.5 per cent. All values at six hours are below the 24 hour volume although three are within 3 per cent, which is within the analytical errors. It is likely that six hours is not sufficient time always to achieve a stable equilibrium after oral administration of bromide, although three hours is suffi-

TABLE V

Distribution of chloride and bromide in tissues (from the literature) *

Subject	Route	Time	Serum conc.		Ratio R_s to R_t§					Reference
			Cl^-	Br^-	Liver	Striate muscle	Smooth muscle	Lung	Skin	
			mEq/L							
Dog	Vein	1 hour	72	43		1.01				(5)
Dog	Vein	1 hour	79	34		0.78			0.94	(5)
Dog	Vein	1 hour	72	49		0.92			0.97	(5)
Dog	Vein	1 hour	75	33		0.78			0.95	(5)
Dog	Vein	1 hour	69	40		0.82			0.93	(5)
Dog	Vein	3 hours	96	27	0.95	1.02	1.02	1.08	1.03	(2)
Cat	Vein	3 hours	118	22	0.99	1.11		0.95	1.01	(2)
Dog	Vein	3 hours	96	27	0.96			1.08		(3)
Dog	Oral	24 hours	97	21	0.93					(3)
Dog	Oral	48 hours	82	28	0.86			0.91		(3)
Dog†	Oral	24 hours	88	30	0.86	0.83		0.94		(3)
Dog‡	Oral	15 days	86	28		0.96				(5)
Dog	Oral	4 days	84	22	1.06	1.03	1.05			(5)
Dog	Oral	4 days	84	31		1.38				(5)
Dog	Oral	?	71	40	0.99		1.08	1.08	1.11	(5)
Dog	Oral	?	79	31	1.05	1.11			0.97	(5)

* The first five were studies 45 to 60 minutes after injection. As was noted by the authors this may not be long enough for equilibration.

† Given daily bromide for eight days and samples 24 hours later.

‡ The paper does not state the interval after administering last dose of bromide in the last five listings. The dogs are known to have received bromide for the intervals indicated except for the last two.

§ R_s = ratio of chloride to bromide of serum; R_t = ratio of chloride to bromide of tissues.

cient after intravenous injections (4, 5, 9). In the rat, six hours sufficed after intraperitoneal injection. Assuming that the rise in average bromide space reflects only errors in measuring bromide excretion, the error in these observations is about 1 per cent per day. From previous experience in other types of balance observations, we know that total excretion is seldom measured. Consequently, the errors are accumulative in the direction of indicating retentions. The cumulative error in estimating bromide retention explains the rise in bromide space with time and the rise in estimated total body chloride. The data indicate that bromide may be used to measure total chloride when this systematic error is kept in mind. The error is unlikely to be great for a period of one to three days but it is likely to be serious over longer periods. Successive measurements of body chloride following a single dose of bromide should be most satisfactory when changes in body chloride are large over a short period.

SUMMARY

The ratio of serum chloride to bromide in serum is similar to the ratio of these ions in liver, muscle and skin at 6, 24 and 72 hours after injection of NaBr into the peritoneal cavity of rats in amounts producing a serum concentration of about 3 mEq per L. Bromide space of rats is constant at 6 and 24 hours but is considerably greater at 72 hours. Following oral ingestion of KBr in human adults, bromide space averages 95 per cent of the 24 hour space at 6 hours and slowly increases for 10 days to about 107 per cent of the 24 hour space. In view of the consistent agreement between the ratio of Cl^- to Br^- in serum and tissues of rats for three days, the increase in bromide space is probably erroneous owing to failure completely to measure bromide losses. The studies indicate that the ratio of chloride to bromide in serum, multiplied by body content of bromide, is close to total body chloride. Application of this relationship involves the usual errors of balance techniques.

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