On the Bromide Test of Permeability of the Barrier between Blood and Cerebrospinal Fluid—an Assessment

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The publication of Walter's monograph (1929) on the blood/cerebrospinal-fluid barrier was followed by the widespread use of the bromide test in diseased conditions of the brain and spinal cord. The bromide test consists in the administration of bromide to the patient; after allowing 24 hr. for equilibration with the cerebrospinal fluid (c.s.f.), the content of bromide in the serum and c.s.f. is determined. The permeability of the barrier is then judged by the magnitude of the ratio (bromide/unit vol. serum)/(bromide/unit vol. c.s.f.). Walter found normal control ratios to lie in the range 2·90–3·30 (mean 3·10). In pathological states causing the greatest increase in permeability (syphilitic or tuberculous meningitis) the ratio was near 2·0, although a few values as low as 1·50 have been recorded. On the other hand, ratios over 3·50 were found in a number of conditions.

Walter emphasized certain desirable features of the bromide test. Bromide is excreted very slowly, so the blood level remains relatively constant over long periods (days or weeks), permitting adequate time for the establishment of equilibrium between blood and c.s.f., and making possible several tests without further bromide administration. Bromide does not enter metabolism and might thus be expected to qualify as a test substance for permeability. It is neither bound by, nor adsorbed to, protein. It can be determined with adequate precision at low levels in serum or c.s.f. It is neither disliked by, nor toxic to, patients, and at the levels used is unlikely to affect barrier permeability. Its normal distribution between serum and c.s.f. gives a ratio well removed from unity so that decreases or increases in permeability are readily recognized.

A fairly extensive study of neurological conditions with the bromide test has enabled us to examine a number of points relevant to the test which have received little or no attention. From the beginning we regarded the auric chloride method of bromide determination (Walter, 1919; Malamud, Mullins & Brown, 1933; Katzenelbogen & Czarski, 1934) with some scepticism. Since several workers have questioned its reliability (e.g. Lovell & Brown, 1934; Tod, 1933; Mishkis, Ritchie & Hastings, 1933; Fremont-Smith, Dailey & Sloan, 1935; Gray & Moore, 1942) a method based upon iodometric titration (Hunter, 1953), and regarded as more accurate, has been used throughout. With this method, however, the ratios obtained were markedly lower than those commonly reported, our mean for nearly normal controls being 2·60 (S.D. 0·30). This necessitated the examination of sera and c.s.f. by both methods, and it was found that there is commonly present in human serum a substance that reacts like bromide with auric chloride solutions to give a brown colour. With c.s.f. the two methods give results in near agreement but with serum the result by the auric chloride method is commonly too high, and an erroneously high bromide distribution ratio is obtained.

As bromide tests are carried out under ordinary hospital working conditions, the technique for collecting blood should be as simple as possible. The use of whole blood overcomes analytical uncertainties that may arise from uneven distribution of ions between cells and plasma, but it necessitates an anticoagulant, and is less suitable than plasma or serum for studies on the balance of electrolytes with other body fluids. Therefore we decided to use serum for bromide analysis; this raised the question whether there was a 'shift' of bromide, like that of chloride, with changing pH and degree of oxygenation of haemoglobin. This seemed probable from the little information available, especially on human blood. The normal distribution of administered bromide between plasma and corpuscles also seemed to call for further observation since false bromide distribution ratios might arise through alteration of the serum bromide after taking the blood. Some of our blood samples might be kept several days before the serum was separated from the clot.

In the determination of serum/c.s.f. bromide ratios Walter states that blood and c.s.f. were taken 24 hr. after the last oral dose of bromide. Yet there is little information in the literature as to how long administered bromide takes to reach equilibrium with the c.s.f. Nor is there adequate evidence that

the ratio is consistent from day to day in the same person in similar conditions. This paper describes experiments designed to assess the general reliability of the Walter test.

The convention that Br_s = bromide in serum and $(Br)_s$ = bromide in serum water has been followed. Other subscripts used are p = plasma, c = cells, and c.s.f. = cerebrospinal fluid.

EXPERIMENTAL

Determination of bromide. The iodometric titration method of Hunter (1953) and the colorimetric auric chloride method as modified by Katzenelbogen & Czarski (1934) were used.

Comparison of results from iodometric and auric chloride methods

Table 1 shows bromide values obtained on sera and c.s.f. and the resulting ratios, by the two methods. The colours obtained with auric chloride were read in a photoelectric colorimeter at 450 mμ., in the region of maximum absorption for auric bromide (which is the substance that gives the brown colour), and also at 500 mμ., as likely to accord better with readings in a visual colorimeter. It will be seen that there is fair concordance with both the titrimetric and the colorimetric method, at either wavelength, for c.s.f. With serum, however, the values obtained colorimetrically are higher and often much higher, especially at 500 mμ., than the corresponding titrimetric values. The first four cases listed in Table 1 have about normal permeabilities with a mean ratio, by the titration method of 2·67 or by the colorimetric method at 450 mμ. of 2·79 and at 500 mμ. of 3·11, a value the same as the Walter mean. The lower ratios are similarly increased in the three cases of tuberculous meningitis. Most striking perhaps are the values on the sera of the five individuals not treated with bromide; their sera contained usually less than 1 mg. Br/100 ml. according to the iodometric method but much more according to the colorimetric method. It may be noted that Gray & Moore (1942), in an examination of blood from seventy-six subjects who had received no bromide, obtained by the auric chloride method results indicating 16–53 mg. Br/100 ml. in nine of these cases, although no bromide was detected by other tests.

It seems that human sera, normal and pathological, contain a substance (or substances) which is not bromide but which produces, like bromide, a brown colour in auric chloride solution and that this substance is responsible for the high mean value of the ratio $Br_s/Br_{c.s.f.}$ obtained when auric chloride is used to determine bromide in serum. The nature of the interfering substance in serum is unknown; a variety of substances in dilute solution, such as iodide, sulphide, thiosulphate, guaiacol, increase the brown colour of auric chloride solutions under the conditions of the test, whereas small amounts of uric acid or ascorbic acid nearly completely discharge the colour with formation of metallic gold. On such grounds alone it is not surprising that the use of this reagent leads to uncertain results in blood serum.

Rate of equilibration of bromide between plasma and corpuscles

The experiments recorded in Table 2 show that equilibration of bromide between plasma and corpuscles is established within 5 min. of addition of bromide *in vitro* and within 30 min. *in vivo*.

Table 1. *Bromide distribution ratios in serum and cerebrospinal fluid*

Bromide was determined by iodometric titration and by the auric chloride method. In the latter the absorption was measured at 450 and at 500 mμ.

Patient		Iodometric titration method			Auric chloride method, measurement at					
					450 mμ.			500 mμ.		
		Bromide in			Bromide in			Bromide in		
No.	Diagnosis	Serum (mg./100 ml.)	C.s.f. (mg./100 ml.)	$\frac{Br_s}{Br_{c.s.f.}}$	Serum (mg./100 ml.)	C.s.f. (mg./100 ml.)	$\frac{Br_s}{Br_{c.s.f.}}$	Serum (mg./100 ml.)	C.s.f. (mg./100 ml.)	$\frac{Br_s}{Br_{c.s.f.}}$
94S	Palsy	18·8	7·2	2·61	16·2	7·0	2·31	22·3	7·2	3·10
97S	Pain	18·8	7·0	2·69	18·3	6·6	2·78	23·0	7·3	3·15
98S	Pain	20·7	7·8	2·65	23·8	8·4	2·81	26·1	8·2	3·18
99S	Disseminated sclerosis	18·3	6·7	2·73	22·1	6·8	3·27	25·0	8·3	3·00
128X	Tuberculous meningitis (treated cases)	22·8	15·9	1·43	24·6	14·2	1·73	29·7	15·0	1·98
95X		26·4	15·7	1·68	28·2	15·7	1·79	36·0	15·3	2·35
113X		27·6	13·3	2·07	36·0	14·1	2·55	40·8	14·1	2·90
—	Normal	0·9	—	—	1·8	—	—	5·4	—	—
120X	Meningitis	0·5	—	—	3·8	—	—	4·5	—	—
141X		0·4	—	—	4·7	—	—	8·1	—	—
127X		1·1	—	—	6·3	—	—	18·0	—	—
134X	Transverse myelitis	0·9	—	—	5·7	—	—	17·1	—	—

Table 2. *Rate of equilibration of bromide with blood* in vitro *and* in vivo

In vitro. NaBr (as 0·6 ml. of isotonic glucose solution containing 5·38 mg. Br) was added to 20 ml. heparinized blood. Samples (3 ml.) were taken for analysis at intervals. The packed cell volume was 48·5%.

In vivo. Heparinized blood was obtained after intravenous injection of NaBr.

Time after addition of NaBr (min.)	Bromide (mg./100 ml.) Plasma (*a*)	Cells (*b*)	Whole blood Found	Calc. from *a* and *b*	$\frac{Br_c}{Br_p}$
In vitro					
5	32·1	—	—	—	—
10	32·6	—	—	—	—
15	33·0	—	—	—	—
30	32·5	—	—	—	—
60	32·6	—	—	—	—
17 hr.	32·5	19·3	26·6	26·1	—
In vivo					
30	27·8	15·4	—	—	0·55
26 hr.	23·7	13·3	—	—	0·56

Table 3. *Effect of* pH *on equilibration of blood with bromide added* in vitro *and* in vivo

In vitro. NaBr in isotonic glucose solution was added to fresh, heparinized human blood. The mixed blood was divided into four parts. One part was untreated; N-NaOH or N acetic acid in bromide-free saline was added to the others to give the range of plasma pH shown. After 15 min. the bloods were centrifuged and bromide determined. (The top layer of cells containing 'buffy coat' was removed and discarded.) It was assumed that plasma and cells contained 93 and 70% of water, respectively (Maizels, 1936; Harris & Maizels, 1952).

In vivo. Heparinized blood was taken from two patients who had previously received bromide.

Blood no.	Bromide added	Bromide (mg./100 ml.) Plasma	Cells	Plasma water	Cell water	$\frac{(Br)_c}{(Br)_p}$	Plasma pH at 20°
1 (86S)	*In vitro*	42·0	24·6	45·2	35·1	0·77	8·0
2		40·2	26·4	43·2	37·7	0·87	7·8
3		36·0	28·4	38·7	40·6	1·05	7·5
4		35·1	28·2	37·7	40·2	1·06	7·2
1 (76S)	*In vivo*	18·1	7·6	19·4	10·9	0·56	7·98
2		17·7	9·5	19·0	13·6	0·72	7·57
3		15·8	9·8	17·0	14·0	0·82	7·15
4		15·2	9·9	16·3	14·1	0·86	7·00
1 (75S)	*In vivo*	11·8	5·8	12·7	8·3	0·65	8·00
2		11·7	7·0	12·6	10·0	0·79	7·56
3		10·0	7·2	10·8	10·3	0·96	7·18

Effect of pH *on distribution of bromide between plasma and cells*

Table 3 shows that the ratios of bromide in cell water to bromide in plasma water, $(Br)_c/(Br)_p$, vary inversely as the pH. It may be noted that the change in plasma water through addition of solutions of base and acid is less than 1% and has been neglected in the calculation.

Bromide distribution in blood collected from hospital patients

Venous blood was collected in a syringe and mixed directly with heparin in a corked test tube. The tube, with cork in place to prevent loss of carbon dioxide, was centrifuged. From twenty-four observations on nineteen patients suffering from a variety of neurological conditions and including only one febrile case, the ratio $(Br)_c/(Br)_p$ was found to be 0·84 (S.D. 0·08). The observations are not given separately as there seemed to be no relationship between $(Br)_c/(Br)_p$ and sex, age, pathological condition, or plasma pH.

Shift of bromide in stored blood

It seemed possible that on storage of blood a change in the ratio $(Br)_c/(Br)_p$ might occur even if there was no change in pH. Accordingly the bromide level was followed in heparinized blood kept at room temperature and in clotted blood stored in a refrigerator for several days. The packed cell

Table 4. *Effect of storage on plasma or serum bromide*

Heparinized blood was stored at 20° and clotted blood at 4°. Plasma and cell water were assumed to be 93 and 70%, respectively.

No.	Time of storage of blood (days hr.)	pH of plasma or serum at 20°	Packed cell volume	Bromide (mg./100 ml.) Plasma or serum	Cells	Plasma when $(Br)_c/(Br)_p = 1$	Maximum fall in plasma or serum bromide (%)
			Heparinized blood				
72	0·5	—	37	24·0	13·3	—	—
	4	—	—	21·8	—	—	—
	8	—	—	21·4	—	22·0	10·8
74	0·5	—	42	20·3	12·5	—	—
	4	—	—	20·1	—	—	—
	2	7·58	—	19·1	—	18·8	6
77	17	7·56	41	20·4	13·7	—	—
	2	7·56	—	19·9	—	19·6	2·5
81	1	7·56	44	17·1	12·0	—	—
	4	7·40	—	16·5	—	16·7	3·5
			Clotted blood				
83	4	7·72	—	29·4	—	—	—
	2	7·80	—	29·3	—	—	—
	4	7·60	—	28·3	—	—	3·7
87	1	7·63	—	13·8	—	—	—
	2	7·61	—	13·6	—	—	—
	6	7·64	—	13·3	—	—	3·6
90	2	7·50	—	20·3	—	—	—
	3	7·54	—	19·8	—	—	—
	6	7·71	—	20·3	—	—	—
	12	7·24	—	19·7	—	—	3·0
91	2	7·57	—	18·9	—	—	—
	4	7·57	—	18·9	—	—	—
	10	7·52	—	18·1	—	—	4·2

volume of the former was determined and the bromide level expected in the plasma when the anion becomes evenly distributed in cell and plasma water was calculated. The results from several experiments are given in Table 4 which shows that all plasma or serum values tend to fall towards the calculated level.

DISCUSSION

Equilibration of bromide with blood

It is apparent from Table 2 that bromide added *in vitro* or *in vivo* to human blood rapidly reaches equilbrium with the cells. This finding is in accord with the observations of Hastings & Van Dyke (1931) on dog blood. Table 3 shows that whether bromide is added *in vitro* or *in vivo* to blood its distribution between the cells and plasma varies, like chloride, with pH. This finding is also in accord with those of Hastings & Van Dyke (1931) and of Hastings, Harkins & Liu (1932), when very large amounts of bromide were given to dogs. If the ratios in Table 3 are plotted against pH they have about the same slope, with a shift of 0·3 pH unit causing a change of about 10% in the ratio. The chloride shift with pH as found by Harris & Maizels (1952) has a similar magnitude. At normal blood pH the magnitude of the chloride ratio, $(Cl)_c/(Cl)_p$ is by general consent (see Owen & Power, 1953) close to 0·70 for man. In nine female epileptic patients, Notkin, Garcia & Killian (1933) found a mean bromide ratio of 0·80 from bloods collected without precautions to exclude air. Their corresponding chloride ratio was 0·82. In four patients with bromide intoxication, Mason (1936) found a mean chloride ratio of 0·73 and a corresponding bromide ratio of 0·80. In dogs, Weir & Hastings (1939) found a chloride ratio of 0·72 and a bromide ratio of 0·76 (ten observations). Our bromide ratio, from twenty-four observations on nineteen persons, is 0·84 (S.D. 0·08). This is significantly higher than the few values previously reported for human blood and serves to emphasize that bromide displaces more than its chloride equivalent from erythrocytes.

Hastings and his associates used venous blood collected under anaerobic conditions. Our blood samples were collected with minimum exposure to air, but not with strict anaerobic technique, with some consequent oxidation of haemoglobin and loss of carbon dioxide both of which actions would tend to lower the ratio. From the observed pH's there could not have been serious carbon dioxide loss, but at any rate the ratio of 0·84 found should be

slightly below the physiological. Or, had the blood been collected under anaerobic conditions, we should expect the mean distribution of bromide between cells and plasma to be close to 0·85.

Probable error from bromide shift

Our immediate purpose in determining the distribution ratio of bromide between corpuscles and plasma was to assess the extent of the error that might arise from the bromide shift. As the bromide ratio is much nearer unity than the chloride ratio, the bromide shift is likely to be the source of a smaller error in serum bromide determination than is the chloride shift in serum chloride determination. Also, the pH change in venous blood with loss of a little carbon dioxide would not be expected to be large, as it tends to be compensated by the concurrent formation of more oxyhaemoglobin. If we take an instance when $(Br)_c/(Br)_p = 0{\cdot}84$, the packed cell volume is 45 %, the cells contain 70 % and the plasma 93 % of water, and the cell water and plasma water contain 16·8 and 20 mg. Br/100 ml., respectively, it can readily be calculated that, if the bromide becomes equally distributed between cell and plasma water, the fall in level of plasma bromide would be 1·1 mg. Br or 5·9 %, and a physiological $Br_p/Br_{c.s.f.}$ of 2·50 would be decreased to 2·35. In bloods where the $(Br)_c/(Br)_p$ is appreciably less than 0·84, especially when the packed cell volume is high, the error could be perhaps over 10 %, but with the procedure used in the present research it seems improbable that any error arising from bromide shift is likely to exceed 2–3 %. This estimate of possible error from bromide shifts may be compared with the observed values shown in Table 4. Here the heparinized bloods were kept at room temperature to hasten equilibrium. After 4 days' storage the plasma bromide values were close to the expected equilibrium values, and only one sample had a value greater than 10 % below the initial value. With clotted blood at 4°, as might be expected, the bromide shift to the corpuscles is slower. Even with prolonged storage of up to 12 days, the greatest fall was 4·2 %.

We may thus conclude, on the evidence of rapid equilibration of administered bromide with corpuscles and plasma, and from the magnitude of the ratio of the distribution of bromide between corpuscles and plasma, that, under the conditions of collection and storage of the blood at 4°, no serious errors in the ratio $Br_s/Br_{c.s.f.}$ are likely to arise from bromide shift after the blood has been drawn.

Equilibration of bromide with extracellular fluid

There is evidence in the literature that bromide equilibrates within a few hours with extracellular fluid. For example, Brodie, Friedman & Ferraro (1941) have suggested its use as a measure of extracellular fluid some 3 hr. after oral administration in man. Greenberg *et al.* (1943), from experiments on dogs with ^{82}Br, found it to be completely distributed in the body within 1 or 2 hr. We have followed in a few subjects the blood level at short intervals after intravenous administration of sodium bromide, and a typical case (Table 5) shows that the serum-bromide level falls rather rapidly for about 2 hr. following intravenous administration. After about 2 hr. the rate of fall rapidly decreases. During the first 2–3 hr., presumably, equilibrium is taking place with the body's extracellular fluids.

Table 5. *Rate of fall of blood bromide following intravenous administration*

Time after injection (hr.)	Bromide (mg./100 ml.)	
	Serum	Fall/hr.
0·25	24·8	—
1·0	22·8	2·7
2·0	20·4	2·4
8·0	19·2	0·6
24·0	18·2	0·06

Persistence in blood of administered bromide

Some 24 hr. after giving the bromide we have found the rate of fall to be commonly less than 1·0 mg. Br/100 ml. serum/day. Thus if the initial blood level is over 30 mg./100 ml. serum, determinable amounts of bromide will remain for some weeks. We shall later consider the matter of further administration of bromide in prolonged studies on patients, with the related question of a possible variation of the ratio $Br_s/Br_{c.s.f.}$ with different blood bromide levels, but the persistence of administered bromide in the body fluid is an important aspect of the barrier test, and a matter of some physiological interest. Palmer & Clarke (1933) demonstrated in the dog that the percentage of bromide in urine halides is less than the percentage of bromide in blood halides. They found, however, that the ratio of those urine and blood percentages was constant on a constant chloride intake. On a salt-poor diet it was 0·40 and on a high salt diet it was about 0·70. Similar ratios were found in a few patients with bromide dermatitis. It therefore appeared that the kidney preferentially excretes chloride. A similar conclusion is reached by Smith & Walker (1938) and by Bodansky & Modell (1941). Bromide is not removed from the plasma by increased excretion of urine but only by replacement by dietary chloride. Palmer & Clarke (1933) recorded about 120 mg. Br/100 ml. blood 30 min. after intravenous administration to a dog on a salt-poor diet, and 4·5 months later the blood still contained about 16 mg./100 ml. We have determined serum/c.s.f. ratios on patients who had about 7 mg. Br/100 ml.

serum more than 50 days after they had received bromide to the extent of about 35 mg./100 ml. of serum, and who had had the ordinary hospital salt intake. We commonly find that the relationship between serum bromide and time after bromide administration is of a linear rather than an exponential type, indicating that the rate of bromide loss is largely independent of its level in the serum.

Whether or not the kidney excretes bromide in preference to chloride has long been a vexed question. Mason (1936) supports the findings of Palmer & Clarke (1933) but Frey (1932) and Moller (1932) believe that the kidney does not distinguish between chloride and bromide. The question should, perhaps, be re-examined with modern methods for bromide determination.

Gastric secretion of bromide

Since the observations of Nencki & Schoumov-Simanowsky (1894) on the presence of bromide in the gastric juice of dogs given bromide, there has been repeated mention of gastric secretion as a possible factor in lowering serum bromide. Davenport & Fisher (1940) have clearly shown that in Pavlov pouches in dogs the rate of secretion of bromide is of the same order as the rate of secretion of chloride, and the concentration of bromide in the gastric juice is of the order of 50 % higher than that in plasma. The recent observation of Gabrieli (1950) that this bromide is not secreted with the hydrochloric acid of the juice does not affect this point in our argument, but is suggestive in the study of the physiology of bromide. It seems unlikely that the volume of gastric juice in any short period could be more than 3–4 % of the volume of the extracellular fluid and only the 50 % excess over the plasma level could be regarded as likely to change the plasma level. Thus we should not expect a fall in plasma bromide level of more than 1–2 % following a very active period of gastric secretion and such an alteration is within the usual analytical variation. Fleischacker & Scheiderer (1928) maintain that blood for the bromide barrier test should be taken in the post-absorptive state, but their values (by the auric chloride method) of less than 5 % drop following food can hardly be regarded as significant.

Equilibrium between plasma and cerebrospinal fluid

Walter (1929) recommended an interval of 24 hr. between the last oral dose of bromide and lumbar puncture. This interval seems to have been generally accepted, though there is a paucity of evidence as to the length of time really necessary. Stern & Gautier (1921) demonstrated the presence of bromide in the c.s.f. of animals 1·25, 3 and 16 hr. after intravenous administration of relatively large doses. Wallace & Brodie (1940), after intravenous injection of 22 g. of sodium bromide to a dog weighing 22 kg., found $Br_s/Br_{c.s.f.}$ to be nearly constant after 7 hr. and constant from 24 hr. to 13 days. The c.s.f. was drawn from the *cisterna magna*. With ^{82}Br, Greenberg *et al.* (1943) found a plateau level in about 2 hr. in the dog. Table 6 shows some of our results with human subjects. It is

Table 6. *Equilibrium with cerebrospinal fluid after intravenous injection of sodium bromide*

Values in parentheses are for ventricular fluid.

No.	Diagnosis	$\frac{Br_s}{Br_{c.s.f.}}$	Time after injection of NaBr (days hr.)
56S	Head injury	$\frac{24·3}{5·1}=4·80$	5
		$\frac{23·1}{7·7}=3·00$	28
59S	Subacute combined degeneration of spinal cord	$\frac{22·8}{5·6}=4·07$	5
		$\frac{22·0}{9·6}=2·29$	24
79X	Tuberculous meningitis	$\frac{24·0}{16·9}=1·42$	12
		$\frac{22·6}{15·6}=1·45$	28
34X	Pneumococcal meningitis	$\frac{19·7}{16·0}=1·23$	20
		$\frac{19·0}{15·0}=1·27$	44
90X	Schizophrenia	$\frac{23·8}{7·5}=3·17$	21
		$\frac{22·4}{8·0}=2·80$	48
28X	Pulmonary tuberculosis	$\frac{24·0}{8·7}=2·76$	25
		$\frac{21·4}{8·0}=2·68$	48
105X	Lymphocytic meningitis	$\frac{18·2}{8·0}=2·28$	24
		$\frac{11·1}{5·1}=2·18$	10
98X	Tuberculous meningitis with hydrocephalus	$\frac{27·7}{13·8}=2·01$ (11·10)	5
		$\frac{25·7}{17·7}=1·45$ (3·72)	24
		$\frac{24·5}{18·1}=1·35$ (2·85)	48
		$\frac{11·1}{8·3}=1·34$ (2·31)	7

clear that an interval of about 5 hr., at which we have made many other observations with similar results, is insufficient. In the meningitis cases, 79X, 34X, equilibrium was attained in 12 and 20 hr., respectively, but in this condition, as will be shown elsewhere, the barrier permeability is greatly increased and such cases may give a false impression of the time interval necessary with the barrier normal. In the case of schizophrenia, 90X, 21 hr. was insufficient, and in cases 28X and 105X, 25 and 24 hr. were barely sufficient. In the case of hydrocephalus, 98X, in which we got a more continuous record for both lumbar and ventricular fluids, 24 hr. was insufficient; in 48 hr. equilibrium was reached with the lumbar fluid but not with the ventricular. We have followed another case of hydrocephalus at shorter time intervals up to 24 hr. with similar results.

Although the evidence from Table 6 is not as extensive as might be desired for the more normal cases, it suggests that 24 hr. should be the minimal interval between intravenous bromide dosage and lumbar puncture for the purposes of the bromide test. With oral dosage, a considerably longer interval should be set.

Concentration of bromide in cerebrospinal fluid at different levels in the neural axis

Masserman (1934), in many observations on paretic and schizophrenic patients, found that the lumbar fluid contained slightly more bromide than the cisternal and in seven cases Bau-Prussak & Prussak (1927) reported higher bromide values in the lumbar than in the cisternal fluids. Our own observations are mostly from rather extreme neuropathological conditions which will be discussed elsewhere. However, for Table 7 we have selected six cases where the fluids, as judged from the diagnosis, condition of the patient and concentration of protein in the fluids, are likely to be sufficiently near normal for a comparison of them to be valid. It may be seen that the bromide concentration of the ventricular fluid is about half that of the lumbar, with a resultant distribution ratio between 4·0 and 5·0. In two cases the bromide level of cisternal fluid was about midway between that of lumbar and ventricular fluid. It is clear that values obtained from either ventricular or cisternal fluid should not be compared with those obtained from lumbar fluid.

Table 7. *Bromide and protein concentrations of cerebrospinal fluid at different levels in the neural axis*

Bromide and protein values are expressed as mg./100 ml.

		Cerebrospinal fluid								
		Lumbar (*L*)		Cisternal (*C*)		Ventricular (*V*)				
Patient no. and diagnosis	Serum (*S*) Bromide	Bromide	Protein	Bromide	Protein	Bromide	Protein	*S/L*	*S/C*	*S/V*
36X. Rhinorrhoea	12·0	5·4	48	—	—	2·7	—	2·22	—	4·44
39X. Fits	28·7	12·1	22	—	—	7·1	22	2·37	—	4·04
34X. Intracranial tumour	11·7	4·5	16	—	—	2·8	11	2·60	—	4·18
65X. Psychosis	33·8	12·1	35	—	—	6·9	17	2·71	—	4·90
50X. Cystic glioma	25·6	11·0	60	8·7	60	7·8	92	2·33	2·94	3·28
88X. Tuberculous meningitis, partly healed	35·5	23·5	69	14·6	45	10·5	23	1·51	2·43	3·38

Table 8. *Effect of repeated tappings on bromide level of lumbar cerebrospinal fluid*

Patient no. and diagnosis	Sample no.	Approx. vol. tapped (ml.)	Interval between samples (min.)	Bromide (mg./100 ml.)
36X. Cerebrospinal fluid rhinorrhoea	1	10	—	6·0
	2	10	10	5·3
	3	5	20	4·7
39X. Infantile hemiplegia	1	15	—	8·9
	2	10	20	8·7
	3	10	10	8·7
	4	5	10	8·7
101S. Disseminated sclerosis	1	3	—	10·8
	2*	7	2	—
	3	4	2	9·7

* Not used for bromide determination.

Table 9. *The distribution of bromide between blood and cerebrospinal fluid in control patients*

Condition	No. of cases	$\frac{Br_s}{Br_{c.s.f.}}$ Mean	S.D.	No. of cases with ratio < 2·15	2·15–2·44	2·45–2·74	2·75–3·04	> 3·04
Disseminated sclerosis	20	2·60	0·30	1	5	7	7	0
Psychosis	10	2·60	0·30	0	3	3	3	1

Effect of repeated lumbar punctures on the bromide level of cerebrospinal fluid

Fleischacker & Scheiderer (1930) found a fall of from 4·5 to 9·0 % in withdrawing successively three 5 ml. samples of lumbar fluid, and Walter (1929) has a warning on this point. On two of three patients our observations (Table 8) were made in the course of lumbar air encephalograms (cases 39X, 101S). In one of these and in case 36X there was a marked fall in bromide, while in case 39X the fall was less than might have been expected with the removal of relatively large amounts of fluid. It is clear, however, that a considerable error may arise, where large amounts of fluid are removed, if the first drawn fluid is not used for the bromide analysis.

The normal distribution of bromide between serum and cerebrospinal fluid

With the precautions implied above, the ratio $Br_s/Br_{c.s.f.}$ has been determined in over 200 cases, and in a number of these many times. All the cases, however, were hospital patients and like most workers in this field we have no control values from healthy subjects. From a variety of apparently benign neurological conditions like headache, neuralgia, anxiety neurosis, epilepsy, motor neurone disease, we found rather a high standard deviation and chose as controls a less heterogeneous group consisting of ten psychotic patients with normal c.s.f. protein and twenty cases of disseminated sclerosis, in which the c.s.f. protein did not exceed 50 mg./100 ml. (Table 9). It is no doubt fortuitous that the two groups gave precisely the same ratios and standard deviations. In choosing such patients as controls it may be remarked that, according to Walter (1929), there is a tendency towards high ratios or decreased permeability in schizophrenic patients and the ratio is in the normal range in cases of multiple sclerosis provided the c.s.f. protein is normal. In using cases of psychosis and disseminated sclerosis as controls we are not suggesting that in such conditions the barrier permeability is normal. It would seem in fact that our controls were comparable with those chosen by most workers, in that they gave a mean serum/c.s.f. bromide ratio of about 3·10 by the auric chloride method (2·60 by the iodometric method).

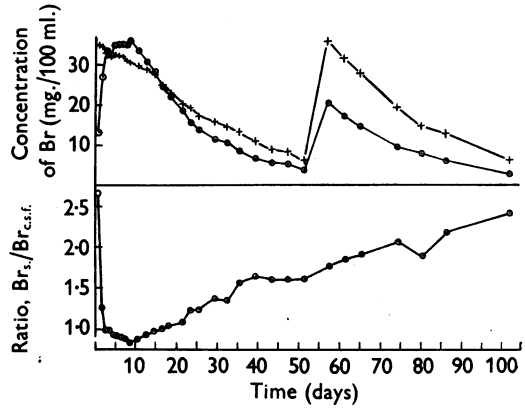

Fig. 1. Case of a psychotic patient who received orally 1·0 g. NaBr thrice daily for 3 days. About 60 hr. after the last dose bromide values in serum and c.s.f. were 35·2 and 13·3 mg. Br/100 ml., respectively (ratio 2·65). The patient was then given tuberculin intrathecally, and the bromide levels in serum and c.s.f. were followed over a period of 102 days. Between 50 and 60 days the patient was given another similar oral course of NaBr. ×—×, serum; ⊙—⊙, c.s.f.

Repeated tests on the same patient

Few repeated Walter bromide tests on the same patient have been recorded. Walter (1925) reported repeat values on four patients at times varying from 9 to 37 days after the initial observations, and concluded that the ratio was very constant and uninfluenced by time and amount of bromide present. Further observations of this kind are desirable. Fig. 1 illustrates our findings in a case where a rapid and profound change in barrier permeability occurred following intrathecal administration of tuberculin; the medical aspects of which will be discussed elsewhere. The main features of this case have been repeatedly observed in other patients. The following points may be observed:

(*a*) The consistently diminishing values obtained on the serum, except at about a week from the start when the blood values remained constant over several days and indeed on one day showed a slight rise. Such changes could well have been due to changes in the water balance as a result of the treatment. As remarked earlier the fall in blood bromide tends to be linear with time. Walter (1929) records a similar curve obtained from his own blood over about 50 days.

(*b*) The c.s.f. values are likewise on a continuous curve with only two points slightly erratic. The peak c.s.f. value of 35·9 mg. Br/100 ml. was the mean of close triplicate determinations. It is not only much higher than the corresponding serum value (30·5) but higher than the serum peak at the start. This surprising bromide distribution will be discussed elsewhere.

(*c*) The values of the ratio $Br_s/Br_{c.s.f.}$ also lie on a smooth curve, despite a large change in the blood level between the 50th and 60th day.

(*d*) When the observations were stopped, the ratio, 2·40, was still somewhat lower than its initial value, indicating that the barrier had not yet quite recovered from its treatment more than 3 months earlier.

From such considerations Fig. 1 is taken as evidence of the general reliability of the bromide test. It also proves, and we have further similar evidence on the same point, that the ratio is independent of the level of bromide in the blood—at least within the limits recommended for the test.

SUMMARY

1. The bromide test of meningeal permeability (Walter, 1929) has been critically examined.

2. The auric chloride method for estimation of bromide in serum is unreliable, and values for the serum/cerebrospinal fluid bromide ratio determined by this method are too high.

3. When bromide is added to human blood an equilibrium between plasma and erythrocytes is established in a few minutes. At this equilibrium the ratio (Br^- in cell water)/(Br^- in plasma water) is 0·84 (S.D. 0·08) and from the magnitude of this ratio it is inferred that administered bromide replaces more than its equivalent of chloride in the human erythrocyte. The ratio also varies inversely with the pH of the blood.

4. On storage of blood the serum bromide tends to fall to a level corresponding to an even distribution throughout the blood water. With clotted blood stored in a refrigerator this process is probably not complete for 1 or 2 weeks. When the serum is separated within 1–2 days the bromide level is not likely to have dropped more than 2–3 %.

5. Intravenously injected bromide takes several hours to equilibrate with the extracellular fluid of the body.

6. Administered bromide persists in the blood for several weeks. Our evidence favours the view that the kidney excretes chloride in preference to bromide.

7. Intravenously injected bromide, except where there is increased barrier permeability, normally takes about 24 hr. to equilibrate with the lumbar cerebrospinal fluid and longer with ventricular fluid.

8. The bromide concentration of cerebrospinal fluid decreases on ascending the neural axis. In nearly normal cases the bromide concentration in ventricular cerebrospinal fluid is about half that of lumbar.

9. A significant error in the bromide distribution ratio between serum and cerebrospinal fluid can arise if much lumbar fluid is removed before the specimen is taken.

10. In thirty cases with nearly normal meninges the bromide distribution ratio between serum and cerebrospinal fluid was 2·60 (S.D. 0·30). This ratio is independent of the level of bromide in the serum.

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The Source of Antibody Globulin in Rabbit Milk and Goat Colostrum

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After the intravenous injection of radioactive amino acids into lactating rabbits, there is an extremely rapid and efficient incorporation of radioactive carbon into milk proteins. The blood plasma-protein radioactivity reached its maximum value 6 or 7 hr. after the injection, but it is only about one-tenth the activity of a milk protein sample collected at the same time (Campbell & Work, 1951, 1952). It has been concluded that blood plasma protein is not a major and direct precursor of milk protein and that most of the milk protein is synthesized in the mammary gland from amino acids or small peptides. Amino acids isolated from radioactive rabbit casein were, however, of slightly higher specific activity than the corresponding amino acids from whey protein, and it seemed possible that this difference might be due to the direct transfer from blood to milk of a fraction of the plasma protein. Campbell & Work (1952) calculated that up to 25% of the total whey protein would have to be so transferred to account for the lowered radioactivity of the whey. Experiments have, therefore, been directed towards identification of the protein transferred from blood to milk in the lactating rabbit. More recently we have been interested in the biosynthesis of milk proteins in the goat, and it was hoped to extend the work to this animal.

Extensive studies on the transmission of immunity from parent to offspring have suggested that antibody may pass across the placental barrier unchanged; high concentrations of antibody being found in colostrum and traces in milk (Ehrlich, 1892; Ratner, Jackson & Gruehl, 1927; Marrack, 1947; McMeekin & Polis, 1949, McCarthy & McDougall, 1953). According to Smith (1948) the immune-globulin fraction of cow colostrum is identical with the immune-globulin of cow milk and closely similar to, but not quite identical with, the T-globulin of cow plasma. The immune-globulins are, however, proteins of high molecular weight, rabbit γ-globulin having a molecular weight of 160000 (Kabat, 1939; Nichol & Deutsch, 1948) and there has been considerable doubt as to whether this whole protein passed across cell barriers or whether it was partially degraded and resynthesized (Cohen, 1950; Calman & Murray, 1951; Brambell, Hemmings & Henderson, 1951).

From the work on immune-globulin, it was clear that in so far as proteins could be transferred directly from rabbit blood to milk, they would probably be found in the immune-globulin fraction. Moreover, any protein transferred directly to the milk during the hours immediately following intravenous injection of a radioactive amino acid should have the radioactivity characteristic of the plasma proteins and, 6 hr. after injection, should have not more than about one-tenth the activity of protein synthesized in the mammary gland. Accordingly, a lactating rabbit was immunized with pneumococcus (type III) and was then given an injection of [^{35}S]methionine. Blood and milk samples were withdrawn at intervals and pneumococcus capsular polysaccharide (SSS III) was used to precipitate the specific antibody from blood and milk. The antibodies thus obtained were compared with one another and with the other milk and blood proteins. A preliminary account of this investigation has been published (Campbell, Humphrey & Work, 1953).

An attempt was then made to reproduce in a goat the experimental conditions already used with rabbits. However, even after a prolonged course of intravenous injections of formalin-treated pneumococcus (type III) the serum antibody level did not rise above 0·4 mg./ml. The level in the milk was much lower, and was too low to permit accurate

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