production of leukemic cells(5,6) and increase the life span of leukemic mice(12,13), (e) the anti-leukemic action of folic acid antagonists may be reversed by folic acid or citrovorum factor(14,15), and partially reversed by DNA or B_{12}(16,17), certain possibilities pertinent to the present discussion would seem to exist. Certainly the present data indicate that leukemic cells are geared to a more active formate metabolism than are normal white blood cells. Since inhibition of formate metabolism via the citrovorum factor coenzyme preferentially inhibits proliferation of leukemic cells, it might be suggested that these malignant cells are utilizing their supplies of this coenzyme to capacity while normal blood elements possess a marginal reserve of this formylating system. If this were true, folic acid antagonists might provide specific anti-leukemic action *in vivo* even though the antagonist concentration in the malignant and normal cell was of the same order because of differential demands on the formylating system.

Further studies are under way in an attempt to determine the significance of this difference in formate incorporation by normal and leukemic cells.

Summary. At one hour after injection of 100 μc of C^{14}-formate into mice with advanced Ak-4 leukemia, it has been shown by means of blood smear autoradiograms that about 90% of the leukemic cells are highly active as compared to about 5% of the more mature lymphoid elements. No active polymorphonuclears, erythrocytes, or thrombocytes were observed within one hour.

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Measurement of Total Body Chloride.* (18947)

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The chloride ion has been considered to be confined for the most part to the extracellular fluid. It has been accepted, nevertheless, that the chloride ion penetrates certain cell walls, in particular the wall of the erythrocyte, the gastric mucosa, the small intestine, the colon mucosa, the liver cell to be excreted in the bile, the cells of sweat and salivary glands, and of the kidney tubule. Precisely how much of the chloride is included in the intracellular water has not been appreciated until it was shown recently that inulin was distributed through a volume of fluid which more closely approximated the anatomical extracellular volume than did the larger "chloride space"(1). When the volume of fluid in which inulin is distributed is taken to be the measure of extracellular fluid, the difference then between the chloride contained in this body of fluid and the total chloride in the body represents intracellular chloride.

Bromide is distributed in tissues in much the same manner as chloride(2-4) and has,

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1. Berger, E. Y., Dunning, M. F., Steele, J. M., Jackenthal, R., and Brodie, B. B., *Am. J. Physiol.,* 1950, v162, 318.

2. Nencki, M., and Schoumow-Simanowsky, E. O., *Arch. f. Exp. Path. und Pharm.,* 1894, v34, 313.

therefore, been used as a measure of what has been called "chloride space"(5). Since it is now apparent that a large proportion of chloride is not extracellular, it is better to consider the bromide as being diluted in the total chloride of the body rather than in a certain volume of water. The dilution of bromide is then a measure of total body chloride in the same way that the dilution of an isotope is a measure of the total quantity of the isotopically normal element; for example, the dilution of radioactive potassium to measure total body potassium(6)†.

Methods. Bromide was administered to hospital patients and personnel in amounts calculated to achieve plasma levels of 2 mEq per liter. To subjects without edema bromide was administered intravenously from a burette as a 4% sodium bromide solution. Subjects with edema received the bromide orally as potassium bromide in capsules. In normal individuals, plasma was sampled after five hours and in those with edema, after 24 hours. Evidence that equilibrium has been established at these times for normal and edematous individuals has been previously documented by Brodie, Brand, and Leshin(5). In 5 edematous patients of the present series, concentration of bromide in ascitic fluid and plasma was in good agreement 24 hours after administration. The urinary excretion of bromide which is small (maximum of 3% of the dose in edematous patients) was measured during the 24 hours equilibration and the amount administered was corrected for that excreted. Bromide was determined by the method of Brodie and Friedman(10); the plasma values were corrected for plasma blanks measured in specimens drawn prior to the administration of bromide. Chloride was determined by the method of Wilson and Ball(11). The bromide administered was considered part of the total body chloride and the total plasma halide determined by precipitation with silver nitrate was not corrected for the small amount of bromide present. Total body chloride was calculated from the relationship

$$\frac{\text{Concentration of chloride in plasma}}{\text{Concentration of bromide in plasma}} = \frac{\text{Total body chloride}‡§}{\text{Retained bromide}}.$$

3. Wallace, G. W. B., and Brodie, B. B., *J. Pharm. Exp. Therap.*, 1939, v65, 214.

4. Weir, E. G., and Hastings, A. B., *J. Biol. Chem.*, 1939, v129, 547.

5. Brodie, B. B., Brand, E., and Leshin, S., *J. Biol. Chem.*, 1939, v130, 555.

6. Hevesy, G., Radioactive Indicators, Interscience Publishers, New York, 1948.

† There are some minor qualifications. One, only part of the chloride in the central nervous system is included because of the barrier to the passage of bromide into the spinal fluid(7). On the other hand the ratio of bromide to chloride in red cell water is somewhat higher than that in plasma water (8, 9). These two discrepancies tend to nullify each other.

7. Wallace, G. B., and Brodie, B. B., *J. Pharm. Exp. Therap.*, 1940, v68, 50.

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9. Smith, P. K., Eisenman, A. J., and Winkler, A. W., *J. Biol. Chem.*, 1941, v141, 555.

10. Brodie, B. B., and Friedman, M. M., *J. Biol. Chem.*, 1938, v124, 511.

11. Wilson, D. W., and Ball, E. G., *J. Biol. Chem.*, 1928, v79, 221.

‡ Total chloride cannot be calculated from urinary concentrations of chloride and bromide after equilibration as might be done with an isotopic element. The kidney tubule preferentially reabsorbs bromide to chloride. The U/P ratio of chloride is 1.5 times the U/P ratio of bromide (mean of 20 comparisons none below 1.0).

§ Thiocyanate presumably distributes in tissues in proportion to their chloride content although direct comparisons of chloride with thiocyanate in tissues have not been reported in the literature(12). Twenty-two comparisons were made between the total chloride as measured by thiocyanate and the total chloride as measured by bromide. The comparisons were made in a group consisting of 16 normal individuals and 6 edematous patients. The ratio of thiocyanate measurement to bromide measurement was 0.99 (mean of 22 comparisons). The individual thiocyanate measurements ranged from —24 to +18% of the bromide; on the average the thiocyanate differed from the bromide by ± 9.5%. Although thiocyanate is much easier to analyze than bromide, bromide was considered preferable for measurement of total body chloride because of the reported metabolism and protein binding of thiocyanate(13, 14, 15).

TABLE I. Total Body Chloride in Males and Females.

Wt group, kg	No. of subjects	Mean age, yr	Mean wt, kg	Mean plasma chloride, mEq/l	Mean total chloride, mEq	Mean chloride/kg body wt, mEq/kg
			Males			
40–49	3	58	46.6	105	1530	32.9
50–59	6	52	56.8	103.7	1997	35.2
60–69	9	39	64.9	103.5	1974	30.6
70–79	5	42	76.8	104.9	2354	30
80+	3	36	82.3	103.5	2305	27.9
Mean	(26)		65.2		2039	31.6
σ					318	3.8
			Females			
40–49	4	42	47.2	104.6	1558	32.9
50–59	6	27	54.8	104.9	1600	29.3
60–69	6	34	63.2	104.8	1812	28.7
70–79	5	42	75.5	104.4	2026	26.9
80+	5	33	97.2	108.6	2484	25.8
Mean	(26)		67.7		1894	28.6
σ					355	3.3
			Combined group males and females			
Mean	(52)		66.6		1967	30.1
σ					345	3.9

Observations. Total body chloride in normal individuals. The normal individuals (26 males, 26 females) consisted of hospital personnel and patients without evident abnormality in their degree of hydration. The mean total body chloride of the males (average weight 65.2 kg) was 2039 mEq, that of females (average weight 67.7 kg) was 1897 mEq (Table I). Using the volume of distribution of inulin as the best measure of extracellular fluid volume, an estimate may be made as to what portion of the total body chloride is inside and what portion is outside the cell. The average extracellular fluid volume, as measured by dilution of inulin is approximately 15% of body weight(1,16-18) or for this group (average weight 66 kg), 10 liters and contains 110 mEq of chloride per liter.¶ Extracellular fluid thus accounts for only 1100 mEq of the total chloride found in the body. The difference between the extracellular chloride and the total body chloride is about 900 mEq, which represents chloride within the cells. It is evident that almost half of the chloride in the body is intracellular. Of interest is the relationship of total body chloride to body weight. In both male and female subjects, the chloride content per kg of body weight tended to decrease as the weight increased. Presumably fat which contains no chloride, or muscle in which the concentration of chloride is quite low compared to other tissues, forms a larger proportion of the body as body weight increases. The average chloride in the males was 32.6 mEq and in females 28.6 mEq per kg. The smaller amount of chloride per unit weight in the female is to be expected since the female contains proportionately more fat(19).

Total body chloride in patients with edema. Twenty patients with abnormal fluid depots were grouped according to the extent of their edema which was rated 1 to 3+ (Table II). The average amount of chloride per kg in each group increased with the extent of the edema. In the group with 1+ edema the

¶ Donnan's equilibrium has been neglected.

12. Wallace, G. B., and Brodie, B. B., *J. Pharm. Exp. Therap.*, 1937, v61, 397.

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18. Schwartz, I. L., Breed, E. S., and Maxwell, M. H., *J. Clin. Invest.*, 1950, v29, 517.

19. Steele, J. M., Berger, E. Y., Dunning, M. F., and Brodie, B. B., *Am. J. Physiol.*, 1950, v162, 313.

TABLE II. Total Chloride in Patients with Clinical Edema.

Degree of edema	Subject	Diagnosis*	Sex	Wt, kg	Plasma chloride, mEq/l	Total chloride, mEq	Chloride per unit wt, mEq/kg
1+	A	CHF	F	50	101.8	1950	39
1+	B	,,	M	51	104.5	2010	39.4
1+	C	,,	M	58.9	108.2	2860	48.6
1+	D	,,	M	75.7	94.5	2770	36.6
		Mean		58.9	102.2	2398	40.9
2+	E	,,	F	45.8	103.1	2260	49.3
2+	F	NEP	M	49.1	101	2180	44.4
2+	G	CHF	F	51.2	113.5	2980	58.2
2+	H	NEP	M	55.6	102	2710	48.7
2+	I	CHF	F	56.8	103.9	2200	38.7
2+	J	,,	F	63	106	2260	35.8
2+	K	,,	F	76	106	2580	33.9
		Mean		56.8	105	2453	44.2
3+	L	,,	F	40	85.6	1530	38.3
3+	M	,,	F	45	89.7	2120	47.2
3+	N	CIR	M	50.9	107.8	3270	64.4
3+	O	,,	M	63	103	2820	44.8
3+	P	CHF	M	67.9	101.8	4740	69.9
3+	Q	CIR	M	68	112	2840	41.9
3+	R	CHF	M	68.3	98.4	3150	46.2
3+	S	NEP	M	73.2	104	2970	40.6
3+	T	CIR	M	78.5	98	3160	40.2
		Mean		61.6	100	2955	48.2

* CHF, congestive heart failure; NEP, nephritis; CIR, cirrhosis.

chloride content averaged 40.9 mEq per kg with 2+ edema 44.2 mEq per kg, and with 3+ edema 48.2 mEq per kg. It may be noted also, that the chloride content of an individual patient may differ markedly from the others in his group. For example, the patient (L) who had been on a low sodium diet (500 mg) and had received frequent injections of mercurial diuretics contained less chloride than the others in the group. High values occurred in patients who had received ammonium chloride (C, G, P). Although the data indicate that the total chloride increased with the extent of the edema, the individual values must be interpreted in terms of the clinical state and prior therapeutic management.

Discussion. Estimates of the chloride content of normal man have been made in the past and the value of 2400 mEq commonly accepted in the literature(20,21) is quoted from work published in 1910 by Magnus-Levy(22). He analyzed the concentration of chloride in various tissues obtained from a 37-year-old male suicide, and multiplied these values by the individual tissue weights proposed by Bischoff in 1863(23). On this basis, he was able to account for 27.4 g (770 mEq) of chloride in the tissues analyzed. He accepted as well known the amount of blood and its chloride content. Blood is recorded as 4.5% of body weight containing 3.5% chloride which brings the total body chloride content to 42.3 g (1190 mEq). For the concentration of chloride in the skin, skeleton, and adipose tissue he "annexed" the values from Nencki (1894) and Wahlgren (1909) who performed their analyses on dog tissues (2,24). The "rest" is 1.32 kg containing 2% chloride. On this basis he estimated total

20. Shohl, Alfred T., Mineral Metabolism, A.C.S. Monograph Series No. 82, Reinhold Publishing Corp., N. Y., 1939, Chap. 2.

21. Sherman, H. C., Chemistry of Food and Nutrition, 6th Ed., Macmillan Co., N. Y., 1941, Chap. 12, Table 32, p. 220.

22. Magnus-Levy, A., *Biochem. Z.*, 1910, v24, 363.

23. Bischoff, E., *Z. f. Rationelle Med.*, 1863, v20, 75.

24. Wahlgren, Valdemar, *Arch. f. Exp. Path. u. Pharm.*, 1909, v61, 97.

body chloride as 85 g (2400 mEq). Considering the evidence from which this value of 2400 was synthesized it is a really good estimate for total body chloride compared with the value of 2000 mEq calculated from the dilution of bromide.

The amount of chloride per unit weight found in man may be compared with that found in other mammals. Weir(25) using the dilution of bromide as a measure of total body chloride in nephrectomized animals, reports 33.3 mEq of chloride per kg as a mean of 9 dogs, 34.4 mEq per kg as a mean of 10 cats, and 30.2 mEq per kg as a mean of 10 rabbits. These values are in agreement with those obtained by the direct analysis of chloride in the total animal. Rosemann extracted the entire animal with alkali and reports 33.6 mEq of chloride per kg as a mean of 3 dogs(26). Harrison, Darrow, and Yannet homogenized the entire animal and report 33.6 mEq per kg as a mean of 2 dogs, 31.2 mEq per kg as a mean of 3 rabbits, and 31.7 mEq per kg in 2 monkeys(27). In the group of 52 individuals in this report the average chloride found was 30.1 mEq per kg. The amount of chloride per unit weight in man, is then quite close to that found in the dog, cat, rabbit, and monkey.‖

25. Weir, E. G., *Am. J. Physiol.*, 1939, v127, 338.

26. Rosemann, C., *Pflugers Arch.*, 1910, v135, 177.

27. Harrison, H. E., Darrow, D. C., and Yannet, H., *J. Biol. Chem.*, 1936, v113, 515.

‖ Recently Burch(28) using the dilution of isotopic Cl^{36} reports 42.8 mEq of chloride per kilo as an average of 5 dogs, the range of values for these 5 dogs, however, was rather wide 29.0 to 60.8 mEq/kg.

28. Burch, G. E., Threefoot, S. A., and Ray, C. T., *J. Lab. Clin. Med.*, 1950, v35, 331.

The present data indicate that about half of the body chloride is within the cells. When a large portion of the chloride is intracellular a change in total chloride does not necessarily represent a change in the volume of extracellular fluid as the change or any portion of it may have taken place within the cells.

If the 900 mEq of intracellular chloride is evenly distributed throughout approximately 25 liters of intracellular water(1), the intracellular concentration of chloride may be estimated at 36 mEq per liter. Of course there is considerable variability of the chloride concentration in the intracellular water of various tissues. In muscle cell water the concentration of chloride seems negligible, whereas in skin it is at least 110 mEq per liter(29). The concentration of chloride in red cell water is 75 mEq per liter(30). In view of this variability it is difficult to give physiological meaning to the "average" value of 36 mEq per liter. It is evident from this data that the volume of dilution of bromide as it is ordinarily calculated does not represent in any sense a volume of water in which chloride is evenly distributed since the anion is present in different tissue cells in varying concentrations.

Summary. Total body chloride was measured in normal man by the dilution of bromide and it has been estimated that nearly half the total body chloride is intracellular.

The authors are indebted to Marvin Fink and Roslyn Jackenthal for their technical assistance.

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