DETERMINATION OF THE BROMIDE SPACE IN MAN BY FLUORESCENT EXCITATION ANALYSIS OF ORAL BROMINE

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The bromide dilutional volume determined by intravenous administration of ^{82}Br has been compared with the corresponding volume determined by oral administration of stable bromide in 11 patients with various medical disorders. Stable bromide was assayed by fluorescent excitation analysis using a ^{109}Cd source and a lithium-drifted silicon detector. The average deviation between the fluorescent and the radiobromide dilutional volumes was 4.2% with a standard deviation of ±8.5%. This substantiates both the accuracy of the fluorescent excitation method as applied to this tracer and the validity of utilizing oral tracer administration in comparison with intravenous administration. The derived estimates of extracellular fluid volume averaged 28.7% of body weight in the entire group of 11 patients and 25.8% in the 4 normal subjects included in the group.

Evaluation of the extracellular fluid space utilizing fluorescent excitation of stable bromide permits high statistical accuracy of sample measurement with great simplicity compared with current chemical methods and with avoidance of the patient radiation exposure associated with ^{82}Br.

Determination of the extracellular fluid volume (ECFV) in man is of considerable interest in evaluating states of physiologic and pathologic fluid shift. Several tracers, both chemical and radioactive, are available for estimation of the ECFV by dilutional assay (*1–3*). These tracers give separate values for the ECFV due to differences in metabolism, specific organ localization, and diffusion into less accessible compartments of the extravascular space (*2*). No single tracer appears to give a truly accurate measurement of the ECFV. It is therefore customary to select one of the common tracers and either perform comparative studies in the same patient or compare values with established norms in the literature.

The bromide volume of distribution, corrected for bromide diffusion into red blood cells and for Gibbs-Donnan and plasma protein effects, is one of the most widely used and accepted values for ECFV even though there is known loss of tracer into gastric juices, thyroid, and salivary glands resulting in a slightly high estimate of the volume (*1–3*). Chemical techniques of assaying stable bromine are used in some laboratories but are lengthy and tedious (*4,5*) while other nonradioactive assay techniques such as the polarographic method (*6*), neutron activation analysis (*7*), and Br^--selective electrodes (*8*) have not gained wide use for a variety of reasons. Bromine-82 offers many advantages in the determination of the bromide volume (*3,9–14*), but its short half-life (36 hr) results in the need for frequent delivery and rapid counting following use and there is a small but definite patient radiation exposure that particularly limits its use in children and during pregnancy.

Fluorescent excitation analysis (FEA) is a simple and relatively new technique in biomedical investigation that permits the highly accurate assay in biologic samples of any element having an intermediate-to-high atomic weight (*15,16*). FEA has been effectively applied to the determination of the bromide space in children, simplifying the assay procedure greatly and eliminating radiation exposure to the subject (*17*). The present study was undertaken to compare FEA of stable bromine with radiobromine (^{82}Br) in the measurement of the bromide space in adults and also to compare the oral with the intravenous routes of tracer administration.

Received Nov. 11, 1974; revision accepted March 6, 1975.

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MATERIALS AND METHODS

Patients. Eleven adult patients, hospitalized for various major and minor medical problems, made up the patient study group (eight male and three female). Informed consent was obtained in each instance prior to undertaking the procedure.

Tracer. Fifty microcuries of sterile ^{82}Br-sodium bromide was administered intravenously to each patient at the beginning of the procedure. In all but two of the patients a standard diagnostic dose of stable sodium bromide, 40 mEq in 10 cc of water, was given orally at the same time. In the remaining two patients the oral bromide was given several hours prior to the radioactive tracer study.

Blood samples were obtained at short intervals beginning 5 min after administration of the radioactive tracer with increasing intervals of sampling out to 24 hr in all patients but one whose study was terminated at 12 hr. The blood samples were heparinized and the plasma separated for assay of ^{82}Br.

Similar aliquots of each plasma sample were assayed for stable bromine content by FEA. The technique has been described in detail elsewhere (*17*). In brief, a 2.5-mCi ^{109}Cd source was collimated and mounted to irradiate the sample of interest. An 80 mm^2 Si(Li) semiconductor detector with a 3-mm depletion depth was also collimated and positioned at right angles to the source, creating a 1-cm^3 region of sensitivity. The cadmium x-rays interact with and eject some of the bromine K shell electrons. Electrons from outer shells, particularly the L shell, then fall into the vacant K shell locations resulting in emission of the characteristic bromine Kα and Kβ x-rays of 11.91 and 13.30 keV, respectively. These x-rays are then detected and quantitated by the Si(Li) detector with its excellent energy resolution and a multichannel analyzer. Bromine concentration is then calculated from the ratio of counts in the Kα peak to counts in a predetermined portion of the ^{109}Cd Compton peak following correction for a corresponding water or plasma background.

As established by repeated testing, the correlation with bromine concentration is linear over a wide range of 0.025–500 mEq/liter, thus encompassing the concentrations both of the administered standard and of the final plasma sample. The assay procedure is independent of sample position and volume. The optimum sample volume for counting is 1–1.5 cc, the minimum approximately 0.5 cc. As illustrated in Table 1, the present ^{109}Cd source of 20 mCi permits quite high accuracy of sample counting at short counting times, particularly advantageous in comparison with the much lengthier chemical method of assay (*5*). The counting procedure is simple and can be performed efficiently by an untrained technician.

TABLE 1. ACCURACY OF BROMINE CONCENTRATION DETERMINATION

Method	Counting time (sec)	Concentration (mEq/liter)	Standard deviation δ (%)
Chemical	8 samples/ 8 hr day	1.42	2.6
Fluorescent	20	292	0.5
	50	17.8	1.1
	100	2.23	1.6
	200	0.365	2.2
	500	0.051	1.1

Calculation of bromide volume of distribution. Since the purpose of the study was to compare the radioactive and stable tracer techniques, the values obtained have been expressed as follows:

$$\text{Bromide dilutional volume (liters)} = \frac{\text{Total tracer introduced}}{\text{Concentration of tracer per liter of plasma at } T_0}$$

The zero time (T_0) concentrations of the stable and the radioactive bromide were derived by least-squares fit of the final slope of the plasma concentration curves both from 3 hr and from 5 hr onward in order to correct for continuous bromide losses. For comparative purposes a third set of dilutional volumes was calculated using the average concentration from 6 to 12 hr. Finally, the extracellular fluid volume itself was estimated by correcting the 5-hr extrapolated space for 5.7% bromide diffusion into red cells and a Gibbs-Donnan and plasma protein correction of 0.93 (*11*).

RESULTS

Figure 1 illustrates the calculated dilutional volumes in two of the patients throughout the entire 24-hr period of sampling, demonstrating the close similarities at all time points between the two techniques and the fact that equilibration occurs by 2 hr with intravenous injection and by 5 hr with oral administration. In patients where the larger percentage deviations occurred, the differences were noted to be systematic throughout the time period of sampling rather than random, suggesting that the error may well have been one of calibration with the dose standard rather than the result of fluctuations in the calculated distribution volumes.

Table 2 compares the three different dilutional volume calculations for the intravenous ^{82}Br with those of the oral stable bromide assayed by fluorescent excitation in each of the 11 patients. The first

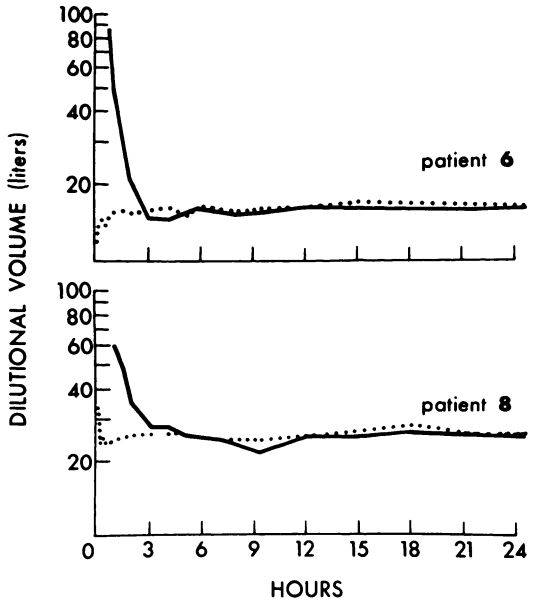

FIG. 1. Serial bromide dilutional volume estimations by fluorescent excitation of stable bromine (solid lines) and by counting of ^{82}Br (dotted lines) in two representative subjects.

two vertical columns represent the simple averages of the 6–12-hr values with standard deviations, the second two columns represent the dilutional volume by single exponential back-extrapolation to time zero of the 3–24-hr values, and the final two columns represent the corresponding zero time extrapolated values using the 5–24-hr data. The average percent deviations of the fluorescent technique compared with the radiobromide technique are expressed at the bottom of the table, including the corresponding standard deviation within each group of 11. In all but four patients the differences between individual studies lie within 9% of each other. Average deviations between the two techniques are 0.9%, 3.2%, and 4.2% by the separate calculation methods, respectively.

Additional clinical data on all patients including age, weight, and clinical status are summarized in Table 3. In addition, the bromide dilutional volume has been corrected by 0.873 for Gibbs-Donnan effect, plasma protein content, and red cell incorporation of the tracer (*11*). Finally, each derived estimate of ECFV has been expressed as a percent of body weight and the mean and standard deviation of each group calculated. In the four patients (numbers 1, 3, 5, and 10) known not to have hepatic or cardiovascular disease potentially predisposing to fluid retention, the average ECFVs by ^{82}Br and stable bromide were 25.7% and 25.8% with standard deviations of 3.5% and 5.3%, respectively.

DISCUSSION

The application of fluorescent excitation analysis to biomedical studies is relatively new but has major clinical potential. Imaging of intrinsic thyroid iodide by fluorescent excitation is being explored by several groups (*15,18,19*), and we have recently begun quantitative in vivo evaluation of the organ kinetics of various iodine- and bromine-containing compounds utilizing fixed solid state detectors (*16,17,19*). In vitro utilization of the technique permits accurate quantitation of elements in biologic samples without the problems of in vivo tissue absorption and by special attention to sample preparation (sample concentration, etc.) allows the use of stable tracers extending to very low atomic weights.

Stable bromine is an advantageous nonradioactive tracer for FEA. Its $K\alpha$ and $K\beta$ characteristic x-rays of 11.91 keV and 13.30 keV can be easily quanti-

TABLE 2. COMPARISON OF FLUORESCENT AND ^{82}Br DETERMINATIONS

	Dilutional volume (liters)					
	6–12 hr average		3–24 hr extrapolation to 0 hr		5–24 hr extrapolation to 0 hr	
Patient	^{82}Br	Fluorescent	^{82}Br	Fluorescent	^{82}Br	Fluorescent
1	19.9 ± 0.5	21.1 ± 0.5	19.9	20.4	19.8	21.0
2	23.7 ± 0.7	26.3 ± 1.3	21.4	24.4	22.6	25.4
3	24.2 ± 0.5	24.0 ± 1.1	24.2	23.1	25.0	23.0
4	21.1 ± 0.4	22.5 ± 0.8	21.4	22.2	21.9	21.5
5	19.6 ± 0.5	19.6 ± 0.6	16.8	16.8	16.6	16.0
6	16.2 ± 0.4	15.9 ± 0.3	15.9	15.1	15.9	15.8
7	21.7 ± 0.7	18.1 ± -0.6	20.8	16.7	20.9	17.5
8	25.2 ± 0.4	25.3 ± 0.5	25.7	26.2	25.3	24.2
9	23.0 ± 0.5	23.6 ± 0.6	22.4	21.7	22.9	21.7
10	22.4 ± 0.9	20.8 ± 1.5	22.1	18.7	22.3	20.3
11	22.3 ± 0.4	20.0 ± 0.4	22.0	19.8	22.1	18.5
All patients	a.d. = 0.9% s.d. = 8.0%		a.d. = 3.2% s.d. = 9.4%		a.d. = 4.2% s.d. = 8.5%	

TABLE 3. CORRECTED EXTRACELLULAR FLUID SPACES

Patient	Sex	Age (yrs)	Weight (kilos)	Diagnosis	^{82}Br ECF (liters)	^{82}Br % Body weight	^{80}Br ECF (liters)	^{80}Br % Body weight
1	M	18	72	Urticaria	17.3	24.0	18.3	25.5
2	M	23	62	Asthma	19.7	31.8	22.2	35.8
3	M	24	87	Obesity	21.8	25.1	20.1	23.1
4	M	42	59	Liver disease	19.1	32.4	18.8	31.8
5	M	29	68	Obesity	14.5	21.3	14.0	20.5
6	F	71	52	Myocardial infarction	13.9	26.7	13.8	26.5
7	F	46	52	Hypertension	18.2	35.1	15.3	29.4
8	F	49	56	Cirrhosis, ascites	22.1	39.4	21.1	37.7
9	M	69	66	Hypertension, CHF	20.0	30.3	18.9	28.7
10	M	72	74	Pneumonia	19.5	26.3	17.7	23.9
11	M	39	49	Sickle cell disease	19.3	39.4	16.2	33.0
				Mean		30.2		28.7
				s.d.		5.8		5.2

tated at the low parts-per-million level, with minimal difficulties from intrinsic sample radiation absorption. The specifics of the FEA technique utilized here have been detailed in a previous publication (*17*). In that study, determination of the bromide space by fluorescent excitation of bromine was compared with a standard chemical method of assay. The present study has extended the comparison to include ^{82}Br with substantiation once again of the validity of the FEA method. The present study has also compared oral administration of stable tracer with intravenous administration of the radioactive tracer, confirming the fact that both methods of administration produce essentially the same bromide volume of distribution as suggested previously by Leth and Binder (*11*).

It is customary to correct for urinary loss of tracer by accurate measurement of this excretion. Since both of the tracers would be lost to the same degree, we did not include such a correction in the calculations. The 24-hr urine loss of ^{82}Br was measured and found to be 1.4–4.7% (mean 2.9%). Back-extrapolation of the 5–24-hr values (Tables 2 and 3) would correct for urinary and any other continuous losses but not for the initially rapid uptake in red cell mass which must be corrected for separately.

Several authors have noted equilibration of the plasma bromine levels in 2–3 hr after intravenous administration (*1,13,20,21*), and in 3–6 hr after oral administration (*1,5,11,12*). Our own observations coincide with these equilibration times. The ECFV in man has been estimated by others to be 26.5% (*13*), 26.4% (*14*), 22.8–23.4% (*22*), 22.0–23.9% (*3*) and 20.4–21.0% (*5*) of body weight, and 23.5% of ideal body weight (*20*), figures with which our average of 25.8% of body weight in the four normal subjects compares quite well. The average deviation of 4.2% between the two techniques in the present study is within the range of 5.3% average deviation noted by Leth and Binder utilizing repeated oral studies in the same subject (*11*).

Our present routine diagnostic procedure for ECFV begins with the oral administration of sodium bromide, 1.75 mEq/kg, in an aqueous solution of 4 mEq/cc concentration, with the subject fasting overnight. Two 5-cc anticoagulated blood samples are drawn 15 min apart at 6 and 6¼ hr, the plasma separated, and the bromine concentrations assayed on 1–2-cc aliquots. These two values are averaged to give the final equilibration concentration. Total urine collection and assay for bromine loss over the 6-hr equilibration time is recommended, although tracer loss during this period amounts to only 0.4–1.2% of the administered dose (average 0.7%). As outlined under MATERIALS AND METHODS, the bromide dilutional volume is then calculated and multiplied by a factor of 0.873 to give the ECFV, thus correcting for diffusion into red blood cells and for Gibbs-Donnan and plasma protein effects (*11*).

The assay of stable bromine by FEA is much simpler and more accurate then the classical chemical technique, permitting determinations at diagnostic levels of 2 mEq/liter with a coefficient of variation of 1.6% in a single 100-sec counting period (Table 1) and requiring no more complex handling of each sample than separation of red cells from plasma. Because of the sensitivity of the FEA technique at these plasma bromine concentrations, it would be possible to give much smaller diagnostic tracer doses, permitting the performance of several sequential studies in the same patient while remaining well below levels of toxicity. This simple nonradioactive procedure is particularly useful for studies in chil-

dren and pregnant women where avoidance of radiation exposure is essential. The present study demonstrates the close correlation between ECF volumes determined by FEA of stable bromine and corresponding dilutional volumes derived from the classical radiobromide technique and additionally substantiates the fact that oral administration results in the same volume of distribution as intravenous administration. The prospect of having many new diagnostic and investigative procedures available through the use of FEA is an intriguing one already under active investigation.

ACKNOWLEDGMENTS

We are grateful to Malcolm Holliday, Nello Pace, Arthur Kodama, and Jack Wang for their assistance in various phases of the study. We wish to thank Richard Frankel and Rolf Woldseth of the KeVex Corporation for the loan of the Si(Li) detector used in this work.

This investigation was supported in part by a Public Health Service Research Career Development Award (Number GM 70598-02) from the NIGMS.

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