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CHLORIDE, BROMIDE, SODIUM, and SUCROSE *
SPACES IN HUMANS

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Introduction

Present methods for measuring the volume of the extracellular fluid have well recognized limitations. There is no known substance that is distributed uniformly throughout the extracellular fluid, including such subdivisions as the gastrointestinal juices and the cerebrospinal fluid, and which also maintains an exclusively extracellular position. Further, it is now realized that when using the dilution methods prolonged time intervals must be allowed before equilibrium can be assumed to be complete; and increased time necessarily magnifies such unavoidable errors as those caused by the metabolism of the agent or by its loss through the skin. In spite of these limitations, the volumes of distribution of certain of these agents provide useful parameters to study variations of the volume of extracellular fluid and to indicate changing distributions, between extra- and intracellular compartments, of substances studied simultaneously.

Of the naturally occurring substances in the body, the

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distribution of the chloride ion most nearly approximates that of the extracellular fluid, and hence the chloride space is an important reference for evaluating measurements of this compartment. The measurement of the dilution of radioactive chloride in the body is a simple and accurate procedure; and owing to the physical properties of two of the isotopes of chlorine (Cl^{36} and Cl^{38}), either of them can be combined in double tracer experiments with any of the isotopes now in clinical use. These double tracer experiments provide dependable and accurate comparisons of the distributions of the two isotopes used. In this study, direct comparisons were made between the radioactive chloride space and the volumes of distribution of bromide, sodium and sucrose in an effort to increase our understanding of the relative significance of these measurements as parameters of the extracellular fluid. Separate determinations of the dilution volumes of these agents have been made previously. The radioactive chloride dilution has been measured by Moore (1) and by Ray, Burch and Threefoot (2);

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radioactive sodium by Forbes and Perley (3); stable bromide
by Brodie, Brand and Leshin (4) and by Dunning, Steele and
Berger (5); and sucrose by Deane, Schreiner and Robertson (6).
Deane and Smith have simultaneously determined the volumes
of distribution of sucrose and sodium (7) and of sucrose and
bromide (8).

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Methods

A previous report from this laboratory (9) has described the methods used in this study to prepare, inject and count Cl^{38} , Br^{82} and Na^{24} ; to count the individual activities when Cl^{38} is used in combination with either Na^{24} or Br^{82} ; and to make corrections for the Donnan equilibrium and for the volume displacement of the plasma proteins. The procedures are briefly reviewed here. When the volumes of distribution of Na^{24} and of Cl^{38} were to be determined simultaneously, a dry salt mixture was prepared which contained 6 mgm/Kg body weight of ammonium chloride and 0.3 mgm/Kg body weight of sodium chloride. The salts were irradiated in the Brookhaven reactor (flux 5×10^{12} neutrons/cm²/sec) for 10 minutes in Lucite cups. The 175 degree temperature and the intense radiation of the interior of the reactor provided sterilization. The contents were dissolved in sterile distilled water, and the preparation was then ready for injection without further processing. An aliquot was taken to serve as the measure of the injected activities. Blood

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samples were drawn at one and at two and one half hours. The separate volumes of distributions were calculated from two counting series as described. When Br^{82} and Cl^{38} were used together, the Br^{82} was prepared and standardized separately.

In experiments on normal subjects the accepted tolerance radiation dose rate of 300 millirep/week should not be exceeded. A total dose of 300 millirep will be delivered in an experiment in which the isotopes or combinations of isotopes are used in the following initial concentrations:

- 2.7 $\mu\text{c}/\text{Kg}$ of Na^{24} ;
- 68.2 $\mu\text{c}/\text{Kg}$ of Cl^{38} ;
- 1.7 $\mu\text{c}/\text{Kg}$ of Br^{82} ;
- 0.5 $\mu\text{c}/\text{Kg}$ of Na^{24} with 56 $\mu\text{c}/\text{Kg}$ of Cl^{38} ;
- 0.4 $\mu\text{c}/\text{Kg}$ of Br^{82} with 52 $\mu\text{c}/\text{Kg}$ of Cl^{38} ;

In the calculations for the above values the definition of the rep as the absorption of 93 ergs/gram of tissue was used.

In this study 5 ml. portions of plasma were counted in a multiple anode gamma sensitive counter*, and Cl^{38} counts were obtained before three hours had elapsed from the time of injection.

*Donated by the Texas Oil Co. (H18-20 TR)

Without exceeding 300 millirep per week, sufficiently high counting rates were obtained so that the maximum standard error of any counting period was less than 2 per cent.

The sucrose spaces were determined using the method described by Deane (6). No priming injection was given. The infusion pump was started 15 minutes after the solution of Na^{24} , Cl^{38} , and stable bromide had been washed in. As all the agents were determined from the same blood samples, the sucrose was analyzed in samples obtained 15 minutes earlier, in relation to the start of the infusion, than is indicated in Table I. Separate volumes of distribution were calculated for each plasma sample. The sucrose was hydrolyzed and the resulting levulose measured as in Schreiner's method for inulin (10). Stable bromide determinations were made using the method of Brodie and Friedman (11). The serum sodium concentrations were measured by flame photometry, serum chloride concentrations by the method of Van Slyke and Hiller (12), and serum proteins by the copper sulphate technique (13). For the purpose of the

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Donnan equilibrium corrections, the plasma volume was assumed to be 4 per cent of the ideal weight. All volume of distribution calculations were corrected for the amount of the agent excreted in the urine.

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Results

The results of the simultaneous determinations of the volumes of distribution of Cl^{38} , Na^{24} , sucrose and stable bromide are tabulated in Table I. The values from the first patient are plotted in figure 1. It is apparent from examination of Table I that equilibrium of sucrose and of Cl^{38} has not been conclusively established. Unfortunately, data that could be used to extend these curves are not readily obtainable. Activities of Cl^{38} that will give the patient less than 0.3 rep per experiment cannot be followed for appreciably longer periods of time with presently available counting equipment. With the Deane sucrose method, accuracy of the volume of distribution measurement becomes progressively more difficult to obtain when, after three to five hours, the amount of sucrose appearing in the urine exceeds half of the amount infused (6). Hence the method is not adaptable to prolonged experiments. For the purposes of the following summary, the $2\frac{1}{4}$ hour and $3\frac{3}{4}$ hour values were averaged to give the "3 hour" sucrose space.

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These dilution measurements (Table I) were performed on 5 chronically ill, non-edematous patients. Only patient was bed-ridden. Converting these volumes to per cent body weight yields the following values:

Cl³⁸ at 2½ hours - 22 to 29% (mean 26.5%)

Stable bromide at 21 hours - 25 to 33% (mean 29.1%)

Na²⁴ at 21 hours - 28 to 35% (mean 31.9%)

Sucrose at 3 hours - 17 to 22% (mean 20.5%)

Although these were hospital patients, the results are in accord with the data of other workers who used normal subjects (2, 3, 4, 5, 6). Further, the "3 hour" sucrose spaces of this study varied between 61 and 79 per cent (mean 70.5 per cent) of the 21 hour bromide space and between 59 and 73 per cent (mean 64.3 per cent) of the 21 hour sodium space. These values are in agreement with those of Deane (7, 8).

In Table II are presented the results of ten double tracer experiments using Cl³⁸ and Br⁸². Patient was edematous, patient had advanced hydrocephaly, and patient

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was bedridden. The other six were ambulatory. The Br⁸² spaces in this series are larger than the Cl³⁸ spaces by an average of only 2.4 per cent. This close correlation was predicted by earlier workers following the observation that the distribution of bromide is closely proportional to that of chloride in animals (14, 15). The values of the distribution of stable bromide (Table I) are larger than those of Cl³⁸ by an average of 7 per cent.

During the course of these experiments, samples of urine, packed red cells (centrifuged at 2,000 g for fifteen minutes), pleural fluid, gastrointestinal juices and cerebrospinal (ventricular) fluid were counted for both Cl³⁸ and Br⁸² in the same manner as were the plasma samples. The results are presented in Table III. Despite the close similarity in the overall dilution values of these two isotopes, there are striking differences in the proportions of the serum concentrations that appear in these samples. The distribution of the two ions relative to each other is described in the sixth column (Table III).

These figures were obtained by dividing the sample:serum ratio of the Br^{82} concentrations by this same ratio for the Cl^{38} concentrations. The ratios were determined from samples taken at the same time after injection.

In additional measurements (not tabulated in Table III), saliva samples were studied 24 hours after the injection of Br^{82} . In two of the patients, and , the saliva: serum ratio for Br^{82} was greater than this ratio for naturally occurring stable chloride by factors of 2.3 and 3.0 respectively.

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Discussion

The Br⁸² and Cl³⁸ concentrations in the various fluid samples listed in Table III are included here as an evaluation of the use of bromide as an indicator of the chloride space. Although bromide and chloride are distributed in most tissues in proportion to their serum concentrations (14, 15), they are not handled "indifferently" by certain of the membranes equipped with active transport systems. The kidney and the choroid plexus apparently show a preference for transporting the chloride ion from the blood stream while the salivary and gastric glands excrete the bromide ion in relatively higher concentrations.

Previous observations comparing the concentrations of bromide and chloride in the blood serum with those found in urine (16, 17) red cells (14, 17, 18, 19), transudates (4, 14) and saliva (17) are in accord with the results of this report. The relatively high concentrations of bromide in the gastric juice observed in this study, however, were not found

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by Mason (17) who studied patients with high levels of serum bromide. In this respect it is interesting to note that the data of Davenport, using both stable bromide (20) and stable iodide (21) predict higher concentration ratios (gastric juice: serum) when the serum concentrations are lower. In the experiments of this study, the amount of carrier for the radioactive bromide was less than 0.1 mgm %. Observations with radioactive iodine, using "tracer" amounts, indicate remarkably high gastric juice: blood ratios (22). Finally, although there is agreement that there is proportionately less bromide in the spinal fluid than there is chloride, there is not agreement as to the degree of this dissimilarity in the distribution of these ions. (23, 24).

With reference to the differences between the volumes of distribution of chloride and of sodium, it is noted that Harrison, Darrow and Yannet (25) described significant amounts of "excess" of sodium over chloride in animal whole-body studies and considered most of this sodium to be located in

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the bone. This observation has been supported by workers using radioactive sodium (26, 27), and the increment of the sodium space between 1 and 24 hours is assumed to be for the most part a measure of the distribution of sodium in bone. The distribution of sodium and chloride in the other tissues has been studied by Manery and Hastings and by Am erson et al with chemical methods (28, 29) and with isotope techniques by Manery (30, 31).

In attempting to evaluate the difference between the Cl^{38} space and the sucrose space, and to clarify the relations of each to the extracellular fluid, it is necessary to consider first, the distribution of these materials under ideal conditions that would allow equilibrium to become complete, and second, the limitations of our presently available methods as regards describing this state. Chloride is known to enter certain cells, particularly the cells of the blood, renal tubules, gastric mucosa, skin and lungs (28, 29); and hence the space that it measures is larger than the extracellular fluid volume.

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Exact data, however, are not available to calculate that

portion of the total body chloride that is inside the cells.

Although direct studies of the tissue distribution of sucrose have not been reported, it is known that sucrose does not enter red cells (32), and because it can be quantitatively recovered from the urine (6, 32), it is believed to maintain an extracellular position. Sucrose does not enter the cerebrospinal fluid (33) or the gastrointestinal juices, and hence the space does not include these subdivisions of the extracellular fluid that are included in the spaces measured by the electrolytes.

Turning to the limitations of the methods, as mentioned previously the time during which the spaces of Cl^{38} and sucrose can be determined is limited. From dilution data using Cl^{36} (2) and bromide (4, and Table I) it appears that by 2.5 hours the volume of distribution of the injected chloride has probably expanded to at least 95 per cent of its maximal value. Deane has reported that maximal values of sucrose are reached in less than three hours (6). Evidence, however, that complete equilibrium

may not be attained by three hours has been presented by Cotlove (34) who has demonstrated increasing amounts of sucrose in rat muscle as constant infusions were maintained for 2, 6 and 15 hours. The sucrose space measurements in Table II show small increases between $2\frac{1}{4}$ and $3\frac{3}{4}$ hours. These considerations of the incompleteness of our knowledge of the distribution of sucrose and of chloride and of the limitations of the available dilution methods discourage quantitative statements relating the extracellular fluid to the volume of distribution of either C^{138} or sucrose.

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Summary

The volumes of distribution of Cl^{38} , Na^{24} , stable bromide, and sucrose were determined simultaneously in five patients. The volumes of distribution of Cl^{38} and Br^{82} were determined simultaneously in ten patients.

Using average values obtained two and one half hours after injection and setting the volume of distribution of chloride at unity, the relative values obtained with the other agents were 1.07 for sodium, 1.07 for stable bromide, 1.02 for radioactive bromide, and 0.77 for sucrose.

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