Volume of Distribution of Radioactive Chloride in Dogs; Comparisons With Sodium, Bromide and Inulin Spaces

J. L. GAMBLE, JR. AND J. S. ROBERTSON

From the Medical Department, Brookhaven National Laboratory, Upton, Long Island, New York

In this study two of the radioactive isotopes of chlorine, Cl$^{36}$ (half life 400,000 years) and Cl$^{38}$ (half life 38 minutes) have been used in an effort to establish more definitively than has been done previously the relationships, in the dog, of the chloride space to the volumes of distribution of sodium, bromide and inulin. The first measurements of the radioactive chloride space in dogs were made by Winkler, Elkington and Eisenman (1) who reported that Cl$^{38}$ distributed through approximately 25 per cent of the body weight compared to 28 per cent for Na$^{24}$. Burch, Ray and Threefoot (2), using Cl$^{36}$, reported volumes of distribution varying from 32 to 38 per cent in five young dogs. Following the observation that the bromide distribution in the plasma and tissues was proportional to that of chloride (3, 4), several workers have used the dilution of stable bromide as a measure of the chloride space. Weir (5), Gaudino, Schwartz and Levitt (6), Brodie, Brand and Leshin (7) have reported bromide spaces of 30 to 32 per cent body weight. Berger, Dunning, Steele and Brodie (8) and Gaudino (6) measured volumes of distribution of bromide that were approximately 150 per cent of simultaneously determined inulin spaces.

Emphasizing direct and simultaneous comparisons in three groups of experiments, the relationships of the radioactive chloride space to those of radioactive sodium (Na$^{24}$), radioactive bromide (Br$^{82}$) and inulin were observed. The radioactive chloride and the substance with which it was being compared were injected simultaneously and the volumes of distribution were calculated from plasma concentrations determined from the same blood sample. As will be described, both the short-lived and the long-lived isotopes of chlorine are easily combined with Na$^{24}$ and Br$^{82}$ in double tracer experiments. These double isotope experiments are simple to carry out and more consistently accurate comparisons of the dilutions of two agents are obtained than when chemical methods are used.

Methods and Procedure

Counting Procedures and Isotope Preparation. Two types of Geiger counters have been used in these studies. The first is an end-window tube¹ having a high efficiency for beta particles and a low efficiency for gamma rays. This counter will be referred to as the beta-sensitive counter. The second counter² is a multiple anode tube with a high efficiency for counting gamma rays. The sensitive volume is a cylinder with a central well which receives the samples. Samples for the beta-sensitive tube are prepared by drying 0.18 ml. of the material on filter paper discs supported by aluminum planchettes. Curling of the discs with drying is prevented by the pre-
liminary addition of two drops of 50 per cent sucrose to each. Samples for the gamma-sensitive tube are counted as liquids in 3 ml. amounts in 15 mm. o.d. test tubes.

Cl$^{36}$ was prepared and purified at the Oak Ridge National Laboratory. The other isotopes were prepared by activation in the Brookhaven reactor: Na$^{24}$ from irradiations of Na$_2$CO$_3$ (or NaCl when to be used in combined studies with Cl$^{38}$); Cl$^{38}$ and Br$^{82}$ from irradiations of NH$_4$Cl and NH$_4$Br, respectively. Neutron bombardment of stable Na$^{23}$ results in the formation of but one radioactive isotope, Na$^{24}$; but during the activations of Cl$^{38}$ and Br$^{82}$, additional radioactive isotopes are formed in small but measurable concentrations. Fortunately, however, errors due to these impurities can be avoided. S$^{35}$ and P$^{32}$, which are present in the Cl$^{38}$ preparation (9), are not sources of counting error when the gamma-sensitive counter is used. These isotopes have no gamma emissions and do not produce significant numbers of counts on this instrument. The Br$^{82}$ preparation has some additional short lived activity detectable with decay studies for the first 24 hours. To avoid errors this preparation was not used until the 5th day following irradiation. To rule out the presence of other adventitious radioactive isotopes in these unpurified Brookhaven preparations, the beta absorption characteristics in aluminum were studied at those times after pile irradiations at which the samples were routinely counted. The decay of the isotopes was then followed until the activity had fallen to less than 1 part in 100. These studies did not indicate other radioactive impurities. All counting of Cl$^{36}$ was done through a 14 mg/cm$^2$ aluminum absorber to eliminate a possible error due to an S$^{35}$ contamination.

All counting procedures were checked by counting plasma and saline standards. From these empirical studies two correction factors were derived and were applied to plasma counts. The first was a factor of 1.035 for Br$^{82}$ when using the beta-sensitive counter and the second was a factor of 1.015 for Cl$^{38}$ when using the gamma-sensitive counter. These factors correct for the lowering of the counts produced by the absorption of beta radiations by the protein in the plasma. The wall of the gamma-sensitive counter shields out beta emissions of average energies but Cl$^{38}$ has beta emissions with such high energies (table 1) that a large percentage of them penetrate the wall and are counted.

**Procedures in Double Tracer Experiments.** The following procedures were used to determine individual activities in double tracer experiments in which either of the chlorine isotopes was combined with either Na$^{24}$ or Br$^{82}$. This separation of the individual activities is possible owing to the large differences between the half lives of the chlorine isotopes and those of sodium and bromide (table 1). The chlorine isotope and the one with which it is to be compared are dissolved in a common solu-

### Table 1. Isotope Physical Characteristics and Dosage

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Cl$^{36}$</th>
<th>Cl$^{38}$</th>
<th>Br$^{82}$</th>
<th>Na$^{24}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half life</td>
<td>$4 \times 10^4$ yr</td>
<td>38 min</td>
<td>35.5 hr</td>
<td>15 hr</td>
</tr>
<tr>
<td>Average beta, mev</td>
<td>0.24</td>
<td>1.30</td>
<td>0.15</td>
<td>0.54</td>
</tr>
<tr>
<td>Gamma, mev</td>
<td>1.16</td>
<td>0.55</td>
<td>2.76</td>
<td>1.38</td>
</tr>
<tr>
<td>$N$</td>
<td>2.15</td>
<td>0.79</td>
<td>1.35</td>
<td></td>
</tr>
</tbody>
</table>

**Maximum Doses Injected**

- Radioactive, μc: 20, 20, 300, 200
- Chemical, mg. includes carrier: 90, 84, 40, 2
tion. From this solution, first, a measured amount is withdrawn and injected into the dog and second, an equal amount is diluted to 2,000 ml., becoming the standard solution to serve as a measure of the injected activities of both isotopes. With each experiment plasma samples and samples of the standard are each counted twice; first, the activity of both when the short-lived isotope is still present and second, the activity of the long-lived isotope alone after a period of time that allows the short-lived isotope to decay to an insignificant proportion (6.5 hours for Cl$^{38}$, 6 days for Na$^{24}$ and 15 days for Br$^{82}$ allows time for these isotopes to decay to less than 1 part in 1,000). The part contributed to the first counting period by the long-lived isotope is calculated by applying decay corrections and subtracted to yield the counts due to the short-lived isotope. Counting both the plasma and the standard samples in this manner, the separate volumes of distribution (in milliliters) can be calculated from the formula (standard counts/plasma counts) $\times$ 2,000.

The adjustment of the dosages of radioactivity is based on the following considerations. In the Cl$^{36}$ studies the problem is a simple one. The Cl$^{36}$ is injected in quantities to provide enough counts for statistical accuracy. On the beta-sensitive counter (background, 30 counts/min.) 300 counts/min. will have a standard error of 2.6 per cent with a 6-minute counting period. As a convenience to reduce counting times, activities of Na$^{24}$ and of Br$^{82}$ were injected in amounts approximately 20 times those of Cl$^{36}$. In the Cl$^{38}$ experiments the relative quantities of the Cl$^{38}$ and of the Na$^{24}$ or Br$^{82}$ must be adjusted with some care if maximum statistical accuracy is to be obtained. In figure 1 the approximate activities used during these experiments are plotted with respect to time after removal from the nuclear reactor. As can be seen the ratio of Cl$^{38}$ activity to Na$^{24}$ activity is close to 250:1 at the time of injection and approximately 3:1 at the end of the first counting period. Injection into the dog is made within the first 30 minutes after activation and blood samples are drawn at 1 and at 3 hours following the injection. The activities of the samples are too high for counting during this period but they fall into the counting range soon after the 3-hour blood sample is drawn. The sample counts at this time represent the activities of both Na$^{24}$ and Cl$^{38}$. Assuming that the Cl$^{38}$ activity during this period falls from 8,000 to 4,000 counts/min. and that the Na$^{24}$ activity is less than 2,000.
counts/min., the standard error of the counting rate should be less than 1.4 per cent with 3-minute counting periods. During the second series of counts with Na\(^{24}\) alone, with total counts of 1,400/min. and considering the high background of the gamma-sensitive counter (300 counts/min.), the standard error should be less than 1.6 per cent with 6-minute counting periods. Almost identical considerations apply to the use of Br\(^{82}\) with Cl\(^{38}\). The standard error figures are those calculated for a single counting period. These countings were repeated twice in the Cl\(^{38}\) experiments and three times in the Cl\(^{36}\) studies.

The calculation may be simplified and the use of large decay corrections for Cl\(^{38}\) avoided by using, in both the first and the second counting series, the following counting sequence: standard, first plasma sample, standard, second plasma sample, standard. Exactly 1-minute intervals are allowed between the 6-minute counting periods. The value for the counts of the standard for the space calculations is obtained by averaging the counts of the two standards taken before and after each plasma sample. Because the decay is exponential this average is in error but by only 0.1 per cent.

As Cl\(^{36}\) is essentially a pure beta emitter, the beta-sensitive counter must be used and, as described previously, with Cl\(^{38}\) the use of the gamma-sensitive counter is desirable to exclude the S\(^{35}\) and P\(^{32}\) contaminants. Thus in these double tracer experiments the choice of the counter used is dictated by the Cl isotope. Referring again to table 1, it can be seen that Na\(^{24}\) and Br\(^{82}\) have both gamma and beta radiations and are satisfactorily measured on either type of counter. Na\(^{24}\) and Cl\(^{38}\) can be prepared simultaneously from irradiations of mixtures of NaCl and NH\(_4\)Cl containing the correct amounts of sodium and chloride to provide the optimal ratio of activities for the counting statistics. When Br\(^{82}\) or Cl\(^{36}\) is used each must be prepared and standardized separately.

Experimental Procedure. The maximum radioactive and chemical doses used are included in table 1. The total radiation received by the dogs during the day of the experiment was approximately 1 r in the Cl\(^{38}\) double tracer experiments and 3 r in the Cl\(^{36}\) studies.

Healthy male and female mongrel dogs who had not had food for 18 hours were used for these studies. Nembutal (80 mg/kg/hr.) was used only in those dogs undergoing the 24-hour experiments. The dogs were restrained on the table and an intravenous infusion started using a foreleg vein. The infusion set consisted of a standard Murphy drip delivering from the barrel of a 50-ml syringe. The dissolved isotopes were then drawn up in volumetric pipettes and delivered to the syringe barrel. The solution was allowed to drip in, being followed by three 10-ml. washes with saline and finally with an air wash as the plunger was inserted. This method of injection has the disadvantage of using additional fluid to wash in the isotope. It is, however, a dependable method of intravenous injection. It offers the advantage of using volumetric pipettes and these are easily handled by remote control (vaselined syringes with rubber tubing to draw up the solution and 20-inch tongs to handle the pipettes). Owing to the short half life of Cl\(^{38}\), the initial activity must be high in the 3-hour experiments. Two millicurie amounts were used which, giving off 15.2 r/hr at 1 cm. (10), should only be handled by remote control.

Blood samples were drawn from the external jugular vein and immediately centrifuged. Catheterized urine collections were obtained and subsequently corrections were made for the excreted activity. With the dogs in a semifasting state, the amount of isotope excreted in the 3-hour urine collections never exceeded 1 per cent of the injected activity.
Serum chloride concentrations were determined by the method of Van Slyke and Hiller. Sodium concentrations were determined by flame photometry. Plasma protein was measured using the copper sulfate technique.

**Determination of Volume of Distribution of Inulin.** The volume of distribution of inulin was measured using the method described by Gaudino, Schwartz and Levitt. The dog stood upright in a Pavlov frame for a 2.5-hour infusion period and the subsequent 5-hour collection period. Urines were collected through a metal bladder cannula fixed in place surgically by Dr. R. N. Watman of this department. Using a Bowman pump a 1.0 gm. per cent solution of inulin was infused at 1.2 ml/min. following a 40-ml. priming injection. An initial 250 ml. of 5 per cent glucose was infused i.v. to initiate diuresis and to wash in the Cl	extsuperscript{36}. Since only intravenous hydration was used water balances could be calculated. Urine flows at the termination of the infusion varied from 1.7 to 2.2 ml/min. The renal dead space correction was made, in the first approximation, by terminating the bladder wash 100 seconds after the completion of the infusion. The urine inulin blank correction was determined from 5-hour collections under control conditions. Inulin concentrations were analyzed by the method of Schreiner.

**Corrections Due to the Protein in the Plasma.** Before calculations of the volume of distribution of Na, Cl or Br can be made from counts taken from plasma samples, two corrections due to the protein in the plasma must be considered. In the first place the protein, acting as a solid, reduces for a given volume of plasma the fluid and contained radioactivity. In the second place, the protein acting as an anion causes inequalities in the concentrations of the sodium and chloride between the serum and the interstitial fluid according to the Donnan equilibrium. The equations of Van Slyke, Wu and McLean indicate that if the activity coefficients of the two ions were equal and if the concentrations are expressed in terms of equivalents of electrolyte per kilogram of water, the Donnan corrections should be close to 1.04 for chloride and 0.96 for sodium. However, data summarized by Peters and other investigations recently referred to by Tarail indicate that the mean factors for the distribution ratio between plasma and edema fluid are more nearly 1.04 for the chloride ions and 0.94 for the sodium. The following formula was used to determine a corrected volume of distribution.

\[
\text{Corrected } V_D = \frac{P}{k} (\text{uncorrected } V_D - V_p) + V_p
\]

Where \(V_D\) = volume of distribution

\(k\) = the Donnan factor 1.04 for chloride
0.94 for sodium

\(P\) = protein displacement factor (13)

\[
(100 - \text{grams protein} \times 0.73) / 100
\]

\(V_p\) = plasma volume

For the purposes of this study we have assumed that the plasma volume was one-fourth of the uncorrected inulin space and one-sixth of the uncorrected chloride space.

**RESULTS AND COMMENTS**

The results of the double tracer experiments are summarized in table 2 and in figure 2.
The first experiment in table 2 was performed as a check on the methods. As mentioned, the C[36] is counted using the beta-sensitive counter while Cl[38] is counted on the gamma-sensitive instrument. The resulting chloride spaces closely approximate each other. In the remainder of the double tracer experiments, the individual spaces were calculated from the two series of counts taken from the same sample with the same counter, as described above. The chloride space of dog D, a 4-month old pup, is larger percentage-wise than those of the other dogs, all adults. Similarly, Burch et al. (2), who used young dogs obtained relatively larger spaces than did Winkler (1), who used mature animals. Spaces as large as 45 per cent have been measured in newborn puppies (19). The results of the double tracer experiments comparing the volume of distribution of radioactive chloride to that of radioactive sodium demonstrate that these spaces are almost equal at 1 hour. After this time there is little change in the chloride space but the sodium space continues to expand. The volume of distribution of C[36] at 24 hours was 24.8 per cent of body weight while that of Na[24] was 28.7 per cent. These results agree with those obtained by Winkler et al. (1) but they emphasize the importance of describing these spaces in relation to the time following injection. During this Na[24],Cl[36] experiment the animal received no water or food. One hundred and ten cc. of urine were excreted which contained 9 mEq. of sodium and 6 of chloride. Weight loss was 350 gm. The serum sodium and chloride concentrations were

Table 2. Cl[38] Studies

<table>
<thead>
<tr>
<th>DOG</th>
<th>WT., KG.</th>
<th>SERUM CI CONC. AT 3 HR. MEG/L.</th>
<th>ISOTOPE</th>
<th>VOL. OF DISTRIBUTION</th>
<th>VOL. OF DISTR. OF Cl[38] AT 2 HOURS, % BODY WT.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 hr ml.</td>
<td>ratio</td>
</tr>
<tr>
<td>D</td>
<td>4.05</td>
<td>110</td>
<td>Cl[36]</td>
<td>1485</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cl[38]</td>
<td>1438</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>11.0</td>
<td>113</td>
<td>Na[24]</td>
<td>1940</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cl[38]</td>
<td>1935</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>11.4</td>
<td>113</td>
<td>Na[24]</td>
<td>2065</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cl[38]</td>
<td>2025</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>11.1</td>
<td>112</td>
<td>Na[24]</td>
<td>2370</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cl[38]</td>
<td>2270</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>11.0</td>
<td>114</td>
<td>Na[24]</td>
<td>2250</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cl[38]</td>
<td>2300</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>10.1</td>
<td>115</td>
<td>Br[82]</td>
<td>1910</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cl[38]</td>
<td>1833</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>11.1</td>
<td>117</td>
<td>Br[82]</td>
<td>2600</td>
<td>1.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cl[38]</td>
<td>2525</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>9.7</td>
<td>117</td>
<td>Br[82]</td>
<td>2470</td>
<td>1.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cl[38]</td>
<td>2400</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>7.5</td>
<td>111</td>
<td>Br[82]</td>
<td>2030</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cl[38]</td>
<td>2065</td>
<td></td>
</tr>
</tbody>
</table>

Burch et al. (2), who used young dogs obtained relatively larger spaces than did Winkler (1), who used mature animals. Spaces as large as 45 per cent have been measured in newborn puppies (19).
measured to be 147 and 114.3 mEq/l., respectively, at 3 hours and 146 and 115.7 mEq/l. at 24 hours.

The Br\textsuperscript{82}-Cl\textsuperscript{36} results, in confirmation of the conclusions of previous investigators \((3, 4)\), indicate only small differences between the volumes of distribution of bromide and chloride. The Br\textsuperscript{82}-Cl\textsuperscript{36} 24-hour study does not suggest a changing relationship with time. The 24-hour volumes of distribution for Cl\textsuperscript{36} and Br\textsuperscript{82} were 25.9 per cent and 27 per cent body weight, respectively. In this experiment 1.6 per cent of the injected Cl\textsuperscript{36} appeared in the urine in 24 hours as compared to 0.9 per cent of the injected Br\textsuperscript{82}. Eighty ml. of urine were excreted containing 2.5 mEq. of chloride. As in the previous experiment no intake was provided. The serum chloride concentration, measured only at the end of the 24-hour experiment, was 115 mEq/l.

The results comparing the volume of distribution of inulin with that of Cl\textsuperscript{36}
are summarized in table 3. These direct measurements, in agreement with the conclusions of previous investigators (7, 8), emphasize the large difference between the volumes of distribution of these two substances at 2½ hours.

**DISCUSSION**

A primary aim in this study has been to evaluate the relative merits of these chlorine isotopes as tools in physiological research. Cl\(^{36}\) is expensive at the present time as months of activation and a final chemical purification are required. The hazards of personnel and laboratory contamination must also be considered. On the other hand, since the activity in the blood can be accurately followed for several weeks, measurements of the chloride space can be made when equilibrium is complete. Cl\(^{38}\), owing to its short half life, can be used only close to the site of production and the period of time during which it can be measured may not permit the isotope to come to complete equilibrium. This short half life does, however, allow repeated experiments at short intervals and the results of double tracer experiments are promptly available. Cl\(^{38}\) has proven to be a convenient isotope with which to work as processing is necessary neither in the preparation of the material for injection nor in the preparation of the samples for counting. As described, the decay characteristics of each of these chlorine isotopes give them special value in double tracer experiments.

Dilution measurements do not attain maximum significance until complete equilibrium is reached. In Cl\(^{38}\) experiments, therefore, it is important to have a quantitative understanding of the approach to equilibrium, of both the Cl\(^{38}\) and the agent with which it is being compared, at that time when the Cl\(^{38}\) measurements must be discontinued. The curves of the volume of distribution of Cl\(^{38}\) appear to indicate that equilibrium is reached within the 3-hour interval during which the Cl\(^{38}\) studies are feasible. These curves, however, are distorted by the effects of renal and insensible losses of salt and water. In experiments such as these in which there is no intake of the tracer element it is the specific activity that provides the valid index of the approach to equilibrium. In dog F the serum chloride concentrations remained almost constant and the specific activity of Cl\(^{38}\) in this single experiment fell only 2 per cent between 3 and 24 hours. Data are insufficient to make this calculation for dog G. The volume of distribution curve, however, suggests that the decrease of the specific activity would be larger by a few per cent. The data of Burch et al. (1) from dog experiments indicate that equilibrium is reached in 1 hour by Cl\(^{38}\). More recently however, these same authors (20) have published results from human experiments demonstrating that 2 days are necessary to establish equilibrium. Turning to sodium it is obvious from the Na\(^{24}\)-Cl\(^{38}\) curves (fig. 2) that there is a considerably greater increase of the Na\(^{24}\) space between 3 and 24 hours than there is of the Cl\(^{38}\) space. The sodium specific activity decreased 13 per cent during this time. This is believed

**Table 3. Comparisons of the Volume of Distribution of Inulin and Cl\(^{38}\) at 2.5 Hrs.**

<table>
<thead>
<tr>
<th>DOG</th>
<th>WT., KG.</th>
<th>WATER BALANCE, ML.</th>
<th>% INJECTED INULIN RECOVERED</th>
<th>(V_D) (Cl^{38}) ml</th>
<th>% body wt.</th>
<th>(V_D) INULIN ml</th>
<th>% body wt.</th>
<th>RATIO (V_D) INULIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>9.7</td>
<td>+250</td>
<td>96.4</td>
<td>2770</td>
<td>28.6</td>
<td>1835</td>
<td>18.9</td>
<td>.66</td>
</tr>
<tr>
<td>C</td>
<td>9.8</td>
<td>+100</td>
<td>90.0</td>
<td>2730</td>
<td>27.9</td>
<td>1930</td>
<td>19.7</td>
<td>.71</td>
</tr>
<tr>
<td>C</td>
<td>9.4</td>
<td>+100</td>
<td>102.0</td>
<td>2550</td>
<td>27.1</td>
<td>1575</td>
<td>16.8</td>
<td>.62</td>
</tr>
</tbody>
</table>
to be due in a large part to distribution of sodium in the bone (21–23). The distribution of sodium and chloride in the other individual tissues has been recently reviewed by Manery (9). In the single Br\(^{82}\)-Cl\(^{36}\) experiment, the equilibrium characteristics of bromide appear to parallel those of chloride. The equilibrium characteristics of inulin are under further investigation at this time. Gaudino, Schwartz and Levitt (6) have reported that maximum inulin spaces are reached in dogs 2 hours after the start of a constant infusion. Recently, however, Cotlove (24), analyzing rat muscle, and Nichols, Nichols and Weil (25), studying connective tissue (dog tendon), have reported progressively increasing amounts of inulin in these tissues during periods of time greater than 2 hours.

**SUMMARY**

Methods for using either the short-lived Cl\(^{38}\) or the long-lived Cl\(^{36}\) in conjunction with either Na\(^{24}\) or Br\(^{82}\) in simultaneous measurements of the volumes of distributions of these substances are described. Results obtained using these methods in dogs indicate that the sodium and chloride spaces are nearly equal at 1 hour after the injection of the isotopes. At 24 hours the chloride space has hardly changed but the sodium space has become about 15 per cent greater. The bromide space is consistently a few per cent larger than the chloride space after the 3rd hour after injection. The inulin space, as determined by the constant infusion technique, was found to be only two-thirds as great as the chloride space at 2½ hours after the start of the infusion and the injection of the isotope.

**REFERENCES**