Determination of Total Tissue Sodium Concentrations by Use of Radiosodium

By D. M. Green, M.D., T. B. Reynolds, M.D. and R. J. Gihed, M.D.

A method has been developed for the estimate of total tissue sodium by direct measurement of radiosodium concentrations in undigested samples. Accuracy is comparable to that of flame photometric estimates on tissue digests; time and cost, about one-quarter as great. The method appears particularly useful for experimental designs requiring multiple tissue sodium measurements.

Since the determination of tissue sodium by chemical methods, or by flame photometry, requires the digestion and solution of tissue, a time- and space-consuming procedure when large numbers of samples are involved, this study was made to ascertain if radiosodium would lend itself to a simpler, yet accurate, determination. It was considered that such a method might facilitate studies of tissue sodium metabolism in many circulatory diseases, including hypertension and heart failure.

The basis of this method rests on the presumption that when radiosodium\(^{22}\) is administered to an animal in harmless tracer doses, an exchange occurs between radioactive and non-radioactive sodium ions until a state of equilibrium is attained. At this point, the ratio of radiosodium to total sodium (specific activity) becomes the same in all tissues and body fluids. (Total sodium is considered equivalent to sodium\(^{22}\), since the fraction of sodium\(^{22}\) is negligibly small.) Under these circumstances:

\[
\frac{P_{Na^{22}}}{T_{Na^{22}}} = \frac{T_{Na^{22}}}{P_{Na^{22}}} = T/P_{Na^{22}}
\]

whence:

\[T_{Na^{22}} = T/P_{Na^{22}} \times P_{Na^{22}}\]  \hspace{0.5cm} (2)

where:  
\(T_{Na^{22}} = \text{Tissue radiosodium concentration; } P_{Na^{22}} = \text{Plasma radiosodium concentration; } T_{Na^{22}} = \text{Tissue total sodium concentration;}

From the University of Southern California School of Medicine, Los Angeles.

This study was supported by United States Public Health Service Grant No. H-1403, and Los Angeles County Heart Association Grant No. 88.

Received for publication December 30, 1954.

\(P_{Na^{22}} = \text{Plasma total sodium concentration; } T/P_{Na^{22}} = \text{Tissue-plasma radiosodium concentration ratio.}\)

From equation (2) it will be seen that under conditions of equilibrium the total sodium concentration in any tissue may be determined from measurements of the radiosodium concentrations in tissue and plasma, and the flame photometric measurement of total sodium in the plasma only.

Previous studies, mostly done with sodium\(^{44}\), have shown that the time necessary for equilibration varies somewhat with the species and the tissue. Gellhorn and associates have shown that equilibrium is not complete in the dog after two hours. In the rat, Manery and Bale have found that equilibrium is complete in most tissues within three hours. In the rabbit, it is complete in all tissues, with the exception of brain and bone, after 12 hours. Determinations made in the human by Miller and Wilson have indicated that equilibrium is complete after 12 hours in skin, muscle and gastric juice, but is only 90 per cent complete in cerebrospinal fluid. The same authors, as well as Edelman and coworkers, have reported that equilibration in bone is a slow process, being only 25 to 45 per cent complete in 24 hours. Since the rat was to be the experimental animal in the present studies, the results noted above suggested that a five-hour equilibration period would be adequate as well as practical for soft tissue measurements.

Sodium\(^{22}\) was chosen for these studies, rather than sodium\(^{44}\), because its long half-life (approximately three years as compared to 14.8 hours) allowed considerable flexibility in the planning and conduct of experiments and
Table 1.—A Comparison of the Variability in Measurements of Tissue Concentrations of Sodium\(^{31}\) and Sodium\(^{23}\) as Expressed by the Tissue-Plasma (T/P) Ratios for Each Tissue

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Number of Samples</th>
<th>T/P</th>
<th>(t)</th>
<th>(V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone</td>
<td>5</td>
<td>0.611</td>
<td>19.34</td>
<td>0.083</td>
</tr>
<tr>
<td>Heart</td>
<td>5</td>
<td>0.256</td>
<td>0.68</td>
<td>0.232</td>
</tr>
<tr>
<td>Kidney</td>
<td>5</td>
<td>0.612</td>
<td>0.20</td>
<td>0.146</td>
</tr>
<tr>
<td>Liver</td>
<td>10</td>
<td>0.224</td>
<td>0</td>
<td>0.080</td>
</tr>
<tr>
<td>Lung</td>
<td>5</td>
<td>0.396</td>
<td>0.64</td>
<td>0.104</td>
</tr>
<tr>
<td>Muscle</td>
<td>10</td>
<td>0.151</td>
<td>0.78</td>
<td>0.299</td>
</tr>
<tr>
<td>Brain</td>
<td>5</td>
<td>0.300</td>
<td>1.33</td>
<td>0.114</td>
</tr>
<tr>
<td>Skin</td>
<td>5</td>
<td>0.405</td>
<td>1.28</td>
<td>0.081</td>
</tr>
<tr>
<td>Stomach</td>
<td>4</td>
<td>0.333</td>
<td>0.09</td>
<td>0.143</td>
</tr>
<tr>
<td>Spleen</td>
<td>5</td>
<td>0.184</td>
<td>0.19</td>
<td>0.063</td>
</tr>
<tr>
<td>Average (soft tissues only)</td>
<td>9</td>
<td>0.307</td>
<td>0.315</td>
<td>0.137</td>
</tr>
<tr>
<td>Average (all soft tissue samples)</td>
<td>54</td>
<td>0.283</td>
<td>0.289</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Experimental animals: Five adult albino rats. Diet: Purina laboratory chow. Drinking fluid: Water. Food and fluids withdrawn prior to experiment. Load: Na\(^+\)Cl, 3.25 \(\mu\)g/Kg., in 0.43% Na\(^+\)Cl, 2.5 ml/Kg., I.P., 5 hours prior to sacrifice.

* \(p < 0.01\)

eliminated the need for corrections due to decay over the short period of time required for the analyses.

The method has been applied to measurements of sodium concentrations in bone, heart, kidney, liver, lung, muscle, brain, skin, stomach and spleen. The experience of the past two years in the application of the method indicates that analyses of total soft tissue sodium concentrations can be performed at approximately one-quarter of the time and cost required by digestion and flame photometry, with about the same degree of accuracy.

**METHOD**

*Procedure for Determination of Tissue and Plasma Radiosodium Concentrations*

Adult rats of various sizes, strains and sex were used as experimental animals. They were fed Purina laboratory chow and water and were kept in metabolism cages to lessen the risk of radioactive contamination of the animal room. Sodium\(^{23}\), procured as a concentrated Na\(^+\)Cl solution, was diluted and injected intraperitoneally, usually in a dose of 3.25 \(\mu\)g/Kg., in 2.5 ml per Kg. of 0.43 per cent Na\(^+\)Cl.

Five hours after injection, the animals were anesthetized with ether and bled to death by use of a heparinized 10 ml syringe, either by cardiac puncture or by aspirating the abdominal aorta or a jugular vein. A blood volume for analysis varying between five and 15 ml. could usually be secured. The blood was immediately centrifuged at 2000 RPM for 45 minutes. Two one ml. plasma samples were then pipetted into small, cylindrical glass sample vials of uniform diameter and thickness.*

While the blood was being centrifuged, samples of representative tissues were removed. Due to their small size some organs, including brain, heart, kidney, stomach, lung and spleen, were frequently used in their entirety as samples. From other tissues, like muscle, skin and bone, portions estimated to represent between one and two grams were secured. Skin was taken from the lumbar region after shaving the area with a clipper. Muscle was obtained from the thigh. All soft tissue samples were cut into small fragments with scissors, while resting on an absorbent paper towel. An adequate amount of bony tissue was secured by grouping together pieces from the two tibiae and one femur. These were cut into fragments about two mm. square with a wire cutter.

All fragments of each tissue were firmly packed at the bottom of a sample vial with a wooden applicator. The vial was weighed before and after insertion of the tissue and the sample weight determined by difference. The vial was then sealed with a plastic snap-on cap to prevent evaporation and contamination.

For counting, the vial was placed on the aluminum casing immediately covering the crystal of an inverted scintillation head enclosed in a lead castle one inch thick. A plastic holder centered the vial directly over the crystal. Each sample, including plasma, was counted for 10 minutes with an automatic electric timer. The average of three 10 minute

* Crystal Flint Glass Snap-Cap Vials, three dram capacity, 51 mm. high, 22 mm. diameter, Braun #63483.
background counts taken at three hour intervals was subtracted from each sample count. The resultant value, divided by ten, was considered to represent the radiation intensity of the entire tissue sample, as expressed in counts per minute (CPM). Before this value could be used in any calculations of tissue sodium concentrations, it was necessary to apply a correction factor to adjust for differences in radiation intensity due to variations in the masses of the tissue samples. The derivation of the correction factor and its application are described in the following section.

The two plasma samples, after counting, were analyzed for total sodium concentrations with the flame photometer. The average of the two values, together with the corrected values for plasma and tissue radioactivity (CPM per Gm.), were substituted in equation (2), above, to calculate the total tissue sodium concentration.

RESULTS

Effects of Differences in Mass of Tissue Upon Count Per Unit of Tissue: When using sample vials of uniform diameter, the mean distance of the tissue from the crystal is directly proportional to the tissue mass, as expressed by its weight. Under such circumstances the measured radiation, per unit of weight, decreases with increasing sample mass because of the increasing mean distance of the source from the crystal. A linear relationship was demonstrated by plotting the observed radiation intensity (CPM) in a series of tissues against the square root of the tissue weight. The equation for this relationship (from the method of least squares) was: 
\[ x = -102.6 + 207.1y \]
where: \( y = \text{Square root of sample weight (in grams)} \); and \( x = \text{CPM (expressed as a percentage of count at 1 gm. weight)} \).

A correction table was constructed from this equation, by which any observed count (CPM) was readily converted to the count for a one gram weight of the tissue (CPM/Gm.).

The relationship deviated from a straight line at tissue weights below half a gram, due to incomplete coverage of the vial bottom and increased counting error. In subsequent experiments, tissue samples weighing between one and two grams were used wherever possible. Measurements on small organs, notably the spleen, were carried out by combining the tissues from two animals.

Variations in Total Count Consequent Upon Repacking or Recounting the Same Sample: In applying the procedure for the measurement of plasma and tissue radioactivity to the overall problem of estimating total tissue sodium concentrations, two sources of measurement error were studied. These were: (1) variations due to geometric differences in the packing of successive tissue samples; and (2) variations due to intrinsic counting error.

Studies of these sources of error were made by comparing successive recounts of a group of tissues with and without repacking. The results demonstrated that variations in the counting measurements were due largely to intrinsic counting error, and were relatively little influenced by the minor changes in geometry associated with repacking. The small size of the various coefficients of variation indicated that a 10 minute count provided an adequate accuracy of measurement for all tissues when the sodium concentration in the plasma exceeded about 700 CPM per Gm.

Comparison of Measurements by the Radiosodium and Flame Photometric Procedures:
Since counting and packing errors were negligible, this comparison was primarily
determined as to whether or not sodium$^{22}$
had equilibrated with sodium$^{23}$ in the tissues.
In general, the procedure consisted in measuring
the Na$^{22}$ concentration of each tissue and
applying the necessary correction factor for
tissue weight, as previously described; then
digesting the tissue with nitric acid, and
measuring the total sodium concentration with
the flame photometer. No correction was at-
ttempted for errors in measurement of bone
sodium due to interference by calcium or
potassium.

To estimate whether or not equilibrium
conditions had been attained, the tissue-plasma
(T/P) concentration ratio for Na$^{22}$ was com-
pared with the corresponding T/P ratio for
Na$^{23}$. The total tissue sodium concentration by
the radiosodium method was calculated by
multiplying the T/P$_{Na^{22}}$ for any particular
tissue by the total plasma sodium concentration
(cf. equation 2). This value was compared
with the total tissue sodium concentration
as determined by direct flame photometric
analysis of the tissue digest. The results of two
experiments are detailed below.

**Comparison in Fasting Animals Under Minimum Sodium Load**

**Procedure:** The experimental animals were
five adult albino rats. Food and fluid were
withdrawn the afternoon preceding the ex-
periment. The following morning, Na$^{22}$Cl,
3.25 μg/Kg, was injected intraperitoneally
in 0.43 per cent Na$^{23}$Cl, 2.5 ml. per Kg. Approx-
imately five hours later the animals were
sacrificed. Samples of plasma and of 10 repre-
sentative tissues (a total of 69 samples, in-
cluding duplicates of plasma, muscle and
liver), were analyzed for Na$^{22}$ and Na$^{23}$.

**Results:** The T/P concentration ratios for
the two substances were significantly different
only in the case of bone, where T/P$_{Na^{22}}$ was
approximately half as large as T/P$_{Na^{23}}$ (Table
1). T/P$_{Na^{22}}$ for the nine representative soft
tissues averaged 0.307, compared with 0.315
for T/P$_{Na^{23}}$, a difference of less than three per
cent. The means of the coefficients of variation
for the soft tissues were nearly identical (0.137
for T/P$_{Na^{22}}$ as compared with 0.146 for
T/P$_{Na^{23}}$). The total sodium concentrations of
the solid tissues varied with the type of tissue,
being lowest in striated muscle and highest in
bone. In general, the values obtained agreed
closely with those previously reported for the
rat by Manery and Bale.$^3$

These results were interpreted to indicate
that equilibration between Na$^{22}$ and Na$^{23}$ had
occurred in all soft tissues by the end of five
hours. The lack of significant differences in the
soft tissue comparison was further tested by
considering all such tissues as statistically
comprising a single group. Despite the increase
in total sample number achieved by this ap-
proach, the difference between T/P$_{Na^{22}}$ and
T/P$_{Na^{23}}$ still was not significant ($t = 0.28;
p > 0.05$). The similarity in the coefficients of
variation for T/P$_{Na^{22}}$ and T/P$_{Na^{23}}$ indicated a
comparable degree of accuracy for the two
methods of measurement.

**Comparison in Fasting Animals Under Maximum Sodium Load**

A brief experiment was conducted, similar
in design to the previous one, in which Na$^{22}$Cl
was administered in 5% Na$^{23}$Cl, 25 ml./Kg,
intraperitoneally. Na$^{22}$ and Na$^{23}$ measure-
ments were done on all representative tissues
of one animal and on the plasma and striated
muscle only of six others (a total of 24 samples).
The mean T/P$_{Na^{22}}$ for all soft tissue samples
was approximately eight per cent lower than
T/P$_{Na^{23}}$ (0.241 and 0.261, respectively). This
difference did not prove significant ($t = 0.51;
p > 0.05$). The total sodium value for bone
was again about double the radiosodium value.

**Summary and Conclusions**

A method has been described for the esti-
mate of total sodium concentrations in tissues
by direct measurement of radiosodium con-
centrations in undigested tissue and in plasma
following a tracer dose of sodium$^{22}$, combined
with the flame photometric measurement of the
total plasma sodium concentration only. The
method has been applied to the measurement
of normal total tissue sodium concentrations
in the fasting rat under conditions of minimum
and maximum salt load.
Equilibration between sodium$^{22}$ and sodium$^{23}$ occurred in all soft tissues of the rat by the end of five hours following a single intraperitoneal injection of Na$^{22}$Cl, 3.25 μc./Kg. Equilibration in bone was approximately 50 per cent complete at the end of the same period. The attainment of equilibrium in soft tissues or bone was not appreciably altered by the simultaneous intraperitoneal administration of a maximum salt load.

The use of this method permitted the estimation of total sodium concentrations in soft tissues with about one-quarter of the time and cost required by the method of digestion and subsequent flame photometric analysis. A comparison of coefficients of variation indicated that the two methods possessed approximately the same degree of accuracy. These considerations suggest the usefulness of the method in experimental designs embracing large-scale determinations of total tissue sodium.

REFERENCES


Laminar Flow in the Portal and Caval Systems

The question whether particles of the blood stream flow in straight lines (laminar flow) or in a turbulent fashion in various parts of the circulatory system arises periodically. It is generally believed that all venous flow is laminar.

The question has recently been studied in the portal vein and venae cavae by injecting highly oxygenated blood or Evans blue into a tributary. Sufficient color differentiation was obtained in the veins for photography by a cinecamera operated at speeds of 16 to 64 frames per second.

It was found that strictly laminar flow in the portal vein was disturbed by its lateral displacements during respiratory movements. This usually caused the entire stream to be diverted laterally; but vortices occasionally appeared.

In the abdominal portion of the inferior cava considerable mixing of streams was found. Dyes injected into a femoral vein often followed a helical course so that blood injected into a left femoral vein, for example, appeared on the right side of the cava in mid-abdomen. Such streams were broken up at the diaphragm and no longer were discernible in the thoracic inferior cava.

In the thoracic inferior vena cava, temporary reversal of colored blood injected into a phrenic vein was observed during respiration, and, to a lesser extent, during cardiac cycles. This caused vortex rings to appear which resulted in some mixing of blood. While laminar layers persisted along the wall, often a zone of disturbed flow occurred temporarily in the axial stream. Similar disturbances of flow were observed when the vein was distorted by external forces, which caused its lengthening or constriction, e.g. at the diaphragm. While the returning blood flow is subject to changes it is not proper to designate the disturbances as turbulent—a term defined by Lamb as a condition involving the entire column of blood. In the venae cavae the condition is local and dies away. The critical Reynolds number of 2000 is also not exceeded. It is, therefore, concluded that inferior caval flow is laminar except when momentarily disturbed by pHisic respiratory and cardiac actions, by distortion of the vessel through external forces and by conditions of flow at junctions.

Determination of Total Tissue Sodium Concentrations by Use of Radiosodium

D. M. GREEN, T. B. REYNOLDS and R. J. GIRERD

doi: 10.1161/01.RES.3.4.330

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1955 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/3/4/330

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/