Evidence for a Prolonged Biological Half-Life of Na$^{22}$ in Patients with Hypertension

By Lewis K. Dahl, M.D., Malcolm G. Smilay, M.D., Lawrence Silver, M.D., and Sanford Spraragen, M.D.

In the treatment of human hypertension, the effectiveness of sodium restriction by either dietary means or natriuretic agents is now well established. This response to sodium restriction suggested that hypertensives might suffer from an increase in total body sodium. The first measurements of the sodium content of individuals with hypertension were done, in 1951, by the technique of isotopic dilution using Na$^{24}$. With the exception of one report, the original observations have been confirmed repeatedly. Exchangeable sodium in the hypertensive was not increased. In the single discordant report, some cases of severe hypertension were found to have an increase in (exchangeable) sodium, but this was interpreted to be secondary to the disease, rather than primary, because it occurred almost exclusively in individuals with advanced hypertension, presumably with markedly impaired renal function.

In 1953, it was observed that when the average salt consumption of rats was increased, the mean systolic pressure increased linearly. Humans showed a similar type of correlation: As the average salt consumption of a community increased, the prevalence of hypertension increased linearly. Such observations indicated a fundamental role of salt in the pathogenesis of hypertension. They were interpreted as showing that chronic excess salt consumption plays a primary role in the etiology of human hypertension.

In view of these developments, we were led to reconsider the possibility that there might exist in hypertension some defect in sodium metabolism which was not put into evidence by the earlier isotopic studies. Therefore during the last two years, we have explored further the sodium metabolism of patients with and without hypertension using Na$^{22}$ (physical half-life, 2.6 years) instead of the short-lived Na$^{24}$ (physical half-life, 15 hours). The results suggest that hypertensives probably do have an abnormality in sodium metabolism, as manifested by a prolonged biological half-life for Na$^{22}$. Furthermore, calculations derived from these observations suggest that hypertensive individuals have increased tissue sodium concentrations.

Methods

PATIENTS: CLINICAL DATA

The patients were all adults, 14 white and 1 Negro, of which 7 had so-called "essential hypertension." A summary of age, sex, and diagnosis for each patient is given in table 1. Details of the numerous laboratory studies have been omitted except where clearly pertinent.

HYPERTENSIVE SUBJECTS

Some of the hypertensive subjects had electrocardiographic evidence of mild to moderate myocardial damage, and variable cardiac enlargement was shown to be present by x-ray. None of these patients had a history suggestive of heart failure. All had negative phentolamine tests for pheochromocytoma. One patient (R. B.) had had two strokes in 1951, with total recovery of function, and a bilateral lumbodorsal sympathectomy in 1955, without effect on his blood pressure. All had normal renal function as indicated by urine examination, intravenous urography, urea or creatinine clearance, blood urea nitrogen, serum creatinine, uric acid, and serum electrolyte concentrations.

NORMOTENSIVE SUBJECTS

Normotensive patient (M. W.) had undergone a unilateral adrenalectomy in July, 1959, after studies had indicated the presence of an aldosterone-producing tumor. At operation, a benign adenoma was found and removed. This patient had been studied for intermittent, mild hypertension since 1953, but she had been normotensive following the operation. This was confirmed by daily blood pressure measurements during her hospitalization for the six weeks of the present investigations, and, therefore, she is included in
our normotensive group. Three other normotensive female patients had varying degrees of obesity for which they were undergoing dietary restriction with weight losses averaging about 0.5 to 1 Kg. per week, except for A. E. who had stabilized her weight prior to this study. These women were otherwise healthy. A fifth female was a periodic alcoholic without clinical or laboratory evidence of liver disease. Two males had had schizophrenia for which they had been institutionalized; they had improved markedly on ataractics and were cooperative during this study. One of these men (L. L.), on routine chest x-ray, was found to have several densities in the right lung field. These were similar in appearance to a bronchogenic carcinoma, but numerous studies including bronchoscopy, laminography, and repeated sputum examinations for malignancy and for a wide variety of pulmonary infections failed to establish the diagnosis. The last patient (E. E.) had had a right hemiplegia for two years without antecedent hypertension; he was able to walk with assistance but for the most part was confined to a chair or bed existence. None of these patients had evidence of cardiac or renal disease, either on clinical grounds or by numerous laboratory studies.

REGIMEN AND DIETS

All patients were hospitalized continuously in a metabolic unit while under study. They were weighed daily upon arising, after voiding, before eating, on a scale accurate and sensitive to 25 Gm. Blood pressures were measured under standardized conditions six mornings a week. All except E. E. were ambulatory, and they walked daily on the extensive laboratory grounds. All food was prepared, accurately measured, and weighed in a diet kitchen. The diet was constant for any one patient beginning about one week after admission when calorie needs for weight maintenance could be estimated. No dietary changes were made once the isotopic turnover studies had been started. The basic diet which each patient received was the low sodium diet previously described, which provides a daily intake of about 5 mEq. of sodium, 1 Gm. of protein per Kg. of ideal body weight, 25 per cent fat, and sufficient carbohydrate to maintain weight. Additional dietary sodium was provided by adding enteric-coated NaCl tablets in amounts of 2, 5, or 10 Gm. (34, 86, and 172 mEq., respectively) per day.

Eight patients were studied on all of these salt intakes. In the pages which follow, the sodium content of the basic diet has been disregarded and only the supplementary salt intake is referred to. It should be emphasized that not every patient received the same diet, but that each consumed an invariant menu. Chemical analysis of these individual constant diets showed that the daily sodium and potassium contents of food were within a relatively narrow range for the group (table 1). Tap water was allowed ad libitum, although it was measured; repeated analyses over a number of years have shown it to contain not more than 0.5 to 0.7 mEq. of sodium per L., so that this extra source of sodium could be disregarded also. The patients were required to eat all the food provided. They were not permitted to eat or drink anything else. Observations of the same individual on different daily salt intakes were generally made by starting with the lower and going successively to the higher intakes. Observations made in the reverse order showed no significant difference. Complete absorption of the supplementary NaCl was confirmed in all patients by analysis of 24-hour urine collections for sodium. NaCl intake was continued at any given level for about two weeks, but longer if necessary to establish accurately the linear loss of Na\textsuperscript{22} from the body.

ISOPOE

Na\textsuperscript{22}

The Na\textsuperscript{22} was purchased from Nuclear Science and Engineering Corporation, Pittsburgh, and was received in a HCl-NaCl solution, 0.67 mc. per ml., carrier-free, with a chemical purity of more than 99 per cent. It has a physical half-life of 2.6 years and emits both beta and gamma radiations, of which the gamma emission with an energy of 1.28 Mev* was used for detection in this work.

Administration and Dose of Isotope

Na\textsuperscript{22} was given orally, dissolved in about 50 ml. of water in a beaker with about 1 mEq. of NaCl as carrier. Five rinsings, all drunk by the patient, left no residual counts in the beaker.

The amount of Na\textsuperscript{22} administered ranged from 1 to 10 mc. Only one patient (E. E.) received 10 mc., and the usual quantity was 2 to 4 mc. Calculations of the whole-body radiation received by the single individual, who received 10 mc. taking into consideration the observed biological half-life, indicated a maximum total dose of less than 0.3 rads. The remaining 14 patients received significantly less than this amount, generally below 0.1 or 0.2 rads.

INSTRUMENTATION

Radiation Counting

Radioactivity was assayed with a Harshaw "matched window line" thallium-activated Na\textsubscript{I} crystal, 8 inches in diameter and 4 inches thick. This was housed in stainless steel and connected to three 3-inch photomultiplier tubes. The pulses from this crystal-phototube unit were counted as a function of the pulse-height with a PENCO.

\*Mev = million-electron-volts.
TABLE 1
Clinical Data and Biological Half-Life for Na\textsuperscript{22} in Patients With and Without Hypertension on the Same NaCl Intakes

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Dx.</th>
<th>Weight (Kg.)</th>
<th>Dietary Na\textsuperscript{22} (mEq./day)</th>
<th>Dietary K (mEq./day)</th>
<th>Biological half-life (in days) of Na\textsuperscript{22} on various daily NaCl intakes</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.R.</td>
<td>51</td>
<td>M</td>
<td>EHT</td>
<td>83</td>
<td>5</td>
<td>72</td>
<td>61 (56, 66)§§ 28 (26, 30) 13.5 (13, 14)</td>
</tr>
<tr>
<td>B.C.</td>
<td>42</td>
<td>F</td>
<td>EHT</td>
<td>51</td>
<td>5</td>
<td>56</td>
<td>65</td>
</tr>
<tr>
<td>E.Kr.</td>
<td>45</td>
<td>M</td>
<td>EHT</td>
<td>62</td>
<td>5</td>
<td>66</td>
<td>62</td>
</tr>
<tr>
<td>A.K.</td>
<td>56</td>
<td>F</td>
<td>EHT</td>
<td>73</td>
<td>5</td>
<td>64</td>
<td>65</td>
</tr>
<tr>
<td>M.L.</td>
<td>48</td>
<td>M</td>
<td>EHT</td>
<td>73</td>
<td>5</td>
<td>75</td>
<td>13</td>
</tr>
<tr>
<td>P.R.</td>
<td>58</td>
<td>M</td>
<td>EHT</td>
<td>84</td>
<td>7</td>
<td>71</td>
<td>63</td>
</tr>
<tr>
<td>N.S.</td>
<td>55</td>
<td>M</td>
<td>EHT</td>
<td>63</td>
<td>4</td>
<td>54</td>
<td>10</td>
</tr>
<tr>
<td>A.E.</td>
<td>51</td>
<td>F</td>
<td>Ob§</td>
<td>59</td>
<td>3</td>
<td>45</td>
<td>49 (45, 46, 55) 12 (12, 12)</td>
</tr>
<tr>
<td>R.G.</td>
<td>25</td>
<td>F</td>
<td>Ob</td>
<td>85-79</td>
<td>4</td>
<td>47</td>
<td>41 (40, 42) 20 11</td>
</tr>
<tr>
<td>A.R.</td>
<td>17</td>
<td>F</td>
<td>Ob</td>
<td>55-55</td>
<td>3</td>
<td>35</td>
<td>30 23 11</td>
</tr>
<tr>
<td>M.W.</td>
<td>50</td>
<td>F</td>
<td>N.D.</td>
<td></td>
<td>64</td>
<td>10</td>
<td>34</td>
</tr>
<tr>
<td>M.P.</td>
<td>52</td>
<td>F</td>
<td>A**</td>
<td>47</td>
<td>7</td>
<td>80</td>
<td>41</td>
</tr>
<tr>
<td>L.L.</td>
<td>56</td>
<td>M</td>
<td>Sch†</td>
<td>51</td>
<td>6</td>
<td>85</td>
<td>39 21 11</td>
</tr>
<tr>
<td>C.R.</td>
<td>30</td>
<td>M</td>
<td>Sch</td>
<td>65</td>
<td>7</td>
<td>100</td>
<td>45 22 11</td>
</tr>
<tr>
<td>E.E.</td>
<td>60</td>
<td>M</td>
<td>CVA††</td>
<td>52</td>
<td>6</td>
<td>67</td>
<td>44 12</td>
</tr>
</tbody>
</table>

|       |          |     |         |            | 40.7 ± 5.3                      | 20.5 ± 2.1           | 11.0 ± 1.0                                     |
| 2*     |          |     |         |            | P <0.01                         | P <0.01              | P <0.01                                                 |

*Grains of NaCl per day.
†Essential hypertension.
‡Patient also had Parkinson's disease.
§Obesity. Patient A.E. had occasional blood pressure readings of 150-160/95-100 before study.
||No disease. Mildly hypertensive prior to unilateral adrenalectomy for benign aldosterone-producing tumor.
Completely normotensive since operation (see Methods: Clinical Data).
**Periodic alcoholism. No liver disease.
††Schizophrenia.
†Coronary bypass surgery. No known antecedent hypertension.
§§More than one value indicates additional periods of study. For such individuals, the average of these values was employed in calculating mean and standard deviation of the group.

100-channel pulse-height analyzer, on which the pulse energy spectrum, from 40 kev* (on channel 1) to 2 Mev (on channel 100) was recorded automatically. The gamma emission, with a peak energy of 1.28 Mev, was used for counting. The energy of this peak overflowed slightly into adjacent channels. Therefore, the outputs from the peak at channel 60, as well as from the two channels above and the two channels below this peak, were always included in the calculations: the sum of the counts from these five channels are referred to as "whole-body counts" in this paper.

Since our patients had no other significant radio-isotopic burden, the total counts from all 100 channels could have been used equally well, but by using a window of 1.24 to 1.32 Mev (channels 58 to 62) a maximum signal to noise ratio was obtained. In order to minimize background, crystal and patient were enclosed in a room 6 feet \times 7 feet \times 7 feet, with walls consisting of 6-inch steel lined with one-quarter inch of laminated lead, cadmium, and copper. With this equipment, the machine background in the five channels used was 20 to 30 counts per minute (c.p.m.), and the presence of a patient, before administration of isotope, generally added about 10 c.p.m. to this base level. The 100-channel analyzer was left continuously with the current on in an air conditioned room with a constant temperature of 70 F.

This equipment was checked daily for contamination and sensitivity by making a background...
count followed by counts of both Cs\textsuperscript{37} and Na\textsuperscript{22} standards. Thereafter, the background count was repeated before and after each individual was counted.

The precision revealed by measuring the standards was high; it had a standard deviation of less than 1 per cent whether the counts were made in succession or over successive days. The precision of the measurements on the patients was of the same order by rigidly fixing the counting geometry.

**Counting Technique**

Patients were counted, as nearly as possible, at the same time every day. Before entering the counting room, they voided, removed all items which might be radioactive (e.g. certain luminous watches), and replaced shoes with paper slippers which were worn once and then discarded. Patients reclined in a comfortable contour chair in a rigidly fixed position relative to the scintillation crystal. Depending upon the quantity of radioactivity present, counting periods ranged from 5 to 15 minutes; net counts in the photo-peak ranged from about 30,000 c.p.m. initially to 2,000 c.p.m. toward the end of some experiments. The most frequent measurements were made in the range of about 4,000 to 5,000 c.p.m. during a counting period of five minutes.

In estimating whole-body activity, the counts obtained 24 hours after administration of the isotope were arbitrarily assigned the value of 100 per cent and all later changes were referred to this base. It was found, by repeated whole body counts during the first 24 hours following administration of the isotope, that the counts recorded varied by up to 5 per cent without relation to loss of the isotope. This was presumed to be due to internal shifts in distribution since it also occurred in patients (not included in this study) maintained on the basic low sodium diet without supplementary NaCl, thereby reducing the Na\textsuperscript{22} excretion in the first 24 hours to almost zero.

Patients ordinarily were counted at one- to 3-day intervals for at least 6 to 10 occasions during which the experimental regimen remained unchanged. In many instances, from 10 to 20 counts were made over periods extending for three to four weeks. The whole-body counts, corrected for physical decay, were plotted on semilog paper as per cent of the original 100 per cent base activity against time in days. The decline in whole body activity generally fell along a straight line for any single experimental regimen and from this slope biological half-life was derived.

**CALCULATIONS**

**Total Exchangeable Sodium**

Total exchangeable sodium was determined from the specific activity of Na\textsuperscript{22} in urine as follows:

\[
\text{TENA (in mEq.)} = \frac{\text{total Na}^{22 \text{ counts remaining in body}}}{\text{urine specific activity (c.p.m./mEq. Na)}}
\]

These determinations were made routinely at weekly intervals, but only the final value for each study period has been reported.

**Total Body Potassium**

Total body potassium was determined during the control period by detection of the radioactivity from the naturally occurring potassium isotope, K\textsuperscript{40}. Calculation of total body potassium was made by relating the K\textsuperscript{40} c.p.m. for any single patient to that obtained from a life-size plastic phantom human body, filled with water containing exactly 140.0 Gm. of potassium. Both were counted under identical conditions. From the average specific activity (37.2 c.p.m.) of this potassium-containing standard phantom body, individual total body potassium was calculated by the following formula:

\[
K^{40} \text{ c.p.m. (patient)} = \frac{K^{40} \text{ c.p.m. (phantom)} \times 140}{100} = \text{Gm. K (patient)}.
\]

This method has been found to be in excellent agreement with data obtained in patients after equilibration with K\textsuperscript{40} for 48 hours or more, using the same counting technique.\textsuperscript{10}

**"Desirable" Body Weight**

This was calculated from a recent table prepared by the Metropolitan Life Insurance Company,\textsuperscript{11} based on a large cooperative actuarial study.\textsuperscript{12} For both men and women, "desirable" body weights are somewhat lower than "ideal" weights compiled earlier by the same company.\textsuperscript{15, 20}

**Results**

The addition of salt to the regimen resulted in a prompt increase in Na\textsuperscript{22} excretion as manifested by a fall in total body counts. The rate at which Na\textsuperscript{22} was excreted depended primarily on the amount of salt consumed: as the quantity of ingested salt increased, the rate of Na\textsuperscript{22} loss was enhanced. The rate of decline in counts proved to be steady and relatively predictable for any single patient.
COMPARISON OF THE BIOLOGICAL HALF LIFE (t/2) OF Na\textsuperscript{22} IN 2 HYPERTENSIVE MALES VERSUS 2 NORMOTENSIVE MALES ON DAILY INTAKES OF 2gm NaCI AND 10 gm NaCI

FIGURE 1
Representative whole body counts (as per cent of original count) on four patients, from which slopes for determining biological half-life were determined. All slopes have been shown as originating at zero days for the convenience of the reader, but otherwise the slopes are drawn from original counting data.

Thus, it was possible to plot accurately the decrement with time of total body counts, from which the "biological half-life" of Na\textsuperscript{22} was determined. When this was done, it was of interest to find that at all three levels of salt intake, hypertensive individuals lost Na\textsuperscript{22} significantly more slowly than did those without hypertension ($P < 0.01$). This is summarized in detail in table 1 and figure 2, in which the rate of loss of Na\textsuperscript{22} is described in terms of its biological half-life in the organism.

**Discussion**

There are several possible explanations for the observations that hypertensive subjects have a prolonged biological half-life for Na\textsuperscript{22}:

1. The metabolism of radioactive Na\textsuperscript{22} differs from that of stable Na\textsuperscript{23} in hypertensives only; this is obviously most unlikely.
2. Hypertensives may absorb less of the supplementary NaCl, thereby preventing adequate exchange with and excretion of the Na\textsuperscript{22}; this was directly disproven by the demonstration that 24-hour urinary excretions of sodium in all patients reflected the known NaCl intake.
3. Hypertensives may have more sodium in the body, i.e., they may have a larger "metabolic pool" of sodium; this last hypothesis has been reinvestigated in spite of the previous evidence to the contrary.

In experimental hypertension, the evidence is conflicting. Some observers found an increase in tissue sodium, whereas Grollman did not. Meneely, Ball, and Youmans\textsuperscript{26} failed to find salt retention in rats made hypertensive by chronic excess salt feeding until the highest levels of intake were reached.
Total exchangeable sodium (TENA) calculated by three different techniques: (A) TENA per Kg. of actual weight; cross-hatched circle (2 Gm.) represents patient A. E. (see table 1). (B) TENA per Kg. of desirable weight. (C) Ratio of total exchangeable sodium (in mEq.) to total body potassium (in mEq.).

In human hypertension, by the technique of isotopic dilution using the short-lived isotope Na$^{24}$, an increase in sodium has not been found except by Ross.$^2$ In this report, when an increase in exchangeable sodium was observed, it was present almost exclusively in individuals with advanced hypertension. For this reason, the results had been interpreted as being secondary to the disease rather than primary.

The original calculations of total exchangeable sodium were based on actual weight. However, unpublished observations which we made on obese hypertensive females during sharp decline in weight, frequently had shown...
negligible loss of exchangeable sodium despite weight losses of 20 to 50 Kg. This indicated that the fat depots in such people had a low sodium content. Calculations based on actual weight might have been misleading if the average hypertensive person had been fatter than the average nonhypertensive subject, a supposition in keeping with fact. Such calculations were in agreement with previous experience, in the present study also, since they showed no difference between hypertensive and normotensive patients. However, when body sodium content was recalculated on the basis of two approximations of "lean body mass," it appeared that most of these hypertensive individuals did have an increase in tissue sodium. The first method of calculation consisted of estimating "desirable" weight; in the second, exchangeable sodium was expressed with reference to body potassium which was derived from the amount of naturally occurring K present in each patient as already indicated. The results are summarized graphically in figure 3 (A, B, and C). It was of interest to find that with either technique, the scattered dispersion evident in figure 3 (A) was replaced by a distribution in which the hypertensive tended to be separated from the nonhypertensive subjects by virtue of the greater sodium concentration in the hypertensives (fig. 3, B and C).

Passing consideration must be given to the possibility that the equilibration rates between dietary sodium and the total exchangeable sodium are different in hypertensive and normotensive individuals without significant difference in size of the sodium pools. The fact that only single exponential curves were obtained when the Na retention curves were plotted and that the half-times in all subjects were inversely proportional to the sodium intake argue for, although do not prove, treatment of the exchangeable body sodium as a single component. The presence of a second compartment with different turnover rates in normal and hypertensive individuals might be expected to be demonstrated by a second component in the retention curves; it would be a coincidence if all of the rates involved in the more complex system were uniformly affected by a change in sodium intake. Nevertheless, this possibility exists.

From the data available it has been concluded that hypertensives may have more tissue sodium than do nonhypertensives. From such a small series, it would be unwarranted to conclude that this has been established for all subjects with essential hypertension. Nonetheless, these findings lend additional support to the thesis that sodium is intimately involved in the hypertensive process.

Summary

The biological half-life of Na was found to be significantly longer (P < 0.01) in seven hypertensive adults than in eight normotensive adults on equivalent NaCl intakes. Calculations based on approximations of lean-body mass suggest that the hypertensive subjects may have a larger sodium pool to explain this disparity.

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