associated with changes in the microsomes, because normal rat liver pH 5 fraction was used throughout. An attempt was made to detect the presence of possible inhibitory factors associated with spleen microsomes from cortisol-injected rats by adding such microsomes to a reaction mixture containing spleen microsomes from control rats. This approach seemed reasonable since Macleod *et al*(10) reported that steroid administration to mice resulted in increased ribonuclease activity in lymphosarcoma tumor and Wust and Novelli (4) observed a ribonuclease activity associated with rat spleen ribosomes which was increased by storage. However, addition of microsomes from spleens of cortisol-injected rats caused no inhibition of leucine-C^{14} incorporation by spleen microsomes from control rats.

Summary. L-leucine-C^{14} incorporation has been studied in cell-free systems containing microsomes from spleens of control and cortisol-injected rats and pH 5 fraction from the liver of a control rat. Daily injections of 5 mg of cortisol for three days followed by sacrifice on the fourth day resulted in spleen microsomes that had about 60% of the incorporating activity of microsomes from spleens of control rats.

1. Feigelson, M., Fed. Proc., 1964, v23, 481.
2. Pena, A., Dvorkin, B., White, A., Biochem. Biophys. Res. Comm., 1964, v16, 449.
3. Gabourel, J. D., Comstock, J. P., Biochem. Pharm., 1964, v13, 1369.
4. Wust, C. J., Novelli, G. D., Arch. Biochem. Biophys., 1964, v104, 185.
5. Smith, A. L., Koeppe, O. J., Fed. Proc., 1962, v21, 411.
6. Eichely, H. J., Roth, J. S., J. Cell. Biol., 1962, v12, 263.
7. Layne, E., in Methods in Enzymology, Colowick, S. P., Kaplan, N. O., eds., Academic Press, New York, 1957, v3, 447.
8. Schneider, R., J. Biol. Chem., 1945, v161, 293.
9. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., ibid., 1951, v193, 265.
10. MacLeod, R. M., King, C. E., Hollander, V. P., Cancer Res., 1963, v23, 1045.

Received April 22, 1966 P.S.E.B.M., 1966, v122.

Effects of Chronic Excess Salt Ingestion: Lack of Gross Salt Retention in Salt-Hypertension.* (31298)

ECKART SCHACKOW AND LEWIS K. DAHL
Medical Department, Brookhaven National Laboratory, Upton, N. Y.

Several years ago we reported that in a small series of patients with fixed NaCl intake, most of those with essential hypertension had a longer biological half-life ($T_{½}$) of ^{22}Na than did normotensive subjects on the same regimen(1,2). Indirect calculations suggested that the hypertensives had a larger mass of sodium with which the isotope exchanged, thereby providing an apparent explanation for the prolonged $T_{½}$ observed. This work was in conflict with the overwhelming body of evidence pertaining to essential hypertension in man: from the earliest observations by Dole *et al*(3) and ourselves(4) to the latest by Hollander, Chobanian, and Burrows(5) and Gifford *et al*(6) there had been agreement that patients with uncomplicated hypertension did *not* have an increase in total body sodium, as measured after equilibration with the radioactive isotopes of sodium.

The present report is concerned with our explorations of this subject experimentally. Because of evidence that potassium influences the development of hypertension(7-9), the behavior of potassium as well as sodium has been explored. To this end we have used 2 selectively inbred strains of rats of which one strain (the Sensitive or S strain) is genetically predisposed to develop rapidly severe hypertension from any one of several standard techniques, including a high salt diet, DOCA plus salt, unilateral renal artery compression without salt, cortisone, and ad-

* This work was supported by the U. S. Atomic Energy Commission.

renal regeneration(10-12). By contrast, the other strain (the Resistant or R strain) develops either no hypertension from these techniques, or a much milder variety.

In these experimental studies, despite clear differences in both blood pressure and in genetic propensity for hypertension, we failed to find that the biological $T_{½}$ of ^{22}Na was prolonged in rats with experimental hypertension induced by salt feeding. Furthermore, there was no evidence that during a period when experimental hypertension was still rapidly evolving, significant changes in the whole body content of sodium or potassium occurred.

Materials and methods. Animals. Rats of both sexes from the 6th and 7th generations of our selectively inbred strains(10,11) were tested throughout. All had been weaned at 21 days of age and immediately put on either a high salt (8% NaCl) or a low salt (0.35% NaCl) chow which was maintained without change until observations had been completed. The details of care, feeding, and technique of blood pressure measurement have been reported(13-15).

Methods. 1) Sodium. A. Total exchangeable sodium (TENa). After an overnight fast, with water *ad lib*, each animal was injected intraperitoneally with approximately 125 μc of ^{24}Na. Equilibrium periods of 6 and 24 hours were used for animals on high and low salt intakes, respectively, since pilot studies had shown virtual equilibrium at these times. Specific activity was determined on plasma obtained by nicking the tail. Plasma sodium was estimated with a flame photometer using an internal lithium standard. After appropriate corrections for physical decay and loss of the isotope *via* urine and stool, total exchangeable sodium (TENa) was calculated by the usual procedure, namely

$$\frac{^{24}\text{Na remaining in organism}}{\text{Spec. activ. of plasma (cpm/mEq)}} = \text{TENa (mEq)}$$

B. Whole body sodium. Animals were fasted overnight and sacrificed the following morning with ether anesthesia; the hair was then clipped and discarded, the animal weighed, then skinned and the intestines removed. Skin, intestines and the remainder of the carcass were put separately into 3.2 N HNO_3 for 5 days, then individually homogenized, filtered, and the filtrate made up to an appropriate volume from which aliquots were removed for estimation of sodium. Whole body sodium was considered to be the sum of the 3 samples.

C. Sodium turnover. a. Isotope. Carrier-free ^{22}Na (physical half-life = 2.6 years) with a chemical purity of more than 99% was used and its gamma emission with a peak of 1.28 MEV was counted. Each animal received about 2 μc of ^{22}Na intraperitoneally in 1.0 ml of normal saline.

b. Counting technique. Counts were made in a whole body counter, especially built for rats. This consisted of a horizontal tubular lead chamber closed at one end, with an opening on the under surface for a 3″ × 3″ Harshaw NaI Thalium-activated crystal; this crystal was connected to a 3″ photomultiplier tube which fed into a transistorized scaler *via* a linear amplifier and a pulse height analyzer. To assure constant counting geometry as well as to avoid isotopic contamination of the counting chamber, rats were placed in glass tubes of appropriate size with a small opening at the head end for air and closed at the opposite side with a rubber stopper notched with a V-shaped aperture for the tail. The first count was made 5 hours after the ^{22}Na was injected, then twice daily for 3-7 days, depending on the biological $T_{½}$ and counting rate. Initial counting rates averaged about 100,000 cpm and toward the end of an experiment approximately 1000 cpm; in the latter event, counting was continued until the statistical counting error was less than 2%. Biological $T_{½}$ was obtained by plotting net cpm on semi-log paper against time in days. Until the counting rate had declined below 1% of the initial level, this plot yielded only a single straight line.

2) Potassium. A. Total exchangeable potassium (TEK). The methods were the same as for total exchangeable sodium except that approximately 175 μc ^{42}K (physical half-life — 12.4 hours) were injected intraperitoneally and specific activity determined on urine collected during the period from 12 to 36 hours after injection of the isotope.

TABLE I. Biological Half-Life ($T_{½}$) of ^{22}Na and ^{43}K in Rats on Low (0.38%) and High (8%) NaCl Chow. High NaCl feeding started at weaning—21 days of age. Mean weights of R and S rats within respective groups did not differ significantly ($p > 0.1$).

Strain	Diet	Age (days)*	n	Systolic B.P. (mm Hg)	$T_{½}$ (days) ^{22}Na	$T_{½}$ (days) ^{43}K
R	Low NaCl	25–35	6	128 (10.5)†	7.5 (.86)	1.4 (.14)
S			6	135 (14.8)	5.9[3] (.61)	1.4 (.14)
R	High NaCl	25–35	11	Not measured	.40 (.11)	1.9 (.14)
S			12	" "	.31[1](.05)	1.6[3](.14)
R	High NaCl	50–70	23	123 (16.1)	.41 (.06)	3.7 (.62)
S			20	177[3](21.6)	.35[3](.05)	3.3[1](.61)
R	High NaCl	~100	5	143 (8.4)	.52 (.03)	5.0 (.59)
S			5	205[3](27.5)	.48[1](.03)	4.5 (.48)

* At time of study.
† S.D. in parentheses.
Difference between means of respective R and S groups tested for significance by Student's t test: [1] $.05 > p > .01$ [2] $.01 > p > .001$ [3] $p < .001$

B. Whole body potassium was determined on the same samples used for estimating sodium.

C. Potassium turnover. a. Isotope. Because of its longer physical half-life (22 hours) ^{43}K was used rather than ^{42}K. This isotope was produced in the 60″ Brookhaven cyclotron by an α, p process from $^{40}Argon$. From the gamma spectrum of energies the peak at 0.37 MEV was used for counting. Each rat received approximately 12 μc ^{43}K intraperitoneally in 1.0 ml of Ringer's solution.

b. Counting technique. The techniques and apparatus were the same as those used for studying ^{22}Na turnover; because of the longer biological half-life, counts were made routinely for 7 days. The potassium turnover study was completed, then followed by a sodium ^{22}Na turnover study with the combined elapsed time being about 11 days: residual activity of the ^{43}K made negligible contributions to the ^{22}Na counting data.

3) Miscellaneous. Rats were weighed daily and during a study extending over several days the average of these weights was used in calculations. Blood urea nitrogen (BUN) was determined on 0.1 ml plasma by Conway's microdiffusion technique(16).

Results. Turnover studies. The results are summarized in Table I. Contrary to expectations, in the animals from the S strain with or without hypertension, the biological $T_{½}$ of ^{22}Na was consistently somewhat *shorter* than in members from the R strain. The differences in mean $T_{½}$ values were generally small but statistically significant. A similar but less consistent pattern prevailed in the ^{43}K turnover study.

Total body Na and K (Table II). *Na.* There was no evidence that animals with hypertension had more total body Na, however calculated, than did animals without elevated blood pressure. However, when a difference among the 4 groups existed, it suggested that the animals *least* likely ever to develop hypertension (R-low NaCl) had the *lowest* total body Na.

K. No correlations with either blood pressure or sodium were found.

Discussion. The results of this study with animals are not in agreement with earlier observations on patients: our clinical studies had suggested that hypertensive patients might have a prolonged biological half-life ($T_{½}$) for ^{22}Na, explained by a larger pool of tissue sodium(1,2). However, among rats from a strain genetically predisposed to develop experimental hypertension, the biological $T_{½}$ of ^{22}Na appeared to be *shorter* than among animals from another strain genetically resistant to the development of hypertension. This seemed to be the case both when the diet was low in salt and the animals had no hypertension, and when a high salt diet had caused hypertension in the members of the S strain. Furthermore, neither by equilibration with ^{24}Na nor by total body analysis was there evidence that animals with hypertension had an increase in the size of the ex-

TABLE II

Diet		High NaCl		Low NaCl		Statistical significance‡‡
Strain		S	R	S	R	
n		11	12	11	12	
Systolic B.P. (mm Hg)		184 (20.0)¶	120 (7.1)	137 (11.8)	110 (5.3)	S-hi $>^3$ S-lo $>^3$ R-hi $>^3$ R-lo§§
Weight (g)		237 (30.2)	214 (24.8)	251 (14.8)	225 (28.8)	S-lo $>^3$ R-hi S-lo $>^1$ R-lo
BUN (mg %)		28.9 (15.1)	22.2 (9.4)	17.0 (3.2)	15.3 (2.9)	S-hi $>^1$ S-lo S-hi $>^2$ R-lo R-hi $>^1$ R-lo
Sodium	mEq/l (serum)	146.9 (3.6)	149.3 (2.3)	147.0 (4.5)	150.0 (6.0)	N.S.
	mEq/kg (body wt) TENa*	47.0 (3.2)**	47.8 (3.2)††	47.1 (3.2)**	44.1 (.7)††	S-lo, R-hi $>^1$ R-lo
	Carcass†	47.7 (2.9)	46.6 (2.0)	47.1 (5.2)	44.6 (2.5)	R-hi $>^1$ R-lo
	mEq/kg (LBM)‡ TENa	50.3 (3.4)**	52.3 (3.5)††	49.3 (3.3)**	48.0 (1.7)††	S-hi, R-hi $>^1$ R-lo
	Carcass	53.3 (4.5)	53.0 (2.4)	51.7 (3.8)	50.6 (1.4)	R-hi $>^2$ R-lo
Potassium	mEq/l (serum)	3.97(.68)	4.43(.39)	4.10(.18)	4.31(.55)	R-hi $>^1$ S-lo
	mEq/kg (body wt) TEK§	66.3 (3.5)	66.4 (2.7)	65.7 (3.3)	65.3 (2.1)	N.S.
	Carcass	65.7 (3.3)	64.1 (3.1)	66.7 (3.6)	64.5 (3.2)	N.S.
	mEq/kg (LBM) TEK	71.1 (3.3)	72.7 (2.1)	68.8 (3.8)	71.0 (1.7)	N.S.
	Carcass‖	—	—	—	—	—

* Total exchangeable sodium.
† By analysis.
‡ Lean body mass calculated from carcass K according to Cheek and West(17): $K_t = 0.0747$ LBM—0.330.
§ Total exchangeable potassium.
‖ Not applicable since carcass K appears in definition of LBM (see footnote ‡).
¶ S.D. in parentheses.
** 5 animals.
†† 6 animals.
‡‡ Difference between means tested for significance by Student's t test: N.S. = Not significantly different ($p > .05$) ^{1} $.05 > p > .01$ ^{2} $.01 > p > .001$ ^{3} $p < .001$
§§ hi = high NaCl diet, lo = low NaCl diet.

changeable pool of sodium or in the amount of total body sodium, respectively.

Under normal conditions, the biological half-life of sodium is dependent on the ratio of sodium intake to the metabolic pool with which it actively exchanges. In these rats this pool and total body sodium appeared to be identical. Therefore, our observations that the biological $T_{1/2}$ of ^{22}Na (and to a lesser degree, ^{43}K) was shorter in the strain predisposed to hypertension requires explanation. No final answer is now available but some unpublished data on daily food consumption suggest that the food (hence sodium) intake of animals in the Sensitive strain is slightly greater than that of the animals from the Resistant strain; since the errors in measuring food consumption (*ca* 10 to 20%) are of the same order of magnitude as the differences in $T_{1/2}$, this cannot now be regarded as conclusive. However, a similar conclusion may be deduced from the fact that animals from the S strain generally grow slightly faster than do comparable R animals, again suggesting a somewhat greater food intake.

In earlier studies on experimental hypertension, Laramore and Grollman(18) observed an increase in sodium and a decrease in potassium content in selected tissues of rats with renal hypertension. Using the same technique to produce hypertension in rats but with chemical analysis of the entire animal, Greene and Sapirstein(19) found an increase in tissue sodium but no change in potassium. In rats made hypertensive by feeding excess salt at different levels in the diet for 8-12 months, Lemley-Stone, Darby and Meneely (9) reported that animals on 2% NaCl did not have an increased exchangeable sodium, whereas those on 3 or 4% NaCl did. Exchangeable potassium was measured only in animals on 4% NaCl, but was normal. Tissue analysis of Na and K from selected tissues confirmed the isotopic data. Thus, both hypertension and evidence of sodium retention were present in the groups on the higher salt intakes but, while hypertension was present in animals on 2% NaCl, salt retention was not found. This suggests that chronic gross salt retention did not play a role in the pathogenesis of hypertension induced by salt feeding. Such an interpretation would be compatible with our findings, in that after only 6 weeks on salt, very significant hypertension was observed in rats without evidence of sodium accumulation or potassium depletion in the body as a whole.

It is of interest that in experimental hypertension induced with added dietary NaCl, sodium retention has not been found whereas it has been in hypertension induced by renal manipulation, without added dietary salt. It seems fair to suggest, as did Greene and Sapirstein(19), that the sodium retention observed rather than being primarily involved in the pathogenesis of the hypertension may only reflect a loss of renal excretory function resulting from the technique used for producing hypertension.

It is difficult to assess the significance of the observation that the animals least likely to develop hypertension (those from the Resistant strain, on low NaCl chow) always had the lowest exchangeable and carcass sodium content. However, this was not always statistically significant ($p<0.05$). This observation might be compared with the somewhat similar one that pertains to serum cholesterol concentration and coronary artery disease: individuals with low serum cholesterol concentrations have significantly less evidence of coronary artery disease than do those with high serum cholesterols, although not all of the former are spared nor are all of the latter afflicted.

With the techniques used in our studies, changes in the concentration of sodium or potassium in small but critical tissues would not be revealed. The findings of Tobian and his associates are pertinent in this respect: they have reported increased concentrations of sodium in the aortas(20) and, more recently, arterioles(21) of rats with experimental hypertension. In view of the small mass of tissue involved, such accumulations of sodium could have been present in our animals without being manifest in the measurement of the very much larger mass of total body sodium. If there were other critical areas of accumulation, *e.g.*, in the kidney or central nervous system, they would have been missed as well.

Conclusion. These studies on experimental

hypertension are not in agreement with our clinical research(1,2) in which there was evidence that the biological half-life ($T_{½}$) of ^{22}Na often was prolonged in patients with uncomplicated essential hypertension. The present work on rats from 2 inbred strains having opposite genetic susceptibilities to hypertension induced by salt suggested the contrary: the biological $T_{½}$ of ^{22}Na was found to be *shorter* in the strain predisposed to hypertension, and did not appear to be related to elevations in blood pressure, *per se*. Tissue sodium was not found to be increased in animals with hypertension, nor was there evidence of potassium depletion. It is possible that the shorter biological $T_{½}$ of ^{22}Na in the group predisposed to hypertension was due to a slightly greater food (*i.e.*, NaCl) intake as compared with the animals resistant to the development of hypertension.

In sum, this evidence strongly suggests that however excess salt ingestion induces hypertension, it is not through gross accumulation of sodium, or depletion of potassium, in tissues. However, by the techniques used here, focal accumulation, *e.g.*, in arterioles(21), would not have been found.

We are indebted to Mrs. Lorraine Tassinari and Miss Martha Heine for technical assistance.

1. Dahl, L. K., Smilay, M. D., Silver, L., Spraragen, S. C., Nature, 1961, v192, 267.
2. ———, Circulation Res., 1962, v10, 313.
3. Dole, V. P., Dahl, L. K., Cotzias, G. C., Dziewiatkowski, D. D., Harris, C., J. Clin. Invest., 1951, v30, 584.
4. Dahl, L. K., Stall, B. G., Cotzias, G. C., ibid., 1954, v33, 137.
5. Hollander, W., Chobanian, A. V., Burrows, B. A., ibid., 1961, v40, 408.
6. Gifford, R. W., Mattox, V. R., Orvis, A. L., Sones, D. A., Rosevear, J. W., Circulation, 1961, v24, 1197.
7. Freed, S. C., Am. J. Cardiol., 1961, v8, 737.
8. Meneely, G. R., Ball, C. O. T., Youmans, J. B., Ann. Int. Med., 1957, v47, 263.
9. Lemley-Stone, J., Darby, W. J., Meneely, G. R., Am. J. Cardiol., 1961, v8, 748.
10. Dahl, L. K., Heine, M., Tassinari, L., J. Exp. Med., 1962, v115, 1173.
11. ———, ibid., 1963, v118, 605.
12. ———, ibid., 1965, v122, 533.
13. Dahl, L. K., ibid., 1960, v112, 635.
14. Dahl, L. K., Heine, M., ibid., 1961, v113, 1067.
15. Dahl, L. K., Heine, M., Tassinari, L., ibid., 1962, v115, 1173.
16. Conway, E. J., Microdiffusion Analysis and Volumetric Error, London, Crosby Lockwood & Son, Ltd., 3rd revised edit., 1950, 152.
17. Cheek, D. B., West, C. S., J. Clin. Invest., 1955, v34, 1733.
18. Laramore, D. C., Grollman, A., Am. J. Physiol., 1950, v161, 278.
19. Greene, R. W., Sapirstein, L. A., ibid., 1952, v169, 343.
20. Tobian, L., Binion, J., J. Clin. Invest., 1954, v10, 1407.
21. Tobian, L., Janecek, J., Tomboulian, A., Ferreira, D., ibid., 1961, v40, 1922.

Received May 2, 1966. P.S.E.B.M., 1966, v122.

Binding of Sulfobromophthalein (BSP) Sodium and Indocyanine Green (ICG) by Plasma α_1 Lipoproteins.* (31299)

KATHARINE J. BAKER (Introduced by Stanley E. Bradley)
Department of Medicine, Columbia University College of Physicians and Surgeons, New York City

Sulfobromophthalein (BSP) and the tricarbocyanine dye, indocyanine green (ICG), are water-soluble, anionic dyes. Both are excreted so efficiently by the liver in man and other animals that they may be employed to measure hepatic blood flow and to quantify hepatic function precisely under a wide variety of conditions(1,2). Although the data available on the biliary output of the 2 dyes in dog suggest that they may share, at least in part, a common excretory pathway, BSP and ICG are quite different in their physico-

* Supported by a grant from the John A. Hartford Foundation, Inc.