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LABORATORY INVESTIGATION

Effect of variations in dietary sodium intake on sodium excretion in mature rats

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Effect of variations in dietary sodium intake on sodium excretion in mature rats. Sprague-Dawley rats weighing 400 g or more were studied to determine whether their continued weight gain affects renal sodium handling. Rats maintained on a wide range of sodium intakes gained 3.9 ± 0.4 g/day. The intercept of a linear regression of intake against urinary excretion provided an estimate of the minimum daily requirement for sodium intake of 247 ± 33 μEq/day. When more than this required amount was ingested, the animals excreted the excess quantitatively in the urine. When less was ingested they continued to gain weight at a slower rate, 1.6 ± 0.6 g/day, and remained in positive sodium balance. Nonetheless, they developed a sodium deficit manifested as retention of a sodium challenge. Thus, on an adequate dietary intake the normal physiological state of Sprague-Dawley rats of this size is one of chronic sodium retention rather than neutral sodium balance. In contrast, when inadequate sodium is ingested a deficit develops in the absence of external losses. These observations have important implications for the interpretation of studies of renal sodium handling in these animals.

Effet de variations de l'apport sodé alimentaire sur l'excrétion sodique de rats matures. Des rats Sprague-Dawley pesant 400 g ou plus ont été étudiés pour déterminer si leur prise de poids continue affecte l'élimination sodée rénale. Des rats maintenus à des apports sodés très variés gagnaient 3,9 ± 0,4 g/jour. L'interception de la régression linéaire de l'apport en fonction de l'excrétion sodée fournissait une estimation de l'apport quotidien minimum nécessaire en sodium, 247 ± 33 μEq/jour. Lorsque plus que cette quantité nécessaire était ingéré, les animaux excrétaient quantitativement cet excès dans les urines. Lorsqu'ils ingéraient moins, ils continuaient à prendre du poids plus lentement, 1,6 ± 0,6 g/jour, et restaient en balance sodée positive. Néanmoins, ils développaient un déficit sodé se manifestant par la rétention d'une surcharge sodée. Ainsi, lors d'un apport alimentaire adéquat, l'état physiologique normal des rats Sprague-Dawley de cette taille est une rétention chronique du sodium, plus qu'une balance sodée neutre. A l'opposé, lorsqu'insuffisamment de sodium est ingéré, un déficit se développe en l'absence de pertes externes. Ces observations ont des implications importantes pour l'interprétation des études de l'élimination rénale du sodium chez ces animaux.

Sprague-Dawley rats weighing 250 to 400 g have long been used as a convenient model for the study of renal sodium handling. Although these animals continue to gain weight, they have been considered mature for the purpose of sodium handling studies and are typically referred to as adults [1]. Their maturity is well established from the point of view of the development of renal structure and function, and their continued weight gain is widely assumed to consist of adipose tissue by analogy with changes in body composition in adult humans. However, skeletal growth continues throughout most of the life span of the rat [2], and bone contains large amounts of sodium [3].

Since renal sodium handling is extremely sensitive to variations in both body sodium content and distribution, we reasoned that continued weight gain might affect urinary sodium excretion. If such an effect existed, it could alter the interpretation of the numerous studies which utilize these animals. Accordingly, we measured both urinary and fecal sodium excretion in Sprague-Dawley rats, weighing more than 400 g, which were maintained on a wide range of dietary sodium intakes. The renal response to a sodium challenge was then determined in the same animals to evaluate the status of their sodium stores.

Methods

Balance technique. Sodium balance studies were conducted on three groups of male Sprague-Dawley rats, weighing more than 400 g and housed in individual metabolic cages. Room lighting was controlled with lights on from 6 A.M. to 6 P.M. daily, and room temperature was monitored and remained between 22 and 26°C. Analysis of several commercially available "low salt" rat chows indicated that all contained more sodium than was represented by the suppliers; accordingly, all diets were prepared on site. A modification (see Table 1) of the diet described by Mohring and Mohring [4] was offered ad libitum, and the animals were initially given free access to demineralized water. Each morning from 9 to 11 A.M. a sweetened solution, 5% dextrose in demineralized water (D5W), was offered to encourage the rats to drink a bolus. Prior to use the sodium concentration of all solutions and each batch of food was determined by flame photometry using a lithium internal standard. The rats were weighed daily, and their food and fluid consumption were determined daily by weight. Sodium intake was estimated from the products of the weights of fluid and food consumed and their respective measured sodium contents.

After several days of acclimation to the experimental routine, 24-hr serial urine collections were begun. Urine was collected

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Table 1. Composition of diet, *g*

Dextrose	281.0
Crisco	100.0
High nitrogen casein[a]	100.0
Raw wheat bran[a]	50.0
Vitamin diet fortification mixture[b]	10.0
DL-methionine	2.5
$K_2HPO_4 \cdot 3\ H_2O$	5.1
$CaCO_3$	4.5
$MgSO_4 \cdot 7\ H_2O$	4.1
$CaHPO_4$	3.3
$FePO_4 \cdot H_2O$	0.3
$CuSO_4 \cdot 5H_2O$	0.015
$MnSO_4 \cdot H_2O$	0.004

[a] Bioserv, Frenchtown, New Jersey, USA
[b] ICN Nutritional Biochemicals, Cleveland, Ohio, USA

by funnel at the bottom of the cage into dry, tared vessels. At the completion of a collection period each cage was washed with demineralized water, delivered as a fine spray over the entire cage. Urinary sodium excretion was estimated from the product of the sodium concentration of the wash water and its weight.

Preliminary studies were undertaken to determine the recovery of known amounts of sodium applied to the metabolic cages and funnels. Experiments were conducted washing the cages with 25 ml ($N = 9$), 50 ml ($N = 9$), and 100 ml ($N = 14$) of demineralized water, and the respective percent recoveries (mean ± SE) were 95.5 ± 1.5, 99.2 ± 1.5, and 100.2 ± 1.5. Therefore, during the balance studies 100 ml of wash water was used.

To evaluate the long-held tenet that stool sodium is negligible, fecal sodium excretion was determined in some of the experiments. Prior to washing the cages, all stools passed by each animal during the previous 24-hr period were recovered. The stool specimens were placed in tared vials and digested overnight in 5 ml of concentrated nitric acid. The following day the liquid digest was diluted with 15 ml of demineralized water and filtered by gravity to remove particulates, and the sodium concentration of the filtrate was determined. Fecal sodium excretion was estimated as the product of the weight of the diluted digest and the sodium concentration of the filtrate.

Experimental groups

Group 1. Sixteen rats were offered food containing 4.6 to 6.3 μEq of sodium per gram ad libitum. To obtain a group of rats ingesting a wide range of sodium each day, the average fluid intake of each rat during the last 3 days of acclimation was determined, and specific amounts of sodium (see Table 2) were then added to the drinking water to provide each rat with a different fixed sodium intake in the range of 50 to 1,900 μEq/day. In addition approximately 100 μEq of sodium per day was consumed by each rat in the food. Sodium solutions were removed from 11 P.M. to 9 A.M. daily to encourage prompt consumption of the D5W offered the next morning. After an 8-day acclimation, 24-hr urine collections were obtained on 12 consecutive days, and on day 13 700 μEq of sodium were added to the 20 ml of D5W offered. Urine samples were collected during this 24-hr period, and the experiment was then terminated. Stool collections were made on 6 days in the eight rats with the highest sodium intakes.

Group 2. Eight rats were studied on a sodium deficient diet. After 14 days on a diet containing 95 to 110 μEq of sodium per gram of food, they were given food containing 5.1 to 5.5 μEq/g. Demineralized water was available throughout the experiment, except from 9 to 11 A.M. daily when D5W was offered. During 8 days of ad libitum fluid intake while on the high sodium diet, the average fluid consumption of each rat was determined. Beginning 6 days prior to the institution of the low sodium diet regimen, 25% less than the previous average ad libitum fluid intake was offered over the 24-hr period to encourage consumption of the D5W when offered. After a 3-day equilibration on the low sodium diet, three 24-hr urine collections were made, and on the next day 20 ml of D5W, to which 1,000 μEq of sodium had been added, was offered from 9 to 11 A.M. Urine samples were collected during this 24-hr period, and the experiment was then terminated.

Group 3. Eight rats were studied exclusively to determine fecal sodium excretion. A diet containing 5.7 to 7.1 μEq of sodium per gram was offered ad libitum, and the daily ad libitum fluid consumption was determined for each rat on 7 days. Four rats (group 3A) were then offered a sodium solution containing 100 μEq of sodium in their previous average daily fluid consumption, and the others (group 3B) were offered a solution containing 700 μEq of sodium in their previous average daily fluid consumption. After a 2-day acclimation fecal sodium excretion was measured for 6 days, and the experiment was then terminated.

Statistical analysis

The relationship between sodium intake and both urinary and fecal sodium excretion was analyzed by least squares linear regression. The significance of the slope estimates obtained was evaluated and 95% confidence bands for the regression parameters were computed using the Student *t* test. Independent relationships between weight gain, food intake, sodium intake, and urinary sodium excretion were examined by multivariate analysis. Partial correlation coefficients were computed and compared with zero using the Student *t* test. The response to the sodium bolus was analyzed using the Wilcoxon two-sample rank test. Animals were divided into groups based on their dietary sodium intake; those ingesting more than the minimum daily requirement (see below) were compared with those ingesting less.

Results

The data (means ± SE) obtained on daily food, fluid, and sodium intake and weight gains for the rats in groups 1, 2, and 3 were summarized in Tables 2 and 3, respectively. All the animals in all the groups were gaining weight throughout the study. The only statistically significant differences in daily weight gains, as judged by the overlap of 95% confidence bands for the means, was growth retardation in the sodium deficient rats in groups 2 compared with both the rats in group 1 and the high salt rats in group 3.

Multivariate analysis of the data obtained in group 1 was used to identify significant independent relationships between the variables weight gain, food intake, sodium intake, and urinary sodium excretion, and the results are presented in Table 4.

Table 2. Summary of observations in group 1

Rat	Sodium in water *mEq/liter*	Intake: Food *g/day*	Intake: Fluid *g/day*	Intake: Sodium *μEq/day*	Weight gain *g/day*
1	1.7	20.9 ± 0.5	36.5 ± 1.2	164 ± 6	4.2 ± 0.5
2	3.5	22.1 ± 0.8	35.9 ± 1.7	201 ± 9	4.1 ± 1.3
3	4.6	21.2 ± 0.5	43.6 ± 1.9	263 ± 10	2.6 ± 0.7
4	8.5	20.8 ± 0.3	24.9 ± 0.9	272 ± 10	4.3 ± 0.6
5	8.5	23.8 ± 0.6	30.4 ± 1.6	313 ± 10	3.8 ± 0.6
6	10.4	29.5 ± 0.5	32.2 ± 1.4	380 ± 15	7.0 ± 0.8
7	10.8	25.0 ± 0.5	35.0 ± 1.4	384 ± 16	6.1 ± 0.8
8	13.5	22.7 ± 0.6	29.0 ± 1.8	415 ± 26	4.0 ± 0.6
9	21.1	18.4 ± 0.7	21.1 ± 1.5	472 ± 27	1.8 ± 1.3
10	23.7	20.9 ± 0.5	35.2 ± 1.3	682 ± 46	2.1 ± 0.6
11	28.7	25.3 ± 0.6	30.7 ± 2.0	751 ± 32	3.5 ± 0.9
12	29.0	29.7 ± 0.7	39.9 ± 1.4	925 ± 40	5.8 ± 0.8
13	45.6	24.1 ± 0.7	32.7 ± 1.6	1,173 ± 64	3.1 ± 1.1
14	48.5	21.9 ± 0.5	27.8 ± 1.4	1,204 ± 66	2.8 ± 0.7
15	42.1	27.6 ± 0.9	41.6 ± 2.3	1,313 ± 79	4.1 ± 1.0
16	82.6	23.8 ± 0.6	27.6 ± 1.4	1,694 ± 114	2.9 ± 0.6

Table 3. Summary of observations in groups 2 and 3

Group	Intake: Food *g/day*	Intake: Fluid *g/day*	Intake: Sodium *μEq/day*	Weight gain *g/day*
2	20.2 ± 0.5	28.0 ± 1.6	107 ± 3	1.6 ± 0.6
3A	20.6 ± 0.7	25.2 ± 0.6	208 ± 5	3.5 ± 0.7
3B	23.4 ± 0.6	33.4 ± 1.5	1,042 ± 41	5.2 ± 0.7

Increased weight gains were associated with increased food and sodium intake and decreased urinary sodium excretion. There was no significant association between food intake and either sodium intake or urinary sodium excretion. The correlation between sodium intake and urinary sodium excretion remained significant after corrections for the effects of weight gain and food intake.

The relationship between daily sodium intake and urinary sodium excretion in the rats in group 1 is depicted in Figure 1. Least squares linear regression gave a slope estimate of 0.96 ± 0.05, and the estimated intercept was 247 ± 33 μEq. Because urinary sodium excretion is plotted on the abscissa, the intercept indicates that the urine became virtually sodium-free when dietary sodium intake fell below 250 μEq/day. The fact that the slope of the regression line is close to unity indicates that all sodium ingested in excess of 250 μEq/day was excreted quantitatively. Thus, the intercept provides an estimate of the minimum daily requirement for dietary sodium intake based on urinary sodium excretion patterns.

In contrast with the results obtained in group 1, there was no correlation (P value > 0.2) between daily sodium intake and urinary sodium excretion in the sodium deficient rats in group 2. Accordingly, the daily urinary excretion data for the animals in this group were pooled and averaged to obtain an estimate of obligate urinary sodium losses of 30 ± 6 μEq/day.

The data obtained on urinary sodium excretion following an acute challenge administered as a bolus to the rats in groups 1 and 2 are presented in Figure 2. The animals were divided into two groups based on their average daily sodium intake prior to the challenge. Those receiving less than the estimated minimum daily requirement responded to the bolus by excreting less than 10% (range, 4 to 9%). In sharp contrast, animals previously ingesting more than the minimum daily requirement excreted more than 40% of the bolus promptly (range, 42 to 104%). The response of the two groups to the challenge was significantly different (P value < 0.01) as judged by the Wilcoxon two-sample rank test.

The observed relationship between daily sodium intake and fecal sodium excretion in the rats in groups 1 and 3 is illustrated in Figure 3. Least squares linear regression gave a slope estimate of 0.034 ± 0.009, a value significantly greater than zero (P value < 0.001), and the intercept was 10.4 ± 8.9 μEq. Thus, about 3.4% of ingested sodium was recovered in the stool over the entire range of intakes studied.

Discussion

The mature rats used in this study consistently gained weight as was expected from previous observations [2]. However, in the present study we demonstrated that this weight gain is associated with a requirement for sodium intake of 247 ± 33 μEq/day. This amount, which represents an estimate of the minimum daily requirement for sodium intake based on urinary excretion patterns, is remarkably similar to previous estimates of the sodium intake required to maximize growth rates in younger rats (see **Appendix**) [5].

Although the current study was not designed to examine the nature of the weight gain or the disposition of the sodium retained, some insight can be gained from the data. The ratio of the minimum daily requirement estimate, 247 ± 33 μEq/day, and the mean daily weight gain, 3.9 ± 0.4 g, is 63 μEq/g. This is comparable to the sodium content of whole rats determined in body composition studies [6, 7] and much larger than the sodium content of adipose tissue. This suggests that the observed weight gain includes either extracellular fluid expansion, bone growth, or both, since these are the major sites of sodium storage.

Multivariate analysis provided insight into the relationships among the measured variables. The results showed the ex-

Table 4. Results of multivariate analysis of the data from group 1

Variable 1	Variable 2	Ordinary correlation coefficient	*P* value	Partial correlation coefficient	*P* value
Weight gain	Food intake	0.456	<0.001	0.461	<0.001
Weight gain	Sodium intake	−0.075	>0.1	0.359	<0.001
Weight gain	Urine sodium	−0.242	<0.001	−0.455	<0.001
Food intake	Sodium intake	0.279	<0.001	0.080	>0.1
Food intake	Urine sodium	0.189	<0.01	0.040	>0.1
Sodium intake	Urine sodium	0.937	<0.001	0.942	<0.001

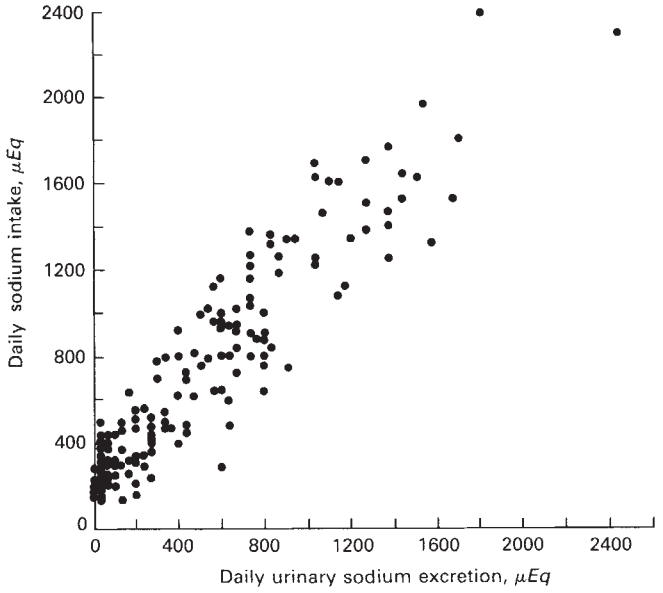

Fig. 1. *Relationship between daily sodium intake and urinary sodium excretion in sixteen rats maintained on different fixed sodium intakes.* The *intercept* indicates the intake necessary for sodium in excess of obligate losses to appear in the urine and provides an estimate of the minimum daily requirement for this essential nutrient based on renal sodium handling.

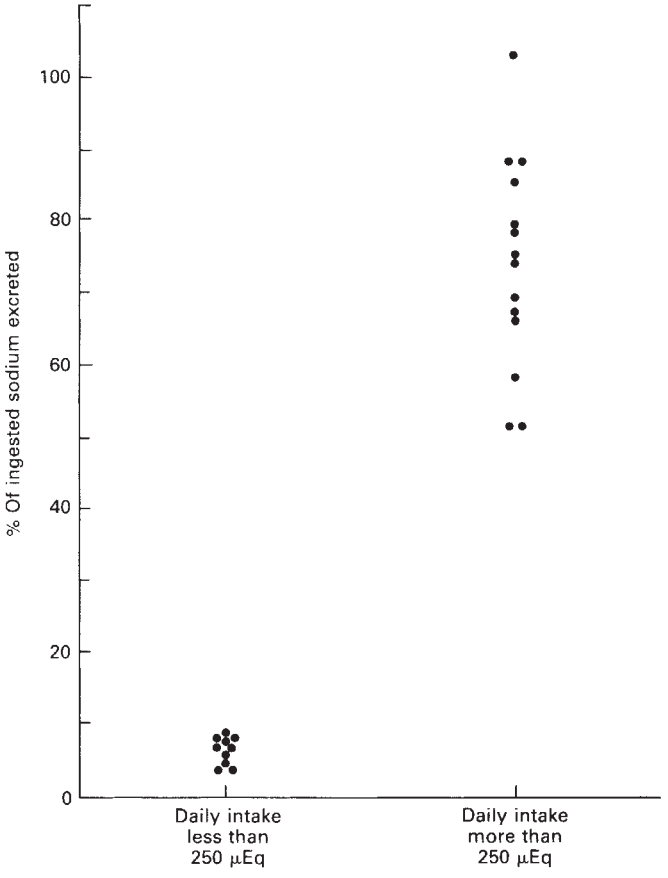

Fig. 2. *Fractional excretion of ingested sodium when excess sodium was offered to rats previously ingesting less than* (left) *and more than* (right) *the estimated minimum daily requirement.*

pected correlation between weight gain and food intake. In addition there was an independent correlation between weight gain and sodium intake, the physiological significance of which is unclear. One can speculate that sodium intake may condition an element of weight gain (for example, extracellular fluid expansion) or that higher weight gain drives the animal to a higher sodium intake. Because a correlation between two variables does not provide insight into cause and effect, either explanation is tenable. Similarly the observed inverse correlation between weight gain and urinary sodium excretion could indicate that increased weight gain causes renal sodium avidity or that increased renal sodium excretion reduces weight gain (for example, sodium excretion lowers extracellular fluid volume). Finally, there was no correlation between sodium intake and food intake, confirming that the experimental design we used permits sodium intake to be varied without affecting the consumption of other nutrients.

It is of interest to note that in three of the six relationships studied, reliance on ordinary correlation coefficients would have lead to erroneous conclusions. The relation between weight gain and sodium intake would not have been revealed, and spurious positive correlations between food intake and both sodium intake and urinary excretion would have been accepted. These observations indicate the importance of using adequate data reduction techniques in the analysis of multifactorial experiments.

The pattern of urinary sodium excretion observed reaffirms the precision with which the kidneys regulate sodium stores. When more than the minimum daily requirement was ingested, the excess was excreted quantitatively. When less was ingested urinary sodium excretion was reduced to a minimal, apparently obligate, loss of 30 ± 6 μEq/day. The linear relation we observed between intake and urinary excretion is substantially different from that previously reported by Mohring and Mohr-

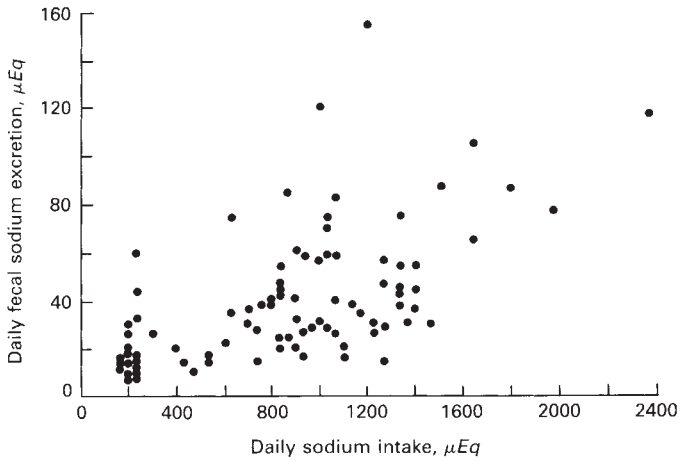

Fig. 3. *Relationship between daily sodium intake and fecal sodium excretion.* About 3.4% of ingested sodium was recovered in the stool.

ing [4]. The regression equation they reported implies a much larger obligate loss of 670 μEq/day and excretion of only 69% of the sodium ingested in excess of 670 μEq/day. These quantitative discrepancies between the two regression equations may derive from the use of smaller rats by Mohring and Mohring, the sham operations to which they subjected their animals, or the higher sodium intakes they used.

As noted above, animals ingesting less than 250 μEq/day excreted very little sodium in the urine. This renal sodium avidity is similar to that reported in smaller, more rapidly growing rats fed a low sodium diet [8]. However, it has been suggested that a low fractional excretion of ingested sodium does not necessarily indicate depletion of sodium stores [9, 10]. Accordingly, we subjected the animals to a sodium challenge to evaluate the status of sodium stores. The response to this challenge clearly segregated the animals into two groups, and the dividing line between the groups coincided with the minimum daily requirement estimate (compare with Figure 2). It is important to emphasize that all of the animals studied were consistently in positive sodium balance. Thus, none had incurred a sodium deficit in the classical sense of losses in excess of intake. Nevertheless, all the animals previously ingesting less than the minimum daily requirement retained nearly all of the challenge, behaving as if their sodium stores were depleted. Thus, a sodium deficit developed endogenously in these animals as they gained weight while ingesting inadequate sodium. Similar observations have previously been reported in pregnant rats maintained on a low sodium intake [11].

Finally, our results indicate that only a small fraction (3%) of ingested sodium is excreted in the stool, confirming earlier less systematic observations [4, 12–14]. For example, rats maintained on a typical sodium ration of 2 mEq/day will excrete about 60 μEq/day in the stool. However, since sodium retention is only about 200 μEq/day [4], fecal excretion may exceed 30% of the net daily retention. Thus, neglecting fecal sodium losses can result in a substantial systematic error in balance calculations in this model.

In conclusion, mature Sprague-Dawley rats maintained on a wide range of sodium intakes continue to gain weight and incorporate a substantial amount of sodium into their bodies each day. The interpretation of studies which use these animals is complicated by the fact that their natural state is one of chronic sodium retention and not neutral balance. For example, experimental maneuvers which modify renal sodium handling in this model might do so merely by modifying weight gain and its attendant sodium retention rather than by a direct renal effect. Furthermore, if these animals ingest less than 250 μEq/day, their continued weight gain generates an effective depletion of sodium stores, which renders them more comparable to nongrowing animals that have sustained external losses than to nongrowing animals ingesting a low sodium diet. This response to dietary sodium restriction contrasts sharply with that in nongrowing animals that can maintain a neutral sodium status when sodium intake is reduced.

Appendix

The dietary sodium requirement of the rat has been studied for at least 65 years [15], but the methods which have been used to estimate this requirement have been rather nonspecific. The adequacy of dietary sodium intake has most often been assessed by comparing growth rates of animals ingesting diets with different sodium contents [5, 6, 8, 16–26], although a variety of other measures have been used, including voluntary intake [27, 28], weight gain per gram of food ingested [17, 24], longevity [25, 26, 29], reproductive performance [22, 24, 30–33], histological abnormalities [19, 29], and changes in both body composition [6, 16, 17, 19, 20, 34, 35] and nitrogen balance [17, 21, 25].

The sodium intake currently recommended for the rat is 0.05% of the diet by weight [2, 36]. This figure is based on the study of Grunert, Meyer, and Phillips [5] who measured weight gains for 6 weeks in rats initially weighing 40 to 45 g that were fed diets with sodium contents ranging from 2.2 to 44 μEq/g. During the first 5 weeks of the study maximal growth rate was observed with diets containing 22 μEq/g or more, but during week 6 only 13 μEq/g were required. Although food intake was not reported these rats should have ingested about 10 g/day at the start of the study and about 20 g/day by the end [2]. Thus, the total sodium intake of the animals with maximal growth rates should have been roughly 220 μEq/day during the first week and 260 μEq/day during the last. Accordingly, the estimate of the minimum daily requirement for sodium intake based on maximum growth rate appears to coincide with the one obtained in the present study based on urinary sodium excretion patterns.

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References

1. SPITZER A: The role of the kidney in sodium homeostasis during maturation. *Kidney Int* 21:539–545, 1982
2. National Research Council: *Nutrient Requirements of Laboratory Animals* (2nd rev ed), Washington, D.C., National Academy of Sciences, 1972, pp 56–93
3. NORMAN N: The participation of bone in the sodium and potassium

metabolism of the rat. I. Simultaneous determinations of the exchangeable body sodium and potassium, and the exchangeable and inexchangeable fractions of these ions in bone in the normal rat. *Acta Physiol Scand* 57:363–372, 1963
4. MOHRING J, MOHRING B: Evaluation of sodium and potassium balance in rats. *J Appl Physiol* 33:688–692, 1972
5. GRUNERT RR, MEYER JH, PHILLIPS PH: The sodium and potassium requirements of the rat for growth. *J Nutr* 42:609–618, 1950
6. MENEELY GR, LEMLEY-STONE J, DARBY WJ: Changes in blood pressure and body sodium of rats fed sodium and potassium chloride. *Am J Cardiol* 8:527–532, 1961
7. SCHACKOW E, DAHL LK: Effects of chronic salt ingestion: lack of gross salt retention in hypertension. *Proc Soc Exp Biol Med* 122:952–957, 1966
8. AVIV A, KOBAYASHI T, HIGASHINO H, BAUMAN JW, YU SS: Chronic sodium deficit in the immature rat: effect on adaptation to sodium excess, *Am J Physiol* 242:E241–E247, 1982
9. STRAUSS MB, LAMDIN E, SMITH WP, BLEIFER DJ: Surfeit and deficit of sodium, a kinetic concept of sodium excretion. *Arch Int Med* 102:527–536, 1958
10. HOLLENBERG NK: Set point for sodium homeostasis: surfeit, deficit, and their implications. *Kidney Int* 17:423–429, 1980
11. PIKE RL, MILES JE, WARDLAW JM: Juxtaglomerular degranulation and zona glomerulosa exhaustion in pregnant rats induced by low sodium intakes and reversed by sodium load. *Am J Obstet Gynecol* 95:604–614, 1966
12. DAY HG, MCCOLLUM EV: Mineral metabolism, growth, and symptomatology of rats on a diet extremely deficient in phosphorus. *J Biol Chem* 130:269–283, 1939
13. CHURCHILL SE, BENGELE HH, ALEXANDER EA: Sodium balance during pregnancy in the rat. *Am J Physiol* 239:R143–R148, 1980
14. EISENSTEIN B, BENGELE HH, ALEXANDER EA: Sodium balance after adrenal enucleation. *Am J Physiol* 228:E220–E222, 1980
15. OSBORNE TB, MENDEL LB: The inorganic elements in nutrition. *J Biol Chem* 34:131–139, 1918
16. FORBES GB: Effect of low sodium diet on sodium content and radiosodium exchange in rat bone. *Proc Soc Exp Bio Med* 98:153–155, 1958
17. KAHLENBERG OJ, BLACK A, FORBES EB: The utilization of energy producing nutriment and protein as affected by sodium deficiency. *J Nutr* 13:97–108, 1937
18. MENEELY GR, TUCKER RG, DARBY WJ: Chronic sodium chloride toxicity in the albino rat. I. Growth on a purified diet containing various levels of sodium chloride. *J Nutr* 48:489–498, 1952
19. MENEELY GR, TUCKER RG, DARBY WJ, AUERBACH SH: Chronic sodium chloride toxicity in the albino rat. II. Occurrence of hypertension and of a syndrome of edema and renal failure. *J Exp Med* 98:71–83, 1953
20. MEYER JH, GRUNERT RR, ZEPPLIN MT, GRUMMER RH, BOHSTEDT G, PHILLIPS PH: Effect of dietary levels of sodium and potassium on growth and on concentrations in blood plasma and tissues of white rat. *Am J Physiol* 162:182–188, 1950
21. MEYER JH: Interactions between a high concentration of dietary sodium chloride and various levels of protein when fed to the growing rat. *J Nutr* 52:137–154, 1954
22. MILLER HG: Sodium deficiency in a corn ration. *J Biol Chem* 70:759–762, 1926
23. MITCHELL HH, CARMAN GG: Does the addition of sodium chloride increase the value of a corn ration for growing animals? *J Biol Chem* 68:165–181, 1926
24. OLSON GA, ST. JOHN JL: The nutritive value of wheat. I. Effect of variation of sodium in a wheat ration. *J Agr Res* 31:365–375, 1925
25. ORENT-KEILES E, MCCOLLUM EV: Mineral metabolism of rats on an extremely sodium-deficient diet. *J Biol Chem* 133:75–81, 1940
26. TUCKER RG, BALL COT, DARBY WJ, EARLY WR, KORY RC, YOUMANS JB, MENEELY GR: Chronic sodium chloride toxicity in the albino rat. III. Maturity characteristics, survivorship, and organ weights. *J Gerontol* 12:182–189, 1957
27. RICHTER CP: Increased salt appetite in adrenalectomized rats. *Am J Physiol* 115:155–161, 1936
28. RICHTER CP, HOLT E JR, BARELARE B JR: Nutritional requirements for normal growth and reproduction in rats studied by the self-selection method. *Am J Physiol* 122:734–744, 1938
29. ORENT-KEILES E, ROBINSON A, MCCOLLUM EV: The effects of sodium deprivation on the animal organism. *Am J Physiol* 119:651–661, 1937
30. GANGULI MC, SMITH JD, HANSON LE: Sodium metabolism and its requirement during reproduction in female rats. *J Nutr* 99:225–234, 1970
31. GANGULI MC, SMITH JD, HANSON LE: Sodium metabolism and requirement in lactating rats. *J Nutr* 99:395–400, 1970
32. KIRKSEY A, PIKE RL: Some effects of high and low sodium intakes during pregnancy in the rat. I. Food consumption, weight gain, reproductive performance, electrolyte balances, plasma total protein and protein fractions in normal pregnancy. *J Nutr* 77:33–42, 1962
33. ST JOHN JL: Growth on a synthetic ration containing small amounts of sodium. *J Biol Chem* 77:27–32, 1928
34. NORMAN N: The participation of bone in the sodium and potassium metabolism of the rat. II. The effect of variation of electrolyte intake, acidosis and alkalosis. *Acta Physiol Scand* 57:373–383, 1963
35. SMITH JD, MEYER JH: Interactions of dietary sodium and potassium and their influence on energy metabolism. *Am J Physiol* 203:1081–1085, 1962
36. ROGERS AE: Nutrition, in *The Laboratory Rat, Volume I, Biology and Diseases*, edited by BAKER HJ, LINDSEY JR, WEIROTH SH, New York, Academic Press, 1979, pp 123–152