Effect of sodium chloride on longevity in mice

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ROSENTHAL, SANFORD M. *Effect of sodium chloride on longevity in mice.* Am. J. Physiol. 243 (Renal Fluid Electrolyte Physiol. 12): F549–F552, 1982.—The effects of dietary sodium chloride on the longevity, body weight, and food consumption in a normotensive strain (A/J) of mice was studied. Four groups of 50 mice each were fed diets containing 0.5 (control group), 1.5, 2.5, and 4.5% sodium chloride. The mean life span was 115.2 wk in the control group, and 115.5, 110.5, and 116.1 wk for *groups 2, 3,* and *4,* with no significant differences from the control group. No differences were present in food consumption, but a graded weight loss was present after the 20th wk, which persisted throughout the experiment. No significant effect of salt loading was demonstrable on the extravascular fluid movement in either acute experiments or in the long-term dietary sodium chloride groups.

transvascular fluid movement; sodium chloride toxicity in normotensive mice

THESE EXPERIMENTS WERE DESIGNED to determine whether increased amounts of sodium chloride consumed throughout the life of normotensive mice would influence their life span. Although it is well established that excess sodium chloride is deleterious in the presence of cardiovascular or renal disease, the tolerance in animals not genetically selected for hypertension has received less attention. Inasmuch as dietary restriction of sodium chloride has received wide application in clinical practice, this study was undertaken to determine whether the administration of large amounts of sodium chloride in the diet of normotensive mice throughout their life would affect their longevity, employing longevity as an index of toxicity.

Richter and Mosier (5) have shown in short-term experiments in rats that a high tolerance to sodium chloride exists if adequate water is supplied. Numerous experiments on hypertensive rats have demonstrated that control normotensive animals show no untoward effects of sodium chloride loading for periods up to 1 yr (for review, see Ref. 12). Torii (12) carried out a comprehensive study on normotensive Wistar/SLC rats as controls for hypertensive SHR rats. For periods of up to 2 yr they were fed diets containing 0.28, 4.8, and 9.6% sodium chloride, in most cases beginning at 7 mo of age. No differences in mortality were observed and hypertension did not result, but at the 9.6% sodium chloride level extensive renal pathology occurred. No untoward effects were observed at the 4.8% sodium chloride level. However, Torii did not report mortality data in the normotensive rats beyond 13 mo of age, at which time only one death had occurred.

Meneeley et al. (3) fed Sprague-Dawley rats (hypertensive-prone) 1.1 and 5.6% sodium chloride in the diet over a period of 3 yr and demonstrated a shortened life span at the high sodium chloride level; this could be partially prevented by increasing the potassium in the diet.

This study was initially suggested by the finding in mice that transvascular fluid movement decreased with age (7); the possibility existed that sodium chloride loading, by increasing extracellular fluid volume, might augment this movement and have a positive effect on longevity.

The present study was carried out on an inbred strain (A/J) of mice subjected to four levels of dietary sodium chloride intake beginning at 3 wk of age and continued until death.

MATERIALS AND METHODS

Two hundred female A/J mice 3 wk of age from the Jackson Laboratory were divided into four groups of 50 each. The A/J strain has been shown to be normotensive (9). They were placed on Ralston mouse and rat meal no. 63-8780, which contains 0.5% NaCl and 0.8% K. *Group 1* served as the control group, while *groups 2, 3,* and *4* received 1, 2, and 4% (wt/wt) NaCl, respectively, thoroughly incorporated into the diet. They were kept, in groups of five, on sawdust in plastic boxes 28 x 18 x 12 cm. The boxes were changed weekly, and the food jars were cleaned weekly and changed monthly. Food was supplied in glass jars with perforated metal tops to prevent scattering. Water was available at all times but intake was not measured. Food consumption and body weights were measured weekly. Autopsies were performed at death.

For the first 8 mo transvascular fluid movement (7) was measured in the tails of selected animals from each group. A separate acute experiment was also carried out on another group of mice (NIH Swiss Webster strain) to determine whether an increase in transvascular fluid movement could be demonstrated following the administration of a single dose of 0.85% NaCl.

The transvascular fluid movement was estimated by a plethysmographic method (6) based on the measurement of the local edema in the mouse tail after the application of a mild vacuum (80 mmHg for 15 min). The accuracy of the method, based on the results in 73 normal mice of 18–23 g weight, was shown in an average tail volume of 591 μl (SE = 5.0, with a range of SE 9–35 in groups of five to ten).

RESULTS

Mortality. Under the conditions of free access to water, NaCl concentrations of 1.5, 2.5, and 4.5% in the diet did not significantly affect the longevity of A/J mice as compared with the control group with 0.5% NaCl.

With the diet begun at 3 wk of age and continued until death, the mean length of life in weeks was 115.2 ± 26.6 (SD) for *group 1*, 115.5 ± 15.9 for *group 2*, 110.5 ± 22.6 for *group 3*, and 116.1 ± 20.3 for *group 4*.

The mortality curves (Fig. 1) reveal that the mortality rate was lower in *groups 2* and *4* between weeks 50 and 112, but no dose-response correlation was present.

The mortality data were submitted to Dr. H. Pettigrew of the Statistics Section, National Cancer Institute, who gave the following analysis.

The survival curves in Fig. 1 were computed by the method of Kaplan and Meier (2), using time to death and adjusting to censored observations, i.e., animals that were withdrawn from the experiment for extraneous reasons.

No significant differences were observed among the four groups by any of the methods described in Thomas et al. (11) nor was any dose-related trend observed.

Body weight. An inverse relationship between salt intake and body weight was observed. After the 20th wk a reduction in weight occurred that correlated with the dosage of NaCl loading and persisted throughout the experiment (Fig. 2). Statistical analysis (Table 1) comparing *groups 2, 3,* and *4* with control *group 1* revealed that the difference in *group 2* were mostly not significant, whereas nearly all the differences in *groups 3* and *4* were significant except at the end of the experiment, when the number of survivors was few.

While the differences between *groups 2* and *3* and *groups 3* and *4* were not statistically significant, a graded dose response is present throughout the experiment.

A similar result in normotensive Wistar rats fed 0.7, 4.8, and 9.6% NaCl for 13 mo was reported by Torii (12). The mechanism of this effect has not been determined. No significant differences in food consumption were present, and Torii found no differences in various hematological and enzymatic analyses.

A possible explanation of the weight loss with NaCl loading is afforded by the experiments of Ogata et al. (4) on human volunteers that showed an increase in basal metabolic rate from 35–36 $cal \cdot m^{-2} \cdot h^{-1}$ in controls to 38–47 under a dietary salt load of 50–60 g daily. The reason for this was not determined, except that peripheral circulation was improved, suggesting increased heat loss.

Food consumption. Food intake was measured weekly. For purposes of charting, the averages were calculated at approximately 4 wk. The results are shown in Fig. 3. No significant differences were present in the four groups.

Transvascular fluid movement. Swelling of the tail was measured by the plethysmographic method previously described (6). Measurements were made immediately before and after the application to the tail of a vacuum 80 mmHg for 15 min. Values were expressed as the percentage of increase in tail volume. In the acute experiments six female mice (NIH strain, Swiss Webster) of 18–22 g weight were given a single dose of 0.85% NaCl. With 1 ml orally or intraperitoneally, measurements were made 0.5 h later and compared with six mice receiving no therapy; with 3 ml orally, measurements were made 1.5 h later. No significant differences were observed (Table 2).

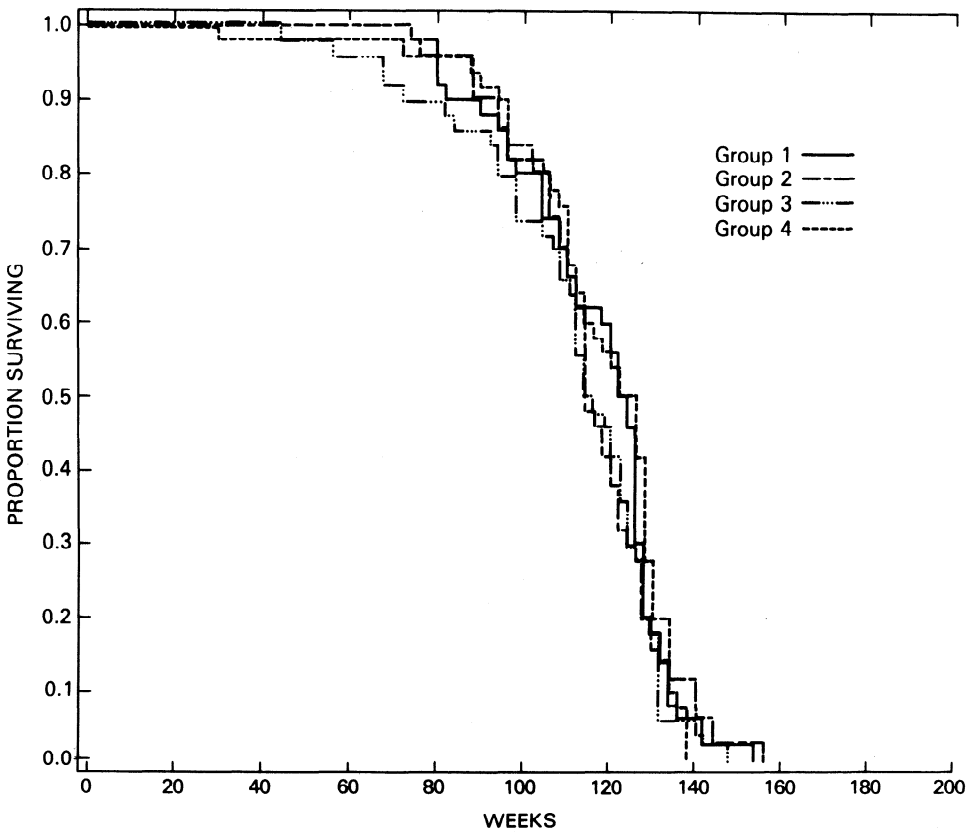

FIG. 1. Survival curves of 4 groups of mice receiving dietary NaCl from 3 wk of age until death. *Group 1*, 0.5% NaCl; *group 2*, 1.5% NaCl; *group 3*, 2.5% NaCl; *group 4*, 4.5% NaCl. All groups started at time 0.

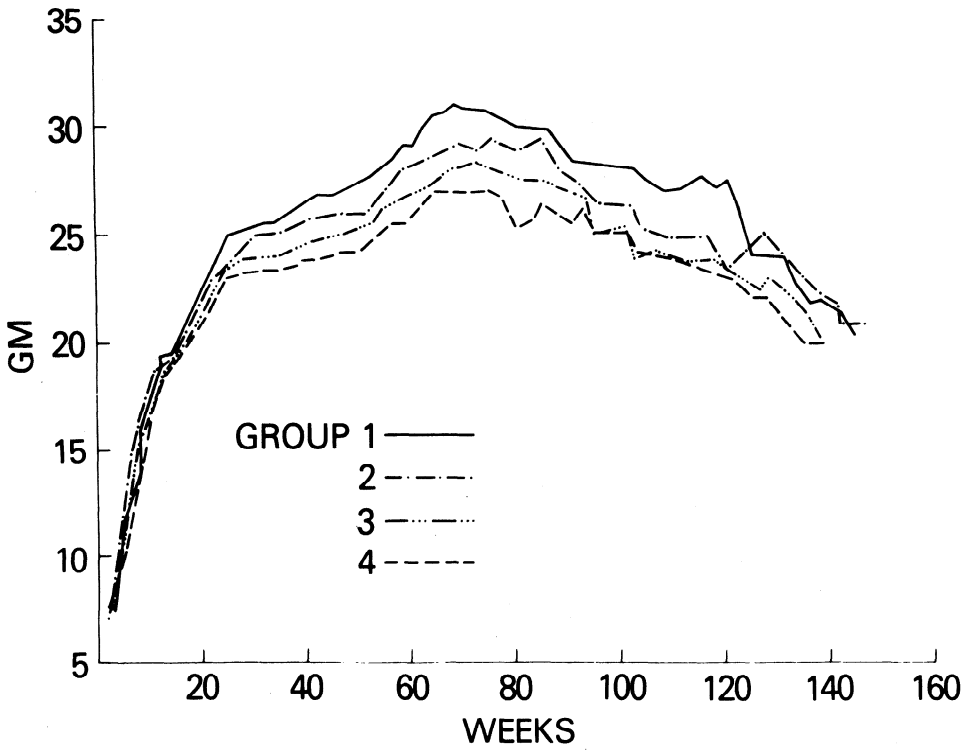

FIG. 2. Average weight of mice on 0.5, 1.5, 2.5, and 4.5% NaCl (*groups 1* to *4*, respectively) from 3 wk of age until death. Decline in weight in *groups 3* and *4* is significant (see Table 1).

TABLE 1. *Significance of weight loss in groups 2, 3, and 4 compared with control group 1*

Age, wk	Group 2		Group 3		Group 4	
	t	*P*	*t*	*P*	*t*	*P*
36	0.491	>0.60	0.653	>0.40	2.088	<0.05*
40	2.529	<0.025	1.089	>0.20	2.848	<0.025*
45	1.616	>0.10	2.082	<0.05*	3.647	<0.005*
50	1.180	>0.05	1.502	>0.10	3.524	<0.005*
54	1.165	>0.05	1.914	>0.05	3.602	<0.005*
58	1.430	>0.10	1.794	>0.05	3.398	<0.005*
62	1.959	>0.05	2.306	<0.02*	4.435	<0.001*
66	1.375	>0.10	1.744	>0.05	4.615	<0.001*
71	1.582	>0.10	2.151	<0.05*	5.173	<0.001*
75	1.473	>0.10	2.111	<0.05*	4.674	<0.001*
81	1.682	>0.10	2.840	<0.01*	6.142	<0.001*
86	0.810	>0.20	1.700	>0.10	4.035	<0.001*
91	1.341	>0.10	3.313	<0.005*	5.962	<0.001*
95	1.061	>0.10	2.902	<0.01	5.785	<0.001*
100	1.626	>0.10	3.243	<0.005*	4.582	<0.001*
105	1.732	>0.10	4.682	<0.001*	4.406	<0.001*
109	1.825	>0.05	4.204	<0.001*	4.356	<O.001
114	2.733	<0.05*	4.268	<0.001*	4.181	<0.001*
118	4.150	<0.01*	4.497	<0.001*	5.467	<0.001*
123	1.137	>0.10	1.864	>0.05	2.301	<0.010
127	0.905	>0.20	2.717	<0.02*	2.213	<0.5*
131	1.554	>0.10	3.789	<0.01*	1.823	>0.05
135	0.655	>0.50	0.646	>0.40	0.138	>0.20

An asterisk indicates significance.

Measurements were made on the A/J mice beginning 7 wk after the diet was started and they were continued at intervals of 2–3 wk for 47 wk. Five mice from each group were selected at each comparison, and no measurements on the same mouse were made for 1–2 mo. A total of 11 comparisons (55 mice from each group) were made, with *group 1* (0.5% dietary NaCl) serving as the control. Measurements were made without knowledge of the group being measured. Although there was a slight increase in swelling with increasing salt load, the differences were not significant (Table 2).

These experiments were discontinued after 8 mo because the procedure, which involved placing the mice in plastic cylinders for the application of negative pressure to the tail, caused a temporary lowering of food consumption, weight loss, and an occasional death from suffocation. Since the results were not significant, the experiments were discontinued in order not to influence the principal objective of this study.

It can be concluded that acute or chronic loading with NaCl does not bring about a significant increase in transvascular fluid movement as measured by the method employed.

The method is based on the local edema produced by a mild vacuum; the mechanisms by which this rate decreases with age are not known (changes in capillary permeability to crystalloids or decrease in the size of the capillary bed), but increasing the extracellular fluid volume by salt loading affects this rate only to a small extent as detectable by the method used. It is most likely that homeostatic mechanisms adequately control the rate of transvascular fluid movement under salt loading, as edema does not result in normal mice from any amount of NaCl.

Autopsies. Analysis of the data from gross autopsies showed no significant differences, except in splenomegaly, in the four groups. The numbers of autopsies were: *group 1*, 48 mice; *group 2*, 43 mice (one group of five was discarded because an undetected male had impregnated the others); *group 3*, 47 mice; and *group 4*, 46 mice.

The incidence of gross tumors was nine, five, six, and eight, respectively, for the four groups. Infection was present in eight, five, ten, and eight animals, respectively, in the four groups.

Renal pathology was seen in three, six, six, and five

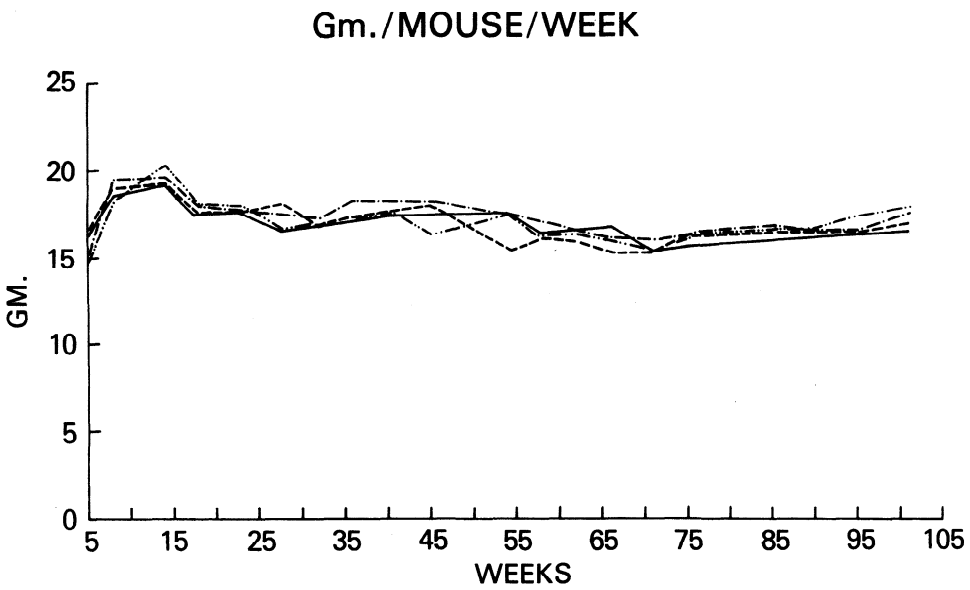

FIG. 3. Average food consumption in the 4 groups of mice. Symbols as in Fig. 1.

TABLE 2. *Transvascular fluid movement*

No. of Mice	0.85% NaCl	Average Increase in Tail Volume	
		%	SE
	Acute NaCl loading		
6	1 ml intraperitoneally	5.2	1.96 ($P > 0.3$)
6	0	5.7	3.06
6	1 ml orally	4.0	1.39 ($P > 0.4$)
6	0	7.0	1.02
6	3 ml orally	10.4	1.90 ($P > 0.2$)
6	0	8.2	1.48
	Dietary NaCl		
55	0.5% in diet (controls)	6.5	0.435
55	1.5%	6.8	0.481 ($P > 0.4$)
55	2.5%	7.5	0.572 ($P > 0.1$)
55	4.5%	7.7	0.828 ($P > 0.1$)

Measurement of transvascular fluid movement (swelling of the tail under decreased atmospheric pressure) in mice following a single dose of 0.85% NaCl and in mice with dietary NaCl loads. The latter results represent 11 comparisons of 5 mice from each group made over a period of 42 wk.

mice, respectively. Liver pathology, chiefly fatty liver, was found in five, three, one, and five mice in each group. Splenomegaly was seen in three, three, one, and zero in *groups 1* to *4* (*groups 1 + 2* versus *groups 3 + 4* SE difference = 2.64; $P = 0.01$). Exploration of this difference must await histologic examination.

DISCUSSION

The A/J strain of mice obtained from the Jackson Laboratory was chosen because of its relatively short life span. Russell (8) reported the mean longevity of 438 females at the Jackson Laboratory to be 62.6 wk [438 days ± 8.3 (SE)]. Other investigators (1, 10) reported life spans of 84.3 and 98.3 wk in female A/J mice. Our animals had a distinctly longer span of 110.5–116.1 wk.

Longevity may be accepted as an index of beneficial or deleterious effects upon the organism. However, an agent may be beneficial or deleterious, dependent on dosage and other factors, and longevity must be accepted as a summation of effects. As an example, if dietary NaCl is restricted below the minimum requirement for the animal or if it is administered in excessively high doses, a decrease in longevity can be expected. In the former case, salt addition would increase longevity. Torii (12) found extensive renal pathology in rats from NaCl loading at the 9.6% level, although no effect was observed on mortality during the period of observation. At the 4.8% dietary level, he reported increases in the size of the heart and kidneys but no pathology.

The largest dose in our experiments, 4.5% dietary NaCl in *group 4*, was calculated, on a basis of the weight of the animals in this group, to average 5.8 $g \cdot kg^{-1} \cdot day^{-1}$ from 20 to 80 wk, and 4.69 $g \cdot kg^{-1} \cdot day^{-1}$ from 81 to 101 wk.

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