Serum Sodium Concentration of Hypertensive Rats: Relation to NaCl Intake, Blood Pressure and Age.* (22139)

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Alterations of sodium metabolism have been studied intensively in both experimental and clinical hypertension. Among the deviations from normal are changes in total body sodium (1), extracellular fluid volume(2), sodium and water contents of aortic walls(3), as well as plasma and serum sodium concentration. With respect to the last, results have ranged from no apparent change in a very small number of experimentally hypertensive rats(1) to slight but definite increases in larger numbers of hypertensive rats(4) and of hypertensive patients(5). In addition, a relative aversion to NaCl, together with polydipsia and polyuria, has been observed in rats made hypertensive by bilateral renal encapsulation with latex envelopes(6-8). The mechanisms by which this relative NaCl aversion is associated with the induced perinephritis and experimental hypertension are still unclear. An elevated serum Na concentration in hypertensive rats might conceivably be one factor in this relative aversion.

With this possibility in view, the studies now reported were done to determine (a) whether serum Na concentration is increased in rats with established hypertension, (b) whether differences in serum Na concentration can be related to known differences in voluntary NaCl intake and/or to systolic blood pressure and (c) whether age at encapsulation affects serum Na concentration. It has been shown previously that age at encapsulation is one of the factors affecting NaCl aversion(8).

Methods. Male rats of the Sprague-Dawley strain, and of similar ages, were kept in individual cages in a thermoregulated room maintained at 25 ± 1°C, illuminated from 8 a.m. to 6 p.m. They were made hypertensive by enclosing both kidneys in latex rubber capsules(9). A group of 31 normal rats (series I) served as controls. In 23 rats (series II) renal encapsulations were done when they were 50 to 90 days old; in 14 rats (series III) when they were 126 to 165 days old. At least 3 months were allowed for development of hypertension(8). All rats were sacrificed when approximately 300 days old. Prior to sacrifice, *ad libitum* intakes of tap water, .15 M NaCl solution and Purina laboratory chow were measured in 27 rats for 5 days as described earlier(8). Blood pressure was measured by microphonic manometer(10) but without anesthesia(7). At the end of the 5th day the rats were lightly anesthetized by ether inhalation and their necks were carefully shaved. On the 7th day groups of normal and hypertensive rats were sacrificed by guillotine decapitation without anesthesia and 5 to 6 ml of blood were collected directly in test tubes by free flow from the neck vessels without anticoagulant. During collection, the shaved skin of the neck was retracted to prevent contamination of the collected blood by Na from skin or hair. The blood was centrifuged, the serum separated and analyzed for Na by a lithium internal standard Baird flame photometer model number DB2. Sera were diluted 1:100 (using pipettes whose delivery volume was individually checked) in order to provide concentrations within the range of maximum accuracy of the photometer (1.0 to 1.5 mEq./L). Dilutions were made with Na-free distilled water in 100 ml volumetric flasks at 22°C. Each serum sample was bracketed by at least 2 standard solutions, with one Na concentration above and one below the unknown. To determine the precision of the photometer and analytic procedure in general, several aqueous test solutions of NaCl were analyzed. Two were prepared gravimetrically from desiccated NaCl by one of us and analyzed as unknowns by another. Two com-

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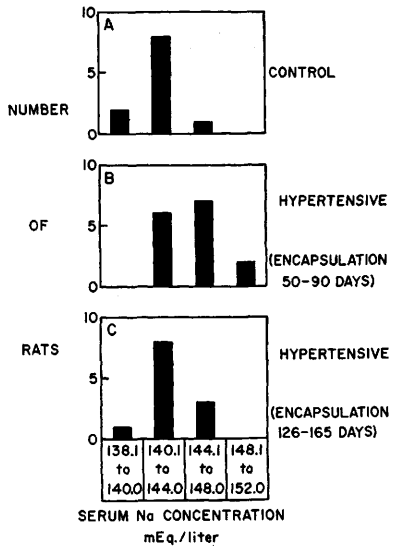

FIG. 1. Frequency distribution of serum Na concentrations (mEq./L) of control and hypertensive rats.

mercially prepared standard solutions‡ of highest accuracy were also analyzed. Typical of these tests are 3 analyses of standards containing 150.3, 100.0 and 5.0 mEq. Na/L which were diluted for analysis 1:100, 1:100, and 1:5 respectively to be in the range of the diluted serum Na. These analyses yielded, in duplicate determinations, final figures of 149.6 and 149.9, 100.0 and 100.0, 5.0 and 5.0 mEq./L respectively. Hence, if each unknown was bracketed by 2 standards, read at least twice and calculated from a separate calibration curve, the precision of the photometer in the range of serum Na concentration was ±0.5 mEq. L. This precision agrees with that reported by others(5). Fisher's test for significance of differences between means was used for statistical comparison of data from any 2 groups(11).

Results. Fig. 1 shows the range and distribution of serum Na concentrations observed in (A) control rats (B) hypertensive rats following encapsulation at 50 to 90 days of age and (C) hypertensive rats following encapsulation at 126 to 165 days of age. Serum Na concentrations tended to be slightly higher than normal in those rats that were made hypertensive by encapsulation while still relatively young (Fig. 1B). As shown in Table I this difference, in terms of mean serum Na concentration, was 2.1 mEq./L. and was statistically significant ($P < .01$). On the other hand, rats made hypertensive by renal encapsulation at 126 to 165 days of age had a mean serum Na concentration which was within the normal range (Fig. 1C and Table I).

The mean *ad libitum* intake of NaCl in the form of .15 M NaCl solution by normal rats in this study was 8.2 ± 1.9 ml/100 g B.W./day. For hypertensive rats encapsulated at 50 to 90 days of age the corresponding figure was 3.0 ± 0.8 ml/100 g B.W./day, showing a highly significant reduction ($P < .01$) in agreement with a number of earlier comparisons(6-8). It is tempting to consider the lower NaCl intake simply a direct effect of the higher serum Na concentration but such an inference is not justifiable. Figure 2 shows the individual intakes of .15 M NaCl solution plotted against serum Na concentrations of the corresponding rats (closed circles for controls, crosses for hypertensive rats). The linear correlation in this plot based on individual rats is not good ($r = -.45$, $P < .05$). Hyperbolic, parabolic, logarithmic and exponential functions did not fit the data any better than the simple linear regression equation. Thus, as a group, rats encapsulated at 50 to 90 days of age presented a significant NaCl aversion together with a slight but significant increase in serum Na concentration. Yet in these same rats, considered singly, no close relationship existed between individual serum Na concentrations and their individual NaCl intakes (Fig. 2).

Mean systolic arterial blood pressures in the 3 groups were 138 (control, Series I), 188 (Series II) and 206 (Series III) mm. Hg respectively. The last two values were significantly higher than the control value ($P < .01$), but did not differ significantly

‡ We are grateful to Mr. Sage and Mr. Lightfoot of Baird Associates, Cambridge, for a sample of their reference standard solution. The second solution was a standard for flame photometry and spectrophotometry prepared by Hartmann Leddon Co., Philadelphia, Pa.

TABLE I. Effect of Age at Encapsulation on Serum Na Concentration and Blood Pressure of Normal and Hypertensive Rats.

	No. of series	Mean age at encapsulation (days)	Mean post-operative age (days)	Mean age at sacrifice (days)	No. of rats	Serum Na conc. (mEq./L)*	P	Systolic blood pres. (mm Hg)	P
Control	I	—	—	350 (268-442)‡	31	143.0 ± .4†		138 ± 2	
Hypertensive	II	63 (50- 90)	246 (160-330)	309 (280-380)	23	145.1 ± .6	<.01	188 ± 7	<.01
	III	150 (126-165)	140 (104-176)	290 (268-345)	14	142.6 ± .7	<.01	206 ± 11	.20

* Each datum can be measured with an accuracy of ± .5 mEq./liter but can be read from a calibration curve with a precision of ± .1 mEq./liter. For purposes of statistical computation the data are tabulated here with 1 more figure than is significant, and the final figure for the mean should be rounded off.

† ± stand. error of the mean.

‡ Range.

from each other (P = .20) and by this criterion the rats of series II and III were equally hypertensive. Yet serum Na concentrations were elevated in one group and normal in the other (Fig. 1, Table I). In both groups plots of individual serum Na concentrations and individual blood pressures (not shown) revealed no close correlation.

Since all groups of rats were sacrificed at approximately the same age, it is evident that those whose kidneys were encapsulated when young were hypertensive somewhat longer than those whose kidneys were encapsulated later in life. Inasmuch as the young group was the one with elevated mean serum Na concentration, a possible relation between duration of hypertension and serum Na concentration was sought (Fig. 3). Earlier observations have shown that blood pressure is maximally elevated in both old and young rats within 70 days after bilateral latex encapsulation of the kidneys and that the earliest rise is detectable within 14 days(8). Therefore, it was possible to express approximately the duration of hypertension by the number of days between the time of operation and the time of sacrifice for both hypertensive groups (Fig. 3). No close correlation could, however, be established between serum Na concentration and the duration of hypertension.

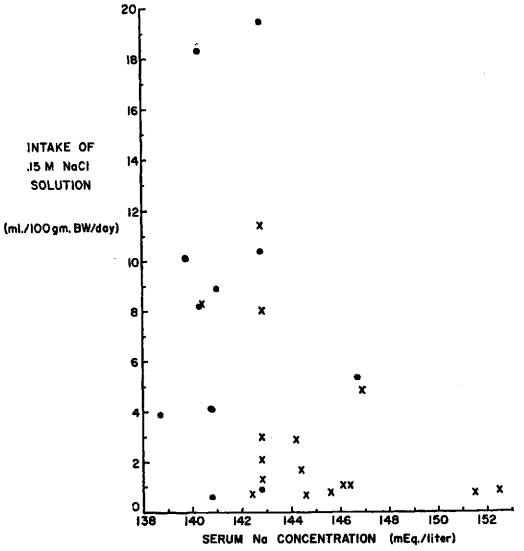

FIG. 2. Individual intakes of .15 M NaCl solution plotted against individual serum Na concentrations of control rats (dot) and rats made hypertensive while young (×).

Discussion. The slight, but significant, elevation of serum Na concentration in rats made hypertensive when young parallels the findings of Holley *et al.*(5) in human essential hypertension and also those of Laramore and Grollman(4) in experimental hypertension in rats with figure of eight ligatures around the kidneys. In the 2 earlier studies, however, age at onset of hypertension and duration of hypertension were not considered with reference to serum Na concentration.

In the present observations, rats whose kidneys were encapsulated when young (50 to 90 days) had a slightly higher mean serum Na concentration while those whose kidneys were encapsulated when older (126 to 165 days) presented a normal distribution of

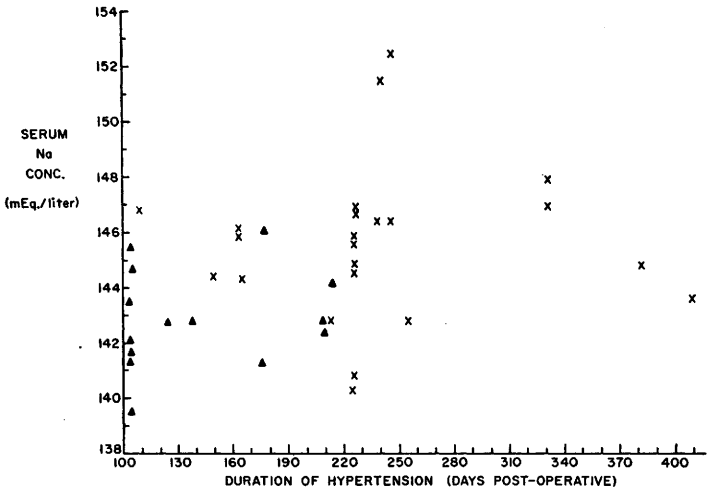

FIG. 3. Serum Na concentration plotted against duration of hypertension for individual rats aged 50 to 90 days (×) and 126 to 165 days (▲) at encapsulation.

serum Na concentrations. A previous study (8) showed that renal encapsulation of younger rats also led to earlier and more definite sodium aversion than did encapsulation later in life. Yet the grade of hypertension was similar with early and late encapsulation. In terms of individual rats, the study reported here has revealed that serum Na concentrations could not be related consistently to the grade of Na aversion, to height of blood pressure, or to duration of hypertension.

It appears, therefore, once more that detailed studies support previous inferences that certain general changes in Na metabolism occur in association with the experimentally hypertensive state under some conditions. Yet a close relationship is not established and further studies are needed to explore possible mechanisms by which the two may conceivably be linked.

Summary. Encapsulating both kidneys of young rats (50 to 90 days) in molded latex envelopes produced hypertension and elevated their mean serum Na concentration slightly (2 mEq./L.) but significantly above that of control rats. The same procedure in older rats (126 to 165 days) produced similar hypertension but mean serum Na concentration remained within normal limits.

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