EFFECTS OF CHRONIC EXCESS SALT FEEDING

Induction of Self-Sustaining Hypertension in Rats

By LEWIS K. DAHL, M.D.

(From the Medical Research Center, Brookhaven National Laboratory, Upton, New York)

(Received for publication, April 10, 1961)

Only about one-fourth to one-third of patients with essential hypertension respond with a significant drop in blood pressure to rigorous restriction of dietary salt (1–8). The lack of response in the majority has been taken to indicate that the hypertension probably was not caused by excessive salt intake, although salt has been conceded as being a possible etiologic factor in the minority who do respond to withdrawal (9). This paper summarizes some experimental evidence which suggests that a lack of therapeutic response to restriction of dietary salt does not rule out an etiologic relationship between excessive intake and the initial development of hypertension.

Experimental

The data in this paper were derived from a study of 35 female rats that became hypertensive [systolic blood pressure of 140 mm Hg or higher (10, 11)] during a year of continuous excess salt feeding. Initially this study comprised 76 animals; the present report, however, includes only the animals that appeared to be in good health at the end of the feeding period. Since both morbidity and mortality rates were highest among the animals that developed severe hypertension, many of the animals with the highest blood pressures were thus excluded.

All animals were Sprague-Dawley females received as 3-week-old weanlings; they were maintained without change for the next 12 to 13 months on one of two sodium-containing diets. The basic diet was Ralston Purina fox chow pellets, without added sodium chloride, containing from 0.5 to 0.75 per cent NaCl and 0.9 per cent potassium by our analyses. To this basic diet, one of two sodium-containing salts had been added by the manufacturer according to our specifications so that the final concentration of NaCl approximated 8 per cent by weight. In one of these diets, the supplementary salt was chemically pure sodium chloride, whereas in the other it was sea salt, obtained by evaporating sea water.[1] Our analyses indicated that the "8 per cent NaCl food" contained an average of 8.1 per cent NaCl whereas the "11.6 per cent sea salt food" contained 7.3 per cent NaCl. Potassium concentration in these two foods was similar, namely 0.90 to 0.95 per cent. The reasons for using sea salt to induce hypertension have been presented elsewhere (11). All animals had free access to food and tap water.

After completion of the salt-feeding regimens, all animals were placed on a sodium-deficient

[1] The authors are indebted to the Trace Elements Corporation, Houston, for sufficient sea salt (Admiral brand trace element sea salt) to initiate these experiments.

TABLE I

Effect of Sodium Restriction on Blood Pressures of Hypertensive Rats

Diet	Rat No.	Before salt restriction		After salt restriction		
		B.P.*	BUN‡	B.P.§		BUN§
		mm Hg	*mg per cent*	*mm Hg*	*per cent*‖	*mg per cent*
8 per cent NaCl chow	533	143	12	**132**¶	92	23
	535	155	15	172	111	27
	536	150	23	142	95	26
	539	150	13	150	100	23
	541	173	11	206	119	21
	542	157	17	142	90	**33****
	543	146	15	**130**	89	21
	546	155	14	**128**	83	25
	550	151	14	214	142	23
	551	153	21	146	95	19
	555	178	14	182	102	22
	574	150	13	151	100	18
	576	175	9	172	98	14
	610	179	19	160	89	15
11.6 per cent sea salt chow	502	153	14	**128**	84	17
	503	169	16	**134**	79	25
	504	152	12	172	113	20
	505	172	16	204	119	17
	507	182	10	172	94	20
	517	150	12	**134**	89	15
	518	170	14	154	91	27
	519	169	14	144	85	18
	520	157	18	192	122	Not done
	521	178	17	195	110	**31**
	522	193	11	204	106	**37**
	523	165	13	154	93	18
	524	183	13	140	77	18
	525	190	15	144	76	17
	526	164	19	154	93	24
	529	194	13	172	89	22
	532	167	12	182	109	22
	569	148	12	163	110	20
	615	166	18	142	85	18
	616	157	19	**136**	87	24
	617	189	20	190	100	18
Mean (and S.D.)		165.2 (±14.6)	14.8 (±3.3)	161.1 (±25.3)	97.6 (±14.7)	21.7 (±5.2)

By Student's t test, the change in mean blood pressure for the entire group of animals after NaCl restriction was not significant ($t = 0.83$, $p > 0.4$) whereas the increase in BUN concentration was significantly higher ($t = 6.98$, $p < 0.01$), than before restriction.

* Average of last 2 monthly systolic blood pressure measurements (approximately 12 and 13 months after salt feeding started).

‡ Blood urea nitrogen concentration, determined at end of salt feeding.

§ After 4 months on low salt diet.

‖ Per cent of asterisk (*).

¶ Indicates "normal" blood pressure (< 140 mm Hg).

** Probably increased concentration (exceeds 2 standard deviations of mean value in control colony).

diet[2] (averaging approximately 0.025 per cent NaCl by our analyses) for 2 months followed by a second period of 2 months during which they received the basic diet chow (0.5 to 0.75 per cent NaCl). Hence, excess dietary sodium was omitted for a total of 4 months. While we have had no previous experience with the sodium-deficient diet, in 3 years of similar investigations, we have not had a single rat become hypertensive among more than one-hundred reared solely on the basic chow. The animals were observed for 4 months in the absence of added dietary salt, a period ordinarily long enough to induce a fall in blood pressure in the majority of people with "essential" hypertension who will respond to salt limitation (1–8). It was therefore assumed that a continued elevation of pressure after this period of restriction indicated a self-sustaining hypertension.

Systolic blood pressures were measured by the microphonic technique of Friedman and Freed (12), as modified by us (10). During the 4 month period when the animals were on the sodium-restricted diets, blood pressure measurements were made at the end of 1, 2, and 4

TABLE II

Group	No. with hypertension	
	Before*	After*
8 per cent NaCl	14	11 (79 per cent)‡
11.6 per cent sea salt	21	17 (81 per cent)
Both groups	35	28 (80 per cent)

Changes in systolic blood pressure among 35 female rats which were hypertensive (systolic blood pressure at least 140 mm Hg) after 12 to 13 months of excess salt feeding. 28 (80 per cent) remained hypertensive after the 4 month period of sodium restriction.

* Salt restriction.

‡ Per cent of original hypertensive animals.

months. These pressures were compared with the average of the readings obtained during the last 2 months of the high salt period. Every value represented the average of at least 4 readings, the variations among which were insignificant. The animals were weighed at the time blood pressure was measured, and urea nitrogen was determined using Conway's micro-diffusion technique (13) on blood obtained by nicking the tail.

RESULTS

Blood Pressure.—The mean systolic pressure of the hypertensive rats was not significantly changed ($t = 0.83$, $p > 0.4$) by sodium restriction; the average pressure was only 2.4 per cent below the prerestriction level (Table I). How-

[2] According to the manufacturer (Nutritional Biochemicals Corp., Cleveland) this diet had the following composition:

	per cent
Sucrose	72
"Vitamin-free" casein	18
Butter fat (salt-free)	5
Sodium-free salt mixture vitamin supplement	5

ever, significant reductions of pressure were observed in some individual cases; seven of the hypertensive animals became normotensive (systolic pressure below 140 mm Hg) (Table II), and a number of others had lower pressures at the end of the treatment period (Table III). Classifying the blood pressures equal to or greater than 90 per cent of prerestriction values as *no decline*, those from 80 to 89 per cent as *moderate decline* and those below 80 per cent as *marked decline*, we find (Table III) that about one-third, 12 of the 35 animals, had a moderate or marked decline in blood pressure and two-thirds, no decline. In six animals the blood pressure increased during the period of sodium restriction; their final readings were from 111 to 142 per cent of the respective prerestriction averages.

Blood Urea Nitrogen.—The data in Table I show normal blood urea nitrogen levels in all animals at the end of the salt-feeding period. With restriction of

TABLE III

Per Cent Change in Systolic Blood Pressure among Hypertensive Rats after 4 Months on Sodium-Restricted Diets

Group	No. in group	Decline in B.P. (per cent of prerestriction level)		
		No decline (90 per cent and above)	Moderate decline (80–89 per cent)	Marked decline (<80 per cent)
8 per cent NaCl	14	11 (79 per cent)*	3 (21 per cent)	0
11.6 per cent sea salt	21	12 (57 per cent)	6 (29 per cent)	3 (14 per cent)
Both groups	35	23 (66 per cent)	9 (26 per cent)	3 (9 per cent)

* Per cent of group.

sodium the mean concentration of the group increased significantly ($t = 6.98$, $p < 0.01$) and in 3 cases rose above normal limits. There was no correlation between blood pressure and concentration of blood urea nitrogen.

Weight.—The mean weight of the group increased from 276 (±20.1) to 310 (±28.2) gm during the 4 month period of sodium limitation. This was a significant increase ($t = 5.80$, $p < 0.01$) for the group. However, there was no correlation between changes in weight and changes in blood pressure. There was no evidence of fluid retention or of abnormal obesity (14). Since the observed mean weights were similar to those of comparable animals in the control colony, it seems probable that the weight changes represented continued normal growth.

DISCUSSION

In the present investigations the elimination of excess dietary sodium failed to cause a significant decline in blood pressure in about two-thirds of the hypertensive animals. Salt-feeding, therefore, can produce a self-sustaining hypertension refractory to treatment with low sodium diet.

These results are in accord with other studies in which hypertension, induced

by different means, continued after the original exciting cause was removed. Grollman, Harrison, and Williams (15), Grollman (16), and Pickering (17) observed that experimental renal hypertension of long standing frequently would not become reversed after removal of a single constricted kidney. Friedman and his associates (18, 19) and Green *et al.* (20) produced a self-sustained hypertension in rats by administration of deoxycorticosterone acetate and salt. In humans, hypertension sometimes develops after unilateral kidney involvement, but removal of the affected kidney may fail to diminish the blood pressure (21, 22), particularly if the condition has been of relatively long duration.

The increase of mean blood urea nitrogen concentration observed in these animals after removal of excess dietary salts appeared to be associated with reduction of fluid intake and consequent reduction of urine volume. The continuous diuresis during salt feeding presumably increased urea clearance; with elimination of the excess salt, the fluid intakes decreased and the BUN returned to higher, but usually normal, levels. The blood urea nitrogen of control animals not given salt at any time was the same as that of the salt-fed animals after elimination of the excess dietary salt.

SUMMARY

Hypertension was induced in female rats by chronic feeding of sodium-containing salts in excess. The hypertension so induced appeared to be self-sustaining since about two-thirds of the animals failed to show a significant fall in blood pressure after withdrawal of these salts from the diet.

Under the conditions of these experiments a lack of response to restriction of dietary sodium does not exclude an etiologic relationship between salt intake and development of hypertension.

The author continues to be grateful to Mrs. Lorraine Tassinari and Miss Martha Heine for their loyal technical assistance with this work.

Addendum. While this manuscript was in preparation, Professor Charles E. Hall of the University of Texas, Medical Branch, Galveston, informed me that he also had induced self-sustaining hypertension in rats by excess salt consumption.

BIBLIOGRAPHY

1. Grollman, A., Harrison, T. R., Mason, M. F., Baxter, J., Crampton, J., and Reichsman, F., Sodium restriction in the diet for hypertension, *J. Am. Med. Assn.*, 1945, **129**, 533.
2. Kert, M. J., Rosenburg, M. J., Coodley, E. L., Murdock, L. J., Hoffman, S. H., Brotman, E. J., and Johnston, W. L., Treatment of hypertension. Experiences with the use of a low sodium diet other than the rice diet: a preliminary report. *J. Am. Med. Assn.*, 1950, **143**, 721.
3. Dole, V. P., Dahl, L. K., Cotzias, G. C., Eder, H. A., and Krebs, M. D., Dietary treatment of hypertension. Clinical and metabolic studies of patients on the rice-fruit diet, *J. Clin. Inv.*, 1950, **29**, 1189.

4. Watkin, D. M., Froeb, H. F., Hatch, F. T., and Gutman, A. B., Effects of diet in essential hypertension, *Am. J. Med.*, 1950, **9,** 428.
5. Corcoran, A. C., Taylor, R. D., and Page, I. H., Controlled observations on the effect of low sodium dietotherapy in essential hypertension, *Circulation*, 1951, **3,** 1.
6. Dole, V. P., Dahl, L. K., Cotzias, G. C., Dziewiatkowski, D. D., and Harris, C., Dietary treatment of hypertension. II. Sodium depletion as related to the therapeutic effect, *J. Clin. Inv.*, 1951, **30,** 584.
7. Dahl, L. K., Stall, B. G., and Cotzias, G. C., Metabolic effects of marked sodium restriction in hypertensive patients: changes in total exchangeable sodium and potassium, *J. Clin. Inv.* 1954, **33,** 1397.
8. Dahl, L. K., Salt intake and salt need, *New England J. Med.* 1958, **258,** 1152, 1205.
9. Corcoran, A. C., Clinical connotation of the experimental hypertensions, *Canad. Med. Assn. J.*, 1959, **81,** 145.
10. Dahl, L. K., Effects of chronic excess salt feeding. Elevation of plasma cholesterol in rats and dogs, *J. Exp. Med.*, 1960, **112,** 635.
11. Dahl, L. K., and Heine, M., Effects of chronic excess salt feeding. Enhanced hypertensogenic effect of sea salt over sodium chloride, *J. Exp. Med.*, 1961, **113,** 1067.
12. Friedman, M., and Freed, S. C., Microphonic manometer for the indirect determination of systolic blood pressure in the rat, *Proc. Soc. Exp. Biol. and Med.*, 1949, **70,** 670.
13. Conway, E. J., Microdiffusion Analysis and Volumetric Error, London, Crosby Lockwood and Son, Ltd., 3rd revised edition, 1950, 152.
14. Meneely, G. R., and Ball, C. O. T., Experimental epidemiology of chronic sodium chloride toxicity and the protective effect of potassium chloride, *Am. J. Med.*, 1958, **25,** 713.
15. Grollman, A., Harrison, T. R., and Williams, J. R., The mechanism of experimental renal hypertension in the rat; the relative significance of pressor and antipressor factors, *Am. J. Physiol.*, 1943, **139,** 293.
16. Grollman, A., Experimental chronic hypertension in the rabbit, *Am. J. Physiol.*, 1944, **142,** 666.
17. Pickering, G. W., Role of the kidney in acute and chronic hypertension following renal artery constriction in the rabbit, *Clin. Sc.*, 1945, **5,** 229.
18. Friedman, S. M., and Friedman, C. L., Self-sustained hypertension in the albino rat: a hypothesis to explain it, *Canad. Med. Assn. J.*, 1949, **61,** 596.
19. Friedman, S. M., Friedman, C. L., and Nakashima, M., Sustained hypertension following the administration of desoxycorticosterone acetate, *J. Exp. Med.*, 1951, **93,** 361.
20. Green, D. M., Saunders, F. J., Wahlgren, N., and Craig, R. L., Self-sustaining, post-DCA hypertensive cardiovascular disease, *Am. J. Physiol.*, 1952, **170,** 94.
21. Smith, H. W., Hypertension and urologic disease, *Am. J. Med.*, 1948, **4,** 724.
22. Page, I. H., Dustan, H. P., and Poutasse, E. F., Mechanisms, diagnosis and treatment of hypertension of renal vascular origin, *Ann. Int. Med.*, 1959, **51,** 196.