

incubation period of the kidney cell cultures during vaccine production and specific variations in the manufacturing process may also be contributing factors.

Preliminary results(9) of studies on antibody to SV₄₀ in normal monkey sera show that rhesus monkeys were infected with SV₄₀ as far back as 1956. This suggests that kidney cell cultures and hence Salk poliomyelitis vaccine may have contained the virus in viable form. The finding(1) that a high percentage of persons following injection of 2 doses of poliomyelitis or adenovirus vaccine developed relatively high antibody titers to SV₄₀, prompted Sweet and Hilleman to comment on the apparent high potency of SV₄₀ antigen in these vaccine preparations. In retrospect, therefore, one can assume that large groups of the population in this country and abroad must have been injected with varying amounts of SV₄₀ during the course of immunization with formalinized poliomyelitis or adenovirus vaccine. Careful clinical studies on selected groups of vaccinees suggest that no harmful effect can be attributed to vacuolating virus within the limits of the observation period. Furthermore, oral† or intranasal(10) administration of SV₄₀ in man results in viral multiplication without causing illness. While short term studies of the pathogenicity of SV₄₀ in laboratory animals

have been reported as negative(1), the long term effect in newborn animals remains to be determined.

Summary. Inactivation of SV₄₀ with formaldehyde followed a biphasic curve with a levelling off after about 40 hours at 37°C leaving a residual fraction of virus which was resistant to further inactivation for the remainder of the 14-day observation period. Viable SV₄₀ was detected in some lots of formalinized poliomyelitis and adenovirus vaccines.

1. Sweet, B. H., Hilleman, M. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1960, v105, 420.
2. Melnick, J. L., *Diagnostic Procedures for Virus and Rickettsial Diseases*, Am. Pub. Hlth. Assn., N. Y., 2nd ed., 1956, 97.
3. Morgan, J. F., Morton, H. J., Parker, R. C., *PROC. SOC. EXP. BIOL. AND MED.*, 1950, v73, 1.
4. Baron, S., *ibid.*, 1957, v95, 760.
5. Böttiger, M., Lycke, E., Melen, B., Wrangle, G., *Arch. Ges. Virusforsch.*, 1958, v8, 267.
6. Hull, R. N., Minner, J. R., Smith, J. W., *Am. J. Hyg.*, 1956, v63, 204.
7. Hull, R. N., Minner, J. R., Mascoli, C. C., *ibid.*, 1958, v68, 31.
8. Heicken, K., Spicher, G., *Zbl. Bakt. Orig. Abt., I.*, 1956, v167, 97.
9. Gerber, P., unpublished data.
10. Morris, J. A., Johnson, K. M., Aulisio, C. G., Channock, R. M., Knight, V., *Fed. Proc.*, 1961, v20, 436.

† Melnick, J. L., personal communication.

Received July 14, 1961. P.S.E.B.M., 1961, v108.

Plasma and Tissue Electrolytes of Rats Given Excess Sodium Chloride in Food.* (26893)

ARTHUR S. HAIGHT AND JOHN M. WELLER

Department of Internal Medicine, The University of Michigan Medical School, Ann Arbor

Elevated blood pressure and changes in electrolytes have been reported in rats given increased quantities of sodium chloride(1). The present study further characterizes the distribution of sodium, potassium, and water in plasma and tissues of rats ingesting excess sodium chloride.

* This work was supported in part by grants from Michigan Heart Assn. and American Heart Assn.

Methods and materials. Sodium chloride was administered as 2.8, 5.6 and 8.4% of a synthetic diet(2) to 3 respective groups totaling 35 rats. Rats were Sprague-Dawley strain males, each weighing 160 to 235 g at onset of the experiment. Tap water was provided *ad libitum*. Blood pressures were determined by the tail-cuff method(3) under light ether anesthesia and body weights re-

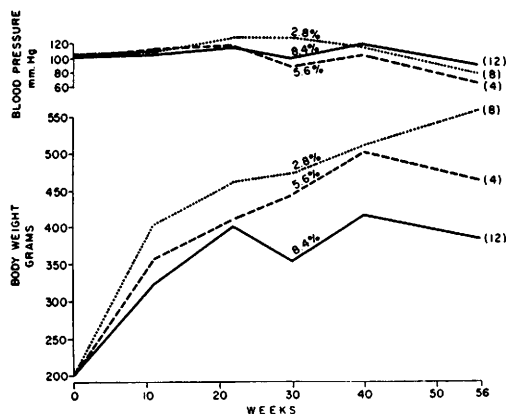


FIG. 1. Changes with time of mean systolic blood pressure (at top) and mean body wt (at bottom) of 3 groups of rats given respectively 2.8, 5.6 and 8.4% NaCl in diet. No. of rats in groups shown in parentheses.

corded every 10 weeks. After 56 weeks on diets rats were stunned and exsanguinated. Gastrocnemius muscle, ventricular heart muscle and aorta (thoracic and abdominal) were removed, immediately dissected free of gross fat and connective tissue and placed in stoppered vessels. Tissues were analyzed by the method of Lowry and Hastings(4), except sodium and potassium were determined by flame photometry. Calculations of extracellular and cellular water phases were based upon extracellular location of chloride.

Carcasses were dried at 105°C for 5-7 days, then ground until they could be sifted through No. 16 mesh. Duplicate samples were subjected to 2 cold petrol ether extractions; differences in dry weight before and after were recorded as neutral fat content. Decanted ether was returned to each vessel containing carcass aliquot, evaporated to dryness and electrolytes extracted with 0.75 N HNO₃ for 24 hours. Before analysis of sodium and potassium by flame photometry, calcium was removed by a modification of Bergstrom's technic(5).

Results. Fig. 1 shows changes in body weights and blood pressures. Failure to gain weight correlated with increasing quantities of sodium chloride in the diet. Compared to rats on 2.8% sodium chloride, fluid intake was increased 2½ times in rats on 8.4% and about 1½ times in rats on 5.6%. Food intake did not differ between groups. No sig-

nificant elevation of mean blood pressure occurred in any of the 3 groups nor was there any significant elevation of blood pressure of any individual rat. Eight of 10 rats ingesting 2.8%, 4 of 11 ingesting 5.6%, and 12 of 14 ingesting 8.4% sodium chloride in food survived 56 weeks. Low survival rate of animals ingesting 5.6% sodium chloride was attributed to a respiratory infection during the 30th week.

Water and electrolyte values are shown in Table I. With increasing amounts of sodium chloride in the diet, changes in total body potassium did not occur. Total body sodium in rats on 8.4% sodium chloride showed an insignificant increase compared with those on 2.8%. Alterations of potassium and chloride levels of plasma and changes in heart and skeletal muscle electrolytes were not statistically significant. In contrast, aorta showed statistically significant changes in rats ingesting 8.4% sodium chloride compared with those on 2.8%. These consisted of increases in sodium, potassium and chloride, and a decrease in water contents.

Discussion. Increases of total exchangeable sodium in rats on 8.4% sodium chloride and increases in total body sodium and total body potassium in rats on diets high in sodium chloride have been reported(1). In the present study with increased quantities of dietary sodium chloride, total body sodium and potassium were not significantly changed.

Slight increases were reported in sodium and potassium contents of heart and skeletal muscle of rats fed diets containing 5.0% sodium chloride(6). Sodium contents of heart and skeletal muscle increased in rats fed diets containing 10% sodium chloride and either 0.7% or 5.6% potassium chloride(7). No statistically significant changes in heart and skeletal muscle electrolytes were found in the present study.

Increases of sodium, potassium, chloride or water contents of aorta have been noted in rats made hypertensive by a variety of methods, but increased aorta potassium has been most consistent(8). Conversely, decreased potassium content of aorta has been associated with decreased blood pressure incident to reserpine administration to rats previously

TABLE I. Water and Electrolyte Contents of Skeletal Muscle, Heart Muscle, Aorta, Plasma, and Whole Animal.

	Plasma			Whole animal	
	Na	K	Cl	Na	K
2.8% NaCl (8)	146 ± .8	5.0 ± .6	103 ± 1	49 ± .6	47 ± 1
5.6% " (4)	145 ± .7	4.0 ± .2	108 ± 2	45 ± .5	47 ± 1
8.4% " (12)	148 ± 1	3.8 ± .2	100 ± .9	52 ± 2	48 ± 1

	Na	K	Cl	(H ₂ O)t	(H ₂ O)e	(H ₂ O)c
Skeletal muscle						
2.8% NaCl (8)	42 ± 1	112 ± 3	12 ± .5	790 ± 8	108 ± 4	681 ± 11
5.6% " (4)	41 ± 1	108 ± 2	12 ± .6	770 ± 5	95 ± 6	676 ± 8
8.4% " (12)	38 ± 2	110 ± .8	12 ± .4	786 ± 6	104 ± 3	681 ± 8
Heart muscle						
2.8% NaCl (8)	56 ± 2	85 ± 1	24 ± 1	804 ± 7	213 ± 12	590 ± 11
5.6% " (4)	52 ± 1	80 ± 2	24 ± 1	770 ± 6	192 ± 9	578 ± 14
8.4% " (12)	54 ± .4	83 ± .8	24 ± 1	785 ± 6	216 ± 11	572 ± 18
Aorta						
2.8% NaCl (8)	115 ± 12	42 ± 3	81 ± 11	651 ± 10		
5.6% " (4)	126 ± 14	48 ± 5	80 ± 12	632 ± 20		
8.4% " (12)	148 ± 7*	52 ± 2*	108 ± 6*	603 ± 9*		

Electrolyte values are expressed as meq/l plasma; meq/kg fat-free wet skeletal muscle, heart muscle, and aorta; and meq/kg fat-free dry whole animal. Water content expressed as g/kg fat-free wet tissue with t = total tissue water, e = extracellular water, and c = cell water. No. of animals analyzed in parentheses.

± stand. error of mean.

* Statistically significant ($p < .02$) from 2.8% NaCl group.

made hypertensive(9). In our study, increases in sodium, potassium, and chloride contents of aorta were associated with increasing quantities of ingested sodium chloride and not with elevation in blood pressure. This finding of increased sodium, potassium, and chloride in aorta of normotensive rats fed excessive sodium chloride, and in aorta of experimentally hypertensive rats, suggests the presence of a common pathogenetic element other than blood pressure level.

Summary. Rats were fed diets containing excess sodium chloride and examined for changes in water and electrolyte contents. Blood pressure elevation did not occur even after 56 weeks, but body weight varied inversely with the quantity of sodium chloride in the diet. Significant water and electrolyte changes were an increase in sodium, po-

tassium and chloride and a decrease in water content of aorta.

1. Meneely, G. R., Ball, C. O. T., *Am. J. Med.*, 1958, v25, 713.
2. Hubbell, R. B., Mendel, L. B., Wakeman, A. J., *J. Nutrition*, 1937, v14, 273.
3. Friedman, M., Freed, C. F., *PROC. SOC. EXP. BIOL. AND MED.*, 1949, v70, 670.
4. Lowry, O. H., Hastings, A. B., *J. Biol. Chem.*, 1942, v144, 637.
5. Bergstrom, W. H., Wallace, W. M., *J. Clin. Invest.*, 1954, v33, 867.
6. Meyer, J. H., Grunert, R. R., Zepplin, M. T., Grummer, R. H., Bohstedt, G., Phillips, P. H., *Am. J. Physiol.*, 1950, v162, 182.
7. Lemley-Stone, J., Darby, W. J., Meneely, G. R., *Fed. Proc.*, 1957, v16, 78.
8. Tobian, L., *Physiol. Rev.*, 1960, v40, 280.
9. Tobian, L., Redleaf, P. D., *Am. J. Physiol.*, 1958, v192, 325.

Received July 24, 1961. P.S.E.B.M., 1961, v108.