PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 170, 15–18 (1982)

# Sodium and Potassium Chloride Preferences in Pyridoxine-Deficient Rats (41388)

CHARLES N. STEWART* AND HEMMIGE N. BHAGAVAN†

*Department of Psychology, Franklin and Marshall College, Lancaster, Pennsylvania 17604, and †Department of Clinical Nutrition, Hoffman-La Roche Inc., Nutley, New Jersey 07110

*Abstract.* Taste preferences for NaCl and KCl were studied in rats reared from weaning on pyridoxine-deficient or control diets. After 30 days on the diet, the deficient rats showed a markedly enhanced preference for KCl (80.1 vs 32.4%). But there was no significant increase in NaCl preference (85.9 vs 77.6%) which was already high in control rats. The increased KCl preference, along with previous reports of enhanced NaCl preference, reduced sodium secretion, and adrenal hypertrophy is suggestive of possible hyperaldosteronism in pyridoxine deficiency.

The most fully documented instance of a single vitamin deficiency producing alterations in taste sensitivity is that of vitamin A (1) where the loss of taste sensitivity has been associated with increased keratinization of the lingual papillae and adjacent structures. Further work (2) indicated that the retinoic acid derivative of vitamin A was sufficient to maintain the functional integrity of the lingual epithelium by preventing keratinization. Of the water soluble vitamins, only pyridoxine (vitamin $B_6$) deficiency has systematically been studied with reference to taste (3). Vitamin $B_6$-deficient rats displayed an increased preference for both 0.1 and 0.3 *M* NaCl, but alterations in their preference for sodium saccharin, quinine sulfate, or HCl was not observed at any of the concentrations used. The $B_6$-deficient animals had normal plasma levels of both Na and Zn and reduced urinary excretion of Na along with a significant hypertrophy of the adrenal glands. The threshold for tympanic nerve response to NaCl was not altered by the $B_6$ deficiency (4). Consistent with the enhanced NaCl preference in pyridoxine deficiency is the earlier report by Kare and Henkin (5) that D-penicillamine also produces a significant increase in NaCl preference. Penicillamine, a vitamin $B_6$ antagonist, inactivates pyridoxal phosphate by forming the thiazolidine derivative (6).

The present investigation reports the effect of $B_6$ deficiency on preference for both sodium and potassium chloride solutions using prolonged preference tests which allow the animals ample opportunity to experience the postingestional consequences of ingesting a particular substance. If as has been suggested by Hsu *et al.* (7), adequate $B_6$ intake is essential for the maintenance of Na and K balance in rats, then there is good reason to expect alterations in K as well as Na preference in the deficient animals.

**Materials and Methods.** Weanling male rats[2] (CD) (49–61 g) were housed individually in stainless steel cages at constant temperature (21–22°) under a 12-hr light/dark cycle. From the day of weaning (21 days) animals were fed either a $B_6$-deficient (*N* =

[1] The paper was written during the senior author's (C.N.S.) tenure as Visiting Scientist at the Monell Chemical Senses Center, Philadelphia, Pa. The research was supported by the Committee on Grants, Franklin and Marshall College.

[2] The rats were offspring from CD rats purchased initially from the Charles River Laboratories, Wilmington, Mass.

[3] Composition of the diet: (g/100 g) vitamin-free casein 22.0; sucrose 69.0; corn oil 4.0; salt mixture No. 446 (H. Spector, *J Biol Chem* 173:659, 1948) 4.0; vitamin fortification mixture (pyridoxine-free), 1.0. The vitamins were provided at the following level (per 100 g diet): (in mg) thiamin hydrochloride 2.2, riboflavin 2.2, nicotinic acid 9.9, calcium pantothenate 6.6, paraaminobenzoic acid 11.0, i-inositol 11.0, choline chloride 165.0, ascorbic acid 99.0, $\alpha$-tocopherol 11.0, menadione 4.9; (in $\mu$g) vitamin $B_{12}$ 2.98, folic acid 198, biotin 44; (in U.S.P. units) vitamin A acetate 1984, vitamin $D_2$ 220. The control diet was supplemented with 3 mg pyridoxine hydrochloride per 100 g diet. These diets were prepared in our laboratory using components purchased from ICN, Cleveland, Ohio.



0037-9727/82/050015-04$01.00/0

10) or control diet[3] ($N$ = 10) *ad libitum*. Body weights were recorded weekly. Pair-feeding was not employed since it has been shown that pair-fed animals do not differ from *ad libitum* controls in their taste preferences (3). During the preweaning periods, pups and dams were fed Purina Lab Chow *ad libitum*.

Preference testing was initiated after the animals had been fed the diets for 30 days. Five deficient and five control animals were given a choice between 0.15 $M$ NaCl vs distilled water and the remaining five deficient and five control animals were given the choice between 0.15 $M$ KCl and distilled water. Preference testing which extended over nine successive 24-hr periods, was conducted in metal test chambers (38 × 31 × 24 cm) with 20-cm lateral separation between drinking tubes. Bottles were weighed at the beginning and end of each period in order to determine the volume of fluid consumed and the positions of the two bottles were alternated according to a right, left sequence at this time. The percentage preference for each solution was calculated and expressed as volume of taste solution/volume of taste solution plus volume of distilled water consumed per 24-hr periods.

Because the animals in this particular study were intended for use in another investigation, they could not be sacrificed for biochemical measures. However, there is ample published data to indicate that the diet used produces severe deficiency (8) after 6–8 weeks.

**Results and Discussion.** The effect of the $B_6$ deficiency upon the growth of the animals is shown in Fig. 1 which indicates that an asymptote of growth was reached after 4 weeks on the deficient diet with the control-diet animals continuing to grow up to the end of the study. By the end of 2 weeks on the diet (35 days of age) there was no overlap between the body weights of the two groups.

Taste preferences for NaCl (0.15 $M$) vs distilled water expressed as the mean percentage of NaCl ingested over the 9 days of testing are shown in Fig. 2. Both groups showed a very strong preference for this concentration of NaCl. With a strong preference present in the control animals, there was less likelihood of an enhanced preference for NaCl being detected in the deficient animals. The mean percentage NaCl preference across the 9 days was 85.9 ± 5.7 (SEM) for deficient and 77.6 ± 4.7 (SEM) for controls, a difference which was not significant ($t(8) = 1.15$, $P > 0.05$).

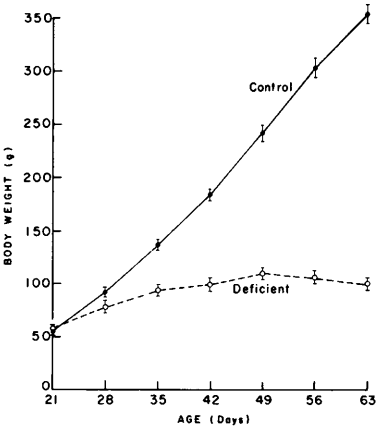


FIG. 1. Mean body weight as a function of age for control ($N$ = 10) and pyridoxine-deficient ($N$ = 10) rats. Each point represents the mean ± SEM. The animals were fed the respective diets from weaning (21 days of age).

The mean percentage of 0.15 $M$ KCl ingested over 9 days of testing for the deficient and control animals is shown in Fig. 3. It is apparent that a strong preference for KCl is present in the deficient animals from Day 1 of testing whereas the control animals show a moderate initial preference which disappears by Day 3 of testing. The mean percentage KCl preference across 9

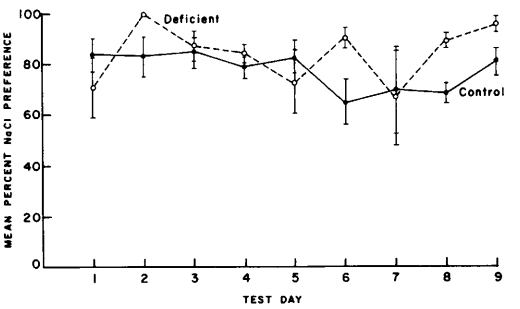


FIG. 2. Mean percentage NaCl (0.15 $M$) ingested for control ($N$ = 5) and pyridoxine-deficient ($N$ = 5) rats. Each point shows mean ± SEM for the day indicated.

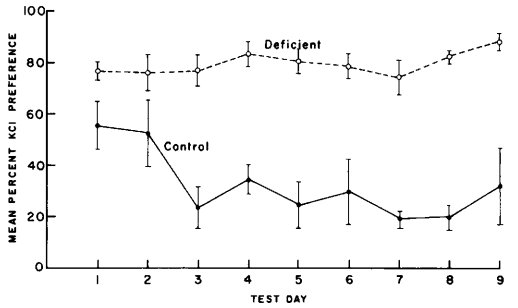


FIG. 3. Mean percentage KCl (0.15 *M*) ingested for control ($N = 5$) and pyridoxine-deficient ($N = 5$) rats. Each point shows the mean ± SEM for the day indicated.

days of testing was 80.1 ± 2.0 (SEM) for deficient and 32.4 ± 1.8 (SEM), a difference which was highly significant ($t(8) = 17.58$, $P < 0.001$). KCl at the concentration used (0.15 *M*) is not normally preferred by rats (9).

The enhanced preference for NaCl reported previously (3), along with the marked increase in KCl preference reported here, is consistent with the hypothesis of Hsu *et al.* (7) that $B_6$ intake is essential for the maintenance of Na and K balance in rats. The failure to find an increased NaCl preference could perhaps be related to the fact that although the differences were in the predicted direction, the control rats in this study drank more 0.15 *M* NaCl (77.6%) than those in the previous investigations (65.6%) using pyridoxine deficiency (3, 4). Also, only a single concentration of NaCl was used in the present study. As suggested by Chan and Kare (3), the enlarged adrenal glands in $B_6$ deficiency may reflect an impairment of the physiological control of Na turnover. Although adrenal gland weight was not determined in this study, previous studies have shown that the diet used does produce adrenal hypertrophy (8), and the enlargement is a well-established component of the deficiency state (10). On the basis of the behavioral data, i.e., an enhanced NaCl preference reported previously (3) and an enhanced KCl preference in this study, it is reasonable to speculate that the adrenal hypertrophy may reflect increased aldosterone secretion and that the hypertrophy may be found in the zona glomerulosa.

Increased aldosterone activity would be consistent with the decreased urinary Na secretion reported by Chan and Kare (3) and would also explain the enhanced NaCl preference they found. As suggested by Ramsay and Ganong (11), this hormone, in addition to promoting the retention of Na by the kidney, may also act on salivary glands to increase reabsorption of Na from saliva causing the taste buds to detect the low Na content of the saliva, signalling for an increase in Na intake. Administration of aldosterone has been reported to increase Na consumption in rats (12). Thrasher and Fregly (13) have shown that the Na preference threshold decreases with lower salivary concentration of this substance. Also consistent with increased aldosterone activity is the enhanced K intake to compensate for the increase in K clearance by the kidneys (14). Direct evidence in support of the hypothesis that there is a hyperaldosteronism associated with $B_6$ deficiency is not available. A detailed histological study of the adrenal cortex in $B_6$ deficiency, measurement of blood levels of aldosterone, and salivary levels of Na and K would appear to be appropriate.

The thoughtful comments of Drs. Morley Kare and Michael Quinn are gratefully acknowledged.

1. Bernard RA, Halpern BP, Kare MR. Effect of Vitamin A deficiency on taste. Proc Soc Exp Biol Med **108**:784–786, 1961.
2. Bernard RA, Halpern BP. Taste changes in vitamin A deficiency. J Gen Physiol **52**:444–463, 1968.
3. Chan MM, Kare MR. Effect of vitamin B-6 deficiency on preference for several taste solutions in the rat. J Nutr **109**:339–344, 1979.
4. Chan MM, Cote J. Fluid intakes and chorda tympani nerve responses in vitamin B-6 deficient rats. Physiol Behav **22**:401–404, 1979.
5. Kare MR, Henkin RI. The effects of D-penicillamine on taste preference and volume intake of sodium chloride by the rat. Proc Soc Exp Biol Med **131**:559–565, 1969.
6. Jaffee IA. The antivitamin B-6 effect of penicillamine: Clinical and immunological implications. Advan Biochem Psychopharmacol **4**:217–226, 1972.

7. Hsu JM, Davis RL, Chow BF. Electrolyte imbalance in vitamin B-6 deficient rats. J Biol Chem 230:889–895, 1958.
8. Bhagavan HN, Koogler JM, Coursin DB. Effect of postweanling pyridoxine deficiency on growth and concentration of the coenzyme pyridoxal-5'-phosphate in heart, kidneys, lungs, and adrenals in rats. Pediat Res 10:730–732, 1976.
9. Bernard RA, Doty RL, Engelman K, Weiss RA. Taste and salt intake in human hypertension. In: Kare MR, Fregly MJ, Bernard RA, eds. Biological and Behavioral Aspects of Salt Intake. New York, Academic Press, p397, 1980.
10. Hsu JM. Interrelations between vitamin B-6 and hormones. Vitam Horm 21:113–134, 1963.
11. Ramsay DJ, Ganong WF. CNS regulation of salt and water intake. In: Kreiger DT, Hughes JC, eds. Neuroendocrinology. Sunderland, Mass, Sinauer Assoc., p129, 1980.
12. Wolf G. Sodium appetite elicited by aldosterone. Psychon Sci 1:211–212, 1964.
13. Thrasher TN, Fregly MJ. Factors affecting salivary sodium concentration, NaCl intake, and preference threshold and their interrelationships. In: Kare MR, Fregly MJ, eds. Biological and Behavioral Aspects of Salt Intake. New York, Academic Press, p145, 1980.
14. Turner CD, Bagnara JT. General Endocrinology. Philadelphia, Saunders, p346, 1976.

---

Received July 21, 1981. P.S.E.B.M. 1982, Vol. 170.