

Influence of Dietary Sodium Intake on Renal Maturation in Unanesthetized Canine Puppies (38623)

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(Introduced by D. L. Kline)

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A major difference between the kidney of the newborn and the adult animal is the reduced ability of the newborn's kidney to adequately excrete an acute salt load (1-4).

When anesthetized newborn dogs were given a normal saline load they excreted only 5% of the infused sodium after 2 hr of sodium infusion. Adult dogs given an equivalent infusion (per kg body wt) excreted 30% of the sodium infused over the same period of time. Puppies excreted only 1-2% of the filtered sodium compared to 5-6% for the adult dog (5).

When premature infants were accidentally given normal saline instead of distilled water in their formula they developed peripheral edema and a large weight gain (6). Infants receiving a relatively high-salt diet prior to this accidental salt load developed less edema and had a smaller weight gain than infants on a lower salt content formula. This finding lead to the speculation that different concentrations of dietary sodium intake may alter the neonatal renal response to an acute salt load and volume expansion.

This study was designed, therefore, to investigate the effect of different concentrations of dietary sodium on neonatal renal function. Specifically, it compares the response to an acute sodium load in canine puppies after a period on either relatively high or a relatively low salt diet.

Materials and Methods. Experiments were carried out in 24 canine puppies 3-28 days of age. Sixteen of the 24 puppies received a regular bitch's milk diet, hereafter referred to as "a low-sodium diet." They were allowed to feed ad lib. from their mothers. Ten of these 16 puppies on the low-sodium diet were studied before and after an acute salt load. The other six puppies had renal

function studies done only under nonsaline expansion conditions. Eight puppies received 6 meq of Na per kg per day in two separate gavage feedings in addition to their regular intake of bitch's milk. This doubled their sodium intake. These puppies will be referred to hereafter as being on "a high-salt diet." These animals were on the high-salt diets for at least 5 days before renal function studies were done. All puppies were weighed on the day of the experiment, brought to the laboratory and placed on a warm table. The experiments were done with the animal unanesthetized. The animals were allowed to move around freely on the warm table, and appeared very comfortable and undisturbed during the experiment. Longdwell, 21-gauge intracatheters (Benton and Dickinson) were inserted transcutaneously into both external jugular veins. One catheter was used for infusion and the other for blood sampling. A priming injection of ^3H -labeled inulin, 3 $\mu\text{Ci}/\text{kg}$, was followed by a constant infusion of labeled inulin (1 $\mu\text{Ci}/\text{ml}$) at a rate of 0.06 ml/min/kg.

After a 60-min equilibration period two 30-min control clearances were obtained. Isotonic saline was then infused at a rate of 2 ml/min/kg for 15 min followed by an infusion at a rate of 0.5 ml/min/kg for the remainder of the experiment. Repeated 30-min sodium and inulin clearances were measured beginning 30 min after the slower saline infusion was started.

Blood samples were collected through one of the jugular catheters. Urine was collected by stimulating the perineal area, which provokes voiding in the newborn puppy (7).

Plasma and urine samples were placed in Bray's (8) solution, and then counted in a

Packard Tricarb Scintillation spectrometer.

Inulin clearance (C_{In}) was equated with glomerular filtration rate (GFR). The fraction of the infused sodium excreted (Na_e/Na_i) was calculated by dividing the amount of sodium excreted (Na_e) with the amount of sodium infused (Na_i). The fraction of the filtered sodium excreted was calculated from the ratio of sodium clearance divided by the inulin clearance, C_{Na}/C_{In} . The sodium concentration of plasma and urine samples were determined using an atomic absorption spectrophotometer (Perkin-Elmer 360). Serial hematocrits were measured. The results were analyzed statistically at the University of Cincinnati computer facilities. Standard t tests, paired t tests, and regression and covariance analyses were used to analyze the results.

Results. Maturation of animals. All animals increased body weight with age. However, there was no difference in growth rate between puppies on a high- or low-salt diet.

GFR (per kg body weight) increased with age in all puppies ($r = 0.78$, $P < 0.01$). However, there was no difference in rate of increase of GFR with age between puppies on the high and the low sodium.

Renal response to an acute sodium load. Puppies on the high-sodium diet responded differently from puppies on the low-sodium diet (Table I). At the end of 2 hr of sodium infusion puppies on a low-sodium diet excreted 4.6% of the infused sodium while puppies on the high-sodium diet excreted 20% ($P < 0.01$). After 3 hr of sodium infusion, puppies on a low sodium diet ex-

creted 10.8% of the infused sodium compared to 29.9% for the puppies on the high-sodium diet ($P < 0.01$). Fractional sodium excretion was less than 1% in both dietary groups during the control period although it was slightly higher in the puppies on the high-salt diet (Table I). Puppies on the high-sodium diet excreted a significantly higher proportion of the filtered sodium in response to a salt load than did the puppies on the low-sodium diet. After 2 hr of saline infusion the puppies on the high-sodium diet excreted 4.8% of the filtered sodium while puppies on the low-sodium diet excreted only 2.6% of the filtered sodium ($P < 0.01$). At the end of 3 hr of sodium load, puppies on a high-sodium diet excreted 6.5% of the filtered sodium while the low-sodium diet group excreted 3.4% ($P < 0.01$) (Table I). Figure 1 shows the effect of salt loading on GFR. There was a significant increase in GFR after saline infusion in the high-salt puppies ($P < 0.01$) but not in the low-salt puppies ($P > .5$). There was no consistent change with age in C_{Na}/C_{In} and Na_e/Na_i for the three puppies on the low-sodium diet that were studied more than once during the first few weeks of life.

There were no significant changes in plasma sodium (P_{Na}) after saline expansion in either diet group. However, plasma sodium concentrations were significantly higher in puppies on the high- than on the low-sodium diet both before saline expansion ($P < 0.025$) as well as after saline expansion ($P < 0.05$) (Table II). Control hematocrits were not significantly different between the two groups of animals. In

TABLE I. NATRIURETIC RESPONSE TO A SALINE LOAD IN NEWBORN DOGS ON HIGH AND LOW SODIUM DIETS.

	Low-sodium diets			High-sodium diets		
	Control	2 hr	3 hr	Control	2 hr	3 hr
$\frac{Na_e}{Na_i} \times 100$		4.6* \pm 2.8	10.8* \pm 2.7		20.8* \pm 2.4	29.4* \pm 2.8
$\frac{C_{Na}}{C_{In}} \times 100$.28* \pm 0.04	2.6* \pm 0.6	3.4* \pm 0.8	.48* \pm 0.05	4.8* \pm 0.8	6.5* \pm 0.9

Values are means \pm SE.

* $P < 0.01$ comparing low and high-sodium diet.

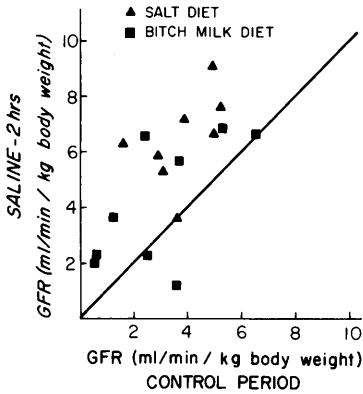


FIG. 1. Glomerular filtration rate during the control period and after 2 hr of saline expansion for puppies on high- (\blacktriangle) and low- (\blacksquare) sodium diets. The line is one of identity.

TABLE II. EFFECT OF SALINE EXPANSION ON HEMATOCRIT AND PLASMA SODIUM CONCENTRATION IN NEWBORN DOGS ON HIGH- AND LOW-SODIUM DIETS.

	Low-sodium diet		High-sodium diet	
	Control	3-hr Saline expansion	Control	3-hr Saline expansion
HCT	29.77 ± 3.39	24.61 ± 3.48	26.93 ± 1.38	21.5 ± 1.4
P_{Na}	136.5* ± 1.68	139** ± 2.04	144.25* ± 2.58	144.81** ± 1.56

Values are means \pm SE.

* $P < 0.025$ comparing high- and low-sodium diet groups.

** $P < 0.05$ comparing high- and low-sodium diet groups.

both groups, however, there was a significant decrease after saline load ($P < 0.01$), but the change in hematocrit was not significantly different between the two groups ($P > 0.05$) (Table II).

Discussion. Results of this study reveal that increased dietary salt intake improves the response of the newborn kidney to an acute salt stress and volume expansion. However, the diet did not affect other renal maturational functions of the newborn dog. Although we have no direct measurement of the extracellular fluid volume the similar weight gain in both groups suggests that there was no water retention associated with the high-sodium diet.

Anesthesia apparently does not signifi-

cantly affect sodium excretion in the newborn dog. C_{Na}/C_{In} during the control period, and Na_e/Na_i and C_{Na}/C_{In} during salt loading were essentially the same for the unanesthetized puppies on a low-sodium diet in the present experiment, as they were for anesthetized puppies on a low-sodium diet from a previously reported similar experiment (5).

Puppies on the high-salt diet excreted a larger proportion of the infused and filtered sodium than did puppies on a low-sodium diet. However, they excreted a smaller fraction of the filtered and infused sodium than did anesthetized adult dogs receiving a similar saline load per kg body weight (5). The reason for the more efficient response to a salt load in the puppies receiving the higher sodium diet is not clear at this time. The filtered sodium after a 2-hr sodium infusion was larger in the puppies on a high-sodium diet. Conceivably the renal tubules of the high-salt puppies may not have been capable of reabsorbing the increased filtered load resulting in increased Na excretion and decreased fractional sodium reabsorption. Alternatively, the high-sodium diet may have reset the glomerular tubular balance sensitivity, resulting in a greater sodium excretory response to sodium load and volume expansion.

Results of the present study do not necessarily imply that newborn infants should be placed on high-sodium diets at birth. However, many newborn infants, especially premature infants and those infants being breast fed, receive a diet, low in sodium. These infants may have difficulties excreting the increased sodium load they receive when they eat high salt-containing commercially prepared baby foods. It may be wise, therefore, to gradually, rather than abruptly, increase the sodium in the diet of small infants.

In conclusion, the present study shows that newborn dogs that receive a chronic increased sodium intake respond more efficiently to an acute salt load than newborn dogs that have not had extra sodium added to their diet. The response is enhanced by an increased GFR and a decreased tubular fractional sodium reabsorption. The mecha-

nism for the enhanced response and its clinical significance still need to be determined.

The authors express their appreciation to Mrs. Ning Hsieh for her excellent technical assistance. This research was supported by U.S. Public Health Service Grants HD-06337 and ES-00159 and H.E.W. Training Grant MCT-000174, and the Fels Division of Pediatric Research.

1. Dean, R. F. A., and McCance, R. A., *J. Physiol.* **109**, 81 (1949).
2. McCance, R. A., and Widdowson, E. M., *J. Physiol.* **129**, 628 (1955).
3. Davies, D. P., Saunders, R., and Gray, O. P., *Arch. Dis. Child.* **47**, 946 (1972).
4. Aperia, A., Broberger, O., Thodenius, K., and Zetterstrom, R., *Acta Paediat. Scand.* **61**, 670 (1972).
5. Kleinman, L. I., and Reuter, J. H., *J. Physiol.* **239**, 225 (1974).
6. Wagner, S. F., Kleinman, L. I., Tsang, R. C., Coen, R. W., and Sutherland, J. M., *Proc. Soc. Pediatr. Res. 40th Annu. Meet.* (1970).
7. McCance, R. A., and Widdowson, E. M., *J. Physiol.* **141**, 81 (1958).
8. Bray, G. A., *Anal. Biochem.* **1**, 279 (1960).

Received September 30, 1974. P.S.E.B.M., 1975. Vol. 148.