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Segmental Nephron Sodium and Potassium Reabsorption in Newborn and Adult Dogs during Saline Expansion (41637)

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Abstract. Studies were carried out in 23 anesthetized neonatal dogs aged 2 to 20 days and in 16 adult dogs to compare the effects of saline volume expansion on renal tubular Na and K reabsorption between newborn and adult animals. Proximal- and distal-tubule function was estimated by the distal-nephron-blockade technique using ethacrynic acid and amiloride. During saline infusion, which increased extracellular volume by approximately 30% for both age groups, total nephron fractional Na reabsorption was 0.91 for the adult but 0.98 for the puppy ($P < 0.01$). However, proximal tubule fractional Na reabsorption was greater in the adult (0.64) than in the puppy (0.48, $P < 0.01$) whereas distal nephron fractional Na reabsorption was much greater in the newborn (0.51) than in the adult (0.26, $P < 0.01$). Sodium reabsorption normalized to kidney weight was lower in all segments of the neonatal kidney than in the adult kidney. The filtered sodium load was lower in the newborn (27.0 μeq $min^{-1}g^{-1}$) than in the adult (105.0, $P < 0.01$), and the Na load to the distal nephron was also lower in the newborn (14.0 μeq $min^{-1}g^{-1}$) than in the adult (37.2, $P < 0.01$). Fractional K excretion was similar in both age groups even though the fraction of filtered K escaping proximal-tubule reabsorption was greater in the newborn than in the adult, indicating greater net K fractional reabsorption in the distal nephron of the newborn than in the adult kidney. These results indicate that in response to saline expansion there is a greater proximal tubule natriuresis in the neonate than in the adult but overall renal Na excretion is less in the newborn animal due to enhanced fractional Na reabsorption in the neonatal distal nephron, particularly in Henle's Loop. This increase in distal nephron fractional sodium reabsorption may be related in part to the relatively small absolute Na load presented to the distal nephron of the neonatal kidney.

The newborn animal does not excrete an imposed sodium load as efficiently as does the mature animal (1–4). For example, when newborn dogs are infused with normal saline in amounts sufficient to increase the extracellular volume by 25–30%, they excrete only 1–2% of the sodium filtered by the kidney. By contrast, adult dogs excrete more than 6% of the filtered sodium when comparably volume expanded (3). Previous studies from our laboratory (5) revealed that when distal nephron function was blocked with ethacrynic acid and chlorothiazide, saline-expanded newborn dogs excreted 50% of the filtered sodium load, compared to only 30% for nonexpanded puppies. Similar results were obtained when amiloride was administered with ethacrynic acid and chlorothiazide (6). Thus, in the newborn, volume expansion results in the inhibition of approximately 20% of the filtered Na load normally reabsorbed in the proximal tubule. Earley *et al.* (7) reported that when distal nephron function in adult dogs was blocked with ethacrynic acid and chlorothiazide, saline expansion inhibited the reabsorption of 12 to 20% of the filtered sodium load, the exact value dependent on the degree of volume expansion. Therefore, we had previously concluded that the proximal tubules of adult and newborn animals responded similarly to saline expansion. However, the magnitude of saline-induced inhibition of proximal-tubule sodium reabsorption appears to be related to the degree of extracellular volume expansion. Therefore, it is important that any comparison between the response of newborn and adult animals to saline infusion be assessed under conditions of identical saline-expansion protocols for the two age groups of animals. Consequently, the present studies were designed to evaluate the effects of equivalent degrees of volume expansion on proximal-tubule function in adult and newborn dogs.

Methods. Studies were performed on 16 adult and 23 mongrel puppies of either sex. The age of the puppies ranged between 2 and 20 days. Animals of this age were chosen because earlier experiments from our labora-

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tories revealed that during the first 3 weeks of life, there is no maturational change in the ability of the puppy to respond to saline expansion (3, 5, 6). The animals were anesthetized by an intravenous injection of pentobarbital, 25 mg/kg, and placed on a temperature-controlled heating board regulated to maintain rectal temperature at 37°C. In puppies, the trachea was exposed and tracheostomized in order to clear the airway and to apply positive-pressure ventilation when necessary. In adults, the trachea was intubated without tracheostomy. Polyethylene catheters of appropriate size were placed in the right jugular vein for infusion of ^{3}H-labeled inulin and maintenance fluids, in the left external jugular vein for infusion of diuretics, in the right femoral vein for infusion of the saline load, in the abdominal aorta via the right femoral artery for blood sampling and recording of arterial blood pressure, and in both ureters for collection of urine. Puppies were infused with a glucose multielectrolyte maintenance solution containing glucose (5 g/dl), sodium (20 meq/liter), potassium (10 meq/liter), and chloride (30 meq/liter) at a rate of 0.06 $ml \cdot min^{-1} \cdot kg^{-1}$. Adult dogs received saline at a rate of 0.06 $ml \cdot min^{-1} \cdot kg^{-1}$ for maintenance fluids. [There was no difference in renal function in adult dogs in the present experiment receiving a maintenance solution of saline and those from a previous study receiving a glucose multielectrolyte maintenance solution (8).] A priming injection of ^{3}H inulin was followed by a constant infusion of labeled inulin. One hour following surgery a control clearance was taken. Subsequent manipulations of the animals are described below.

Protocol 1: distal blockade—nonexpanded animals. The purpose of this protocol was to determine the effect of distal nephron blockade on renal function in nonexpanded newborn (n = 9) and adult dogs (n = 9). After the initial control clearance, priming injections of ethacrynic acid (1.25–2.50 mg/kg) and amiloride (1 mg/kg) were followed by a constant infusion of these diuretics at rates of 1–2 and 2.4 mg $kg^{-1} \cdot hr^{-1}$, respectively, until the completion of the experiment. These doses were similar to the dose rates described in our previous studies on neonatal dogs (5, 6, 8, 9). Urine was collected in a graduated cylinder and urinary fluid losses were replaced continuously and isovolumetrically with a solution containing sodium (140 meq/liter), potassium (5 meq/liter), chloride (140 meq/liter), and bicarbonate (5 meq/liter). After 1 hr of diuretic therapy, the first of two or three consecutive clearances was taken. The results of these clearances were averaged and referred to as the diuretic period.

Protocol 2: distal blockade—saline-expanded animals. The purpose of this protocol was to determine the effect of distal nephron blockade on renal function in saline-expanded newborn (n = 14) and adult (n = 7) dogs. After the initial control clearance, isotonic saline was infused at a rate of 2.0 ml $kg^{-1} \cdot min^{-1}$ for 15 min followed by a rate of 0.5 ml $kg^{-1} \cdot min^{-1}$ to the end of the experiment. One hour after the start of the slower saline infusion, the first of two or three consecutive clearances was started. Results of these clearances were averaged and referred to as the saline period. After the completion of the final clearance of the saline period the saline infusion was continued but, in addition, diuretics and replacement solutions were infused as in Protocol 1. After 1 hr of diuretic therapy, the first of two or three consecutive clearances was started. These clearances were averaged and referred to as the diuretic and saline period.

Plasma and urine samples were placed in Aquasol-2 solution for scintillation counting (Packard) of tritium. Glomerular filtration rate (*GFR*) was equated with the inulin clearance and expressed in units of milliliters per minute per gram wet kidney weight ($ml \cdot min^{-1} \cdot g^{-1}$). Sodium (Na) and potassium (K) concentrations in plasma and urine samples were determined by either atomic absorption spectrometry (Perkin–Elmer) or flame photometry (Advanced Instruments). Chloride concentrations were determined by coulometric–amperometric titration with siver ions (Buchler–Cotlove chloridometer). Urine and plasma osmolalities were measured by water vapor osmometry (Wescor) or by freezing-point depression (Precision Instruments). Sodium and potassium excretions were expressed as fractional excretion of sodium or potassium (C_{Na}/GFR or C_K/GFR). Reabsorbed sodium and potassium were calculated by subtracting the difference between the filtered and excreted ions and normalized either

to wet kidney weight (μeq $min^{-1} \cdot g^{-1}$) or to *GFR* (μeq/ml *GFR*). Hematocrit and plasma protein concentration were measured on all blood samples. Plasma protein concentration was measured with a calibrated, temperature-compensated refractometer (American Optical). All results were calculated with standard statistical computer programs available at the University of Cincinnati. Values are given as means ± SE. Comparison within protocol groups was made with the Wilcoxin sign-rank test. Comparison between protocol groups and between adult and newborn dogs was made with the Wilcoxin rank sum test.

Results. The percentage increase in extracellular fluid volume produced by saline infusion, as calculated from the dilution of the plasma protein, was similar in newborn (32 ± 2.2%) and adult dogs (26 ± 4.7%, $P > 0.20$). Results of the effects of saline expansion on selected variables of renal function are summarized in Table I. Glomerular filtration rate was much lower ($P < 0.01$) in the newborn than in the adult even when normalized to gram kidney weight. This was true for all periods in all protocols. Thus, even though the amount of sodium reabsorbed per gram kidney was lower in the newborn than in the adult, the fact that the filtered sodium load was much lower in the newborn, sodium reabsorption per milliliter *GFR* and fractional sodium reabsorption ($1 - C_{Na}/GFR$) was significantly higher ($P < 0.01$) in the newborn compared to the adult (Fig. 1).

During infusion of the diuretics into either nonexpanded newborn or adult animals, the urine concentrations of sodium and solutes were identical to those of plasma (U_{Na}/P_{Na} and *U*osm/*P*osm were unity) and U_{cl}/P_{cl} was about 1.3 indicating that distal nephron function in these animals was essentially blocked. When diuretics were infused in nonexpanded animals (Table I, Distal blockade) fractional sodium excretion was greater and sodium reabsorption less in the newborn than in the adult animal ($P < 0.01$). There were no differences in potassium excretion or reabsorption (normalized to *GFR*) between the two age groups ($P > 0.20$). When the diuretics were infused into saline expanded animals (Distal blockade and Saline expansion, Table I) the differences in fractional sodium excretion and sodium reabsorption per milliliter

TABLE I. RENAL FUNCTION IN NEWBORN AND ADULT DOGS

	Saline expansion		Distal blockade		Distal blockade and saline expansion	
	Newborn (14)	Adult (7)	Newborn (9)	Adult (9)	Newborn (14)	Adult (7)
GFR (ml · $min^{-1}g^{-1}$)	0.217 ± 0.017	0.705 ± 0.086**	0.208 ± 0.020	0.595 ± 0.048**	0.297 ± 0.025	0.577 ± 0.069**
R_{Na} (μeq $min^{-1}g^{-1}$)	22.6 ± 2.0	94.6 ± 10.8**	17.2 ± 1.9	63.0 ± 4.5**	15.8 ± 1.0	59.8 ± 8.1**
R_{Na} (μeq/ml *GFR*)	129.8 ± 2.4	134.7 ± 2.8	81.3 ± 3.2	108.4 ± 4.3**	58.9 ± 3.3	98.6 ± 4.9**
$C_{Na}/GFR \times 100$	1.51 ± 0.27	9.4 ± 1.4**	33.9 ± 2.4	22.8 ± 2.1**	52.4 ± 2.3	35.8 ± 2.7**
R_K (μeq $min^{-1}g^{-1}$)	0.507 ± 0.05	1.40 ± 0.17**	0.586 ± 0.064	1.71 ± 0.27**	0.318 ± 0.064	0.980 ± 0.160**
R_K (μeq/ml *GFR*)	2.54 ± 0.19	1.96 ± 0.13*	2.78 ± 0.16	2.82 ± 0.29	1.11 ± 0.25	1.67 ± 0.16
$C_K/GFR \times 100$	29.8 ± 3.9	39.6 ± 3.3	40.5 ± 4.0	33.1 ± 3.5	71.9 ± 5.0	53.1 ± 4.3**

Note. Values are means ± SE; Number of animals (*N*) per group in parentheses. R_{Na} and R_K are reabsorbed sodium and potassium, respectively. C_{Na}/GFR and C_K/GFR represent fractional excretion of sodium and potassium, respectively.

* $P < 0.05$ compared to newborn.

** $P < 0.01$ compared to newborn.

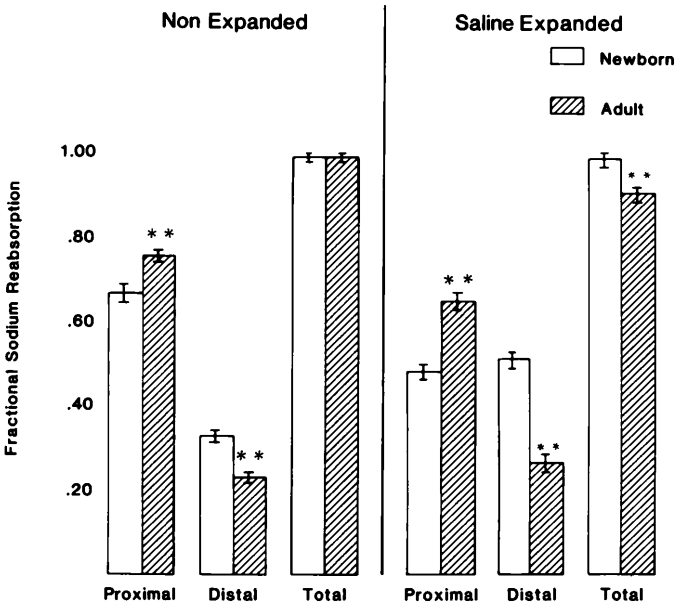

FIG. 1. Fractional reabsorption of sodium (reabsorbed sodium/filtered sodium) in various segments of the nephron in newborn and adult dogs. Bars represent mean and lines represent SE. **, $P < 0.01$ between newborn and adult. Proximal values during nonexpansion were obtained from the diuretic period of Protocol 1, total values were obtained from the control period, and distal values from the difference between these two periods. During saline expansion, proximal values were obtained from the diuretic and saline period of protocol 2, total values were obtained from the saline period, and distal values from the difference between these two periods.

GFR between newborn and adult animals were magnified. In both distally blocked newborn and adult animals, sodium reabsorption per milliliter GFR was less during saline expansion than during nonexpansion. This drop in sodium reabsorption due to saline expansion was greater in the newborn than in the adult animal. In the newborn, sodium reabsorption was reduced from 81.3 μeq/ml *GFR* prior to expansion to 58.9 μeq/ml *GFR* during expansion, a difference of 22.4 μeq/ml *GFR*, while in the adult, sodium reabsorption decreased from 108.4 to 98.6 μeq/ml *GFR*, respectively, a difference of only 9.8 μeq/ml *GFR*. Moreover, there was less sodium reabsorbed per milliliter *GFR* and per gram kidney and more sodium excreted per milliliter *GFR* in the distally blocked saline-expanded newborn animal than in the adult. However, in the newborn dog sodium reabsorption per gram kidney did not change with saline infusion because of the large increase in the filtered sodium load.

Comparisons of fractional sodium reabsorption between newborn and adult dogs during nonexpansion and saline-expansion periods are depicted in Fig. 1. Without volume expansion, fractional sodium reabsorption was slightly but significantly higher in the proximal but lower in the distal nephron of the adult compared to the newborn kidney ($P < 0.01$). During saline expansion these differences between the newborn and adult were magnified. It should be noted that during saline expansion, there was a marked increase in fractional sodium reabsorption in the newborn distal nephron, (from 0.33, nonexpanded to 0.51 saline expanded, $P < 0.01$) while no such increase took place in the adult distal nephron (0.22, nonexpanded, 0.26, saline expanded, $P > 0.20$). Sodium reabsorption expressed in units of microequivalents per minute per gram ($\mu eq\ min^{-1}\ g^{-1}$), in proximal and distal segments of the nephron during saline expansion is shown in Table II. Although fractional sodium reabsorption in the distal nephron was much higher in the newborn than in the adult (Fig. 1), absolute sodium reabsorption (per gram kidney) was lower in the newborn because the filtered so-

TABLE II. REABSORPTION AND LOADS OF SODIUM AND POTASSIUM IN PROXIMAL AND DISTAL SEGMENTS OF THE NEPHRON DURING SALINE EXPANSION

	Sodium		Potassium	
	Newborn	Adult	Newborn	Adult
Filtered load	27.0 ± 2.1	105.0 ± 12.7**	0.74 ± 0.06	2.31 ± 0.22**
Proximal reabsorption	13.0 ± 1.4	67.9 ± 9.5**	0.24 ± 0.05	1.09 ± 0.15**
Distal load	14.0 ± 1.0	37.2 ± 4.3**	0.50 ± 0.04	1.23 ± 0.15**
Distal reabsorption	13.6 ± 1.0	26.7 ± 2.8**	0.30 ± 0.04	0.31 ± 0.06

Note. Values are mean ± SE in units of μeq $min^{-1}g^{-1}$.
** $P < 0.01$ compared to newborns.

dium load was lower. Moreover, even though the proximal tubule rejected a larger fraction of the filtered Na load in the newborn than in the adult, the absolute Na load to the distal nephron remained lower in the newborn.

During saline expansion of nondistally blocked animals (Table I) fractional excretion of potassium was slightly higher in the adult than in the newborn animal, although this difference failed to achieve statistical significance ($0.10 > P > 0.05$). In nonexpanded, distally blocked animals fractional potassium excretion was similar in the newborn and the adult animal ($P > 0.20$). However, during saline expansion of distally blocked animals, fractional potassium excretion was larger in the newborn than in the adult animal ($P < 0.01$).

The filtered load and absolute proximal reabsorption of potassium (milliequivalents per minute per gram), were much less in the newborn than in the adult (Table II). However, although the distal potassium load was much lower in the newborn, the absolute net distal potassium reabsorption was about the same in the newborn and in the adult. Thus, the newborn reabsorbed 60% of the distal potassium load while the adult reabsorbed only 25% ($P < 0.01$).

Discussion. The technique of distal nephron blockade was used in the study to determine whether the attenuated natriuretic response of the newborn is due to differences in proximal or distal nephron function between the immature and mature kidney. The validity of the technique is based upon the premise that ethacrynic acid and amiloride completely inhibit sodium reabsorption in the distal nephron but have no effect on sodium transport proximally so that urine excreted during distal blockade resembles fluid leaving the proximal tubule. Several facts indicate that these criteria were satisfied in our experiments. First, there is very good evidence that ethacrynic acid and amiloride in the dosage used in the present experiments have little or no effect on the proximal tubule (10–13). Second, evidence that the urine obtained during distal blockade resembles fluid leaving the proximal tubule is indicated by the fact that (a) the U_{Na}/P_{Na} and Uosm/Posm ratios were unity, with very little variation (range = 0.95–1.05), and (b) the U_{Cl}/P_{Cl} ratio was approximately 1.3 in nonexpanded distally blocked animals.

The major finding of the present study is that although the overall natriuretic response to saline expansion is less in the newborn than in the adult dog, proximal tubular natriuresis in greater in the newborn animal. Thus, in addition to the previously described differences in distal nephron function between the adult and newborn kidney (5), data from the present study reveal that differences exist also in proximal-tubule function between the two age groups.

During distal blockade, but without saline expansion, fractional sodium excretion in the proximal tubule was slightly greater in the newborn than in the adult (33 vs 23%). Of even more importance, however, is the fact that fractional sodium excretion in distally blocked saline-expanded animals was 52% in the newborn but only 36% in the adult. Thus, saline expansion, to the degree imposed in the current study, results in the inhibition of about 19% of the filtered sodium (22.4 μeq Na/ml *GFR*) in the proximal tubule of the newborn dog. By contrast, in the adult, saline expansion results in the inhibition of only 13% of

the filtered sodium in the proximal tubule, amounting to an inhibition of only 9.8 μeq Na/ml *GFR*.

Proximal-tubule sodium reabsorption, normalized to kidney weight, was also lower in the newborn than in the adult during both nonexpansion and saline-expansion conditions. However, in the newborn dog proximal-tubule sodium reabsorption per gram kidney did not fall during saline expansion. This was due to the larger filtered sodium load in the saline-expanded animals. Nevertheless, these results should not be interpreted as implying that the proximal tubule does not decrease sodium reabsorption in response to saline expansion. As previously demonstrated, when GFR and filtered sodium load are increased in neonatal dogs by a method other than saline expansion, proximal-tubule sodium reabsorption increases proportionately, thereby maintaining proximal fractional sodium reabsorption constant (5). In the present study, when the filtered sodium load was increased in the newborn dog by saline expansion, there was no such proportional increase in proximal sodium reabsorption so that fractional sodium reabsorption in the proximal nephron declined. These results indicate that saline expansion, per se, must have inhibited proximal-tubule sodium reabsorption. Furthermore, when corrected for changes in GFR, the inhibition of proximal sodium reabsorption associated with saline expansion was greater in the newborn than in the adult.

The differences in proximal tubular function between newborn and adult dogs found in the present experiment have important implications toward understanding the mechanisms involved in renal functional maturation. However, the reasons for the lower proximal tubular sodium reabsorption in the newborn than in the adult dog are at the present time unclear. It has been reported that the absolute rate of sodium transport (milliequivalents per cubic millimeter per second) is less in the immature than in the mature proximal tubule (14) and that renal Na-K ATPase activity is lower in the immature than in the mature kidney (15). There may also be a greater backleak of sodium, since intercellular junctions in immature kidneys appear more permeable than those in the adult (16, 17).

Results of the present study shed light also on certain aspects of distal-nephron function during saline expansion that were not apparent from previous studies. During saline infusion some portion or portions of the distal nephron of the immature kidney must reabsorb relatively more sodium than that of the mature kidney (Fig. 1). It has been proposed (18) that the enhanced reabsorption of sodium in the newborn kidney occurs in the aldosterone-sensitive regions of the nephron due to elevated aldosterone levels in the newborn. If this were the case, the increased distal sodium reabsorption would be accompanied by increased distal potassium secretion or by decreased distal net potassium reabsorption. In fact, however, the newborn reabsorbed a greater fraction of the distal load than did the adult (Table II). Thus, it is highly unlikely that elevated levels of aldosterone account for the enhanced distal nephron reabsorption of sodium in the saline-expanded newborn animal or that the enhanced reabsorption occurs primarily in the aldosterone-sensitive region of the nephron. Since there is enhanced fractional reabsorption of both sodium and potassium in the distal nephron of the newborn it is likely that this reabsorption occurs in a region proximal to the distal convoluted tubule, that is, in the loop of Henle.

The sodium load presented to the distal nephron of the newborn is less than that of the adult, even during saline expansion (Table II). Thus, even though fractional sodium reabsorption is high in the distal nephron of the newborn, the absolute amount of Na reabsorbed in this region is low (Table II). Horster (19) demonstrated, that the loop of Henle of the immature rabbit kidney has a lower reabsorptive capacity for sodium compared with that of the mature kidney. Even if these rabbit data can be extrapolated to the newborn dog, the deficiency in loop sodium transport does not appear to be sufficiently large so as to prevent reabsorption of the diminished Na load. These results are consistent with the hypothesis that in the newborn saline-expanded animal the avid distal fractional sodium reabsorption and the resultant attenuated natriuresis may be due in part to the low distal sodium load. Whether the enhanced fractional reabsorption of the immature distal nephron during saline expansion can be explained totally by the relatively small distal

sodium load or whether there are differences between adult and neonatal animals in natriuretic factors or the ability of the collecting duct to excrete sodium requires further investigation.

In summary, the proximal tubule of the newborn dog reabsorbs a smaller fraction of the filtered sodium load than does that of the adult. During saline expansion, fractional sodium reabsorption in the proximal tubule of each age group declines. However, this saline-induced decline is greater in the newborn than in the adult so that the difference in proximal tubular fractional sodium reabsorption between the adult and newborn becomes magnified. Despite the relatively large proximal tubular natriuresis in the neonate, overall renal fractional sodium excretion during saline expansion is less in the newborn than in the adult because of increased fractional reabsorption of sodium in the neonatal distal nephron. Since there is enhanced fractional reabsorption of both sodium and potassium in the distal nephron of the newborn, it is likely that this reabsorption occurs in the loop of Henle. This increase in distal fractional sodium reabsorption may be related in part to the relatively small absolute Na load presented to the distal nephron of the neonatal kidney.

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