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Postnatal Plasma Concentration and Urinary Excretion of Na and K in the Rat (39980)

S. SOLOMON, H. H. BENGELE,[1] AND W. D. SMITH[2]

Department of Physiology, University of New Mexico, School of Medicine, Albuquerque, New Mexico 87131

Introduction. A variety of studies has indicated that in neonatal animals, homeostatic responses resulting in increased excretion of salt and water do not readily occur. Human infants (1) as well as neonates of other species do not increase urinary salt or water excretion as much as adults following loading by water or saline (2-7). Young rats are unable to respond to blood volume expansion until they are between 35 and 40 days old (8). This age is close to that of 33 days when Stopp *et al.* found appropriately increased renal excretion of intraperitoneally administered saline (7). The absence of an increase in renal excretion following saline administration could reflect a change in absorption of the solution, but more recently, it has been established that the response to volume expansion produced by intravenous saline infusion is not as great in the neonate as it is in the adult for the rat (Misanko, Bengele, and Solomon, unpublished) and dog (5), indicating a lack of response by the kidney.

More recently, it has been shown that during postnatal development of the rat, tissue ionic contents change with a critical period (9) which also occurs at around 40 days (10). Relatively little work has been done on age-dependent changes in electrolyte balance and basal urinary excretion of electrolytes during postnatal development in the rat. To this end, both clearance and metabolic studies have been carried out to examine changes in plasma sodium and potassium concentration as well as urinary excretion of these ions during maturation.

Materials and methods. Animals. Wistar rats were used exclusively in this study. Usually, pregnant rats were obtained from our commercial supplier (Simonsen, Gilroy, Calif.) and pups were born in our quarters and then used at specified ages. Three litters of 10 pups each were sent at 15 days of age after being born in California. All animals were kept in a temperature- and humidity-controlled room with a photoperiod of 12-h light and 12-h dark. Food and tap water were available *ad libitum*. Pups were weaned at 21 days of age.

Clearance studies. On the day of study, the animals were anesthetized and clearances were carried out using standard procedures for this laboratory (8, 11). In brief, animals were anesthetized with inactin. The jugular vein was cannulated for infusion of Ringers solution containing [^{3}H]inulin. Infusion rates were 0.5 ml/hr for animals weighing less than 100 g, and 1 ml/hr for all other animals. The bladder was cannulated for collection of urine in plastic tubes. After at least 2 hr of equilibration, or at a longer time when it was required for stabilization of urine flow, we determined three consecutive clearance studies of 20-min duration, with blood samples being collected at midperiod.

Urine volume was estimated as the weight of urine collected. Glomerular filtration rate (GFR) was calculated as the clearance of inulin, the plasma and urine radioactivity being measured by scintillation counting. Statistical analysis was carried out using the paired t test. Some of the data reported here have been taken from controls of other work from this laboratory. They have been reanalyzed to answer the questions considered in this paper.

Metabolic studies. Animals were selected for study at varying times after birth. Three days before the studies, animals were placed in metabolic cages and allowed to acclima-

[1] Present address: Division of Medicine, Boston University School of Medicine, Boston City Hospital, 818 Harrison Ave., Boston, Massachusetts 02118.

[2] Present address: Department of Electrical & Electronic Engineering, California State University, Sacramento, 6000 J Street, Sacramento, California 95819.

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tize. The metabolic cages were modified to contain a cup-within-a-cup of crushed rat food (Zia Brand Mouse and Diet, S.W. Burr & Son, Inc.) and a drinking cup of tap water protected against contamination by a hood and kept at constant level by an external water bottle. Twenty-four-hour food consumption, loss from the water bottle, and urine output ($\dot{V}$), collected under mineral oil, comprised the daily records maintained on these rats. Urinary and plasma sodium and potassium concentrations were determined by flame photometry. The animals in the metabolic studies, as well as those normally housed, were weighed daily.

Results. Hematological values. Age-dependent hematological changes during development of the rat are shown in Fig. 1. The most striking observation is the change in hematocrit. This variable increases with age but does not show a "critical period." The absolute values of hematocrit of mature animals are high as compared to other laboratories. It should be remembered, however, that our studies are carried out at a high elevation and under semiarid conditions. As a result, our control aimals are probably dehydrated by standards in other laboratories. Rather, it appears best fitted by a hyperbolic function. The change cannot be related to a generalized reduction in extracellular fluid since the time course of changes in water content of various tissue is different than found here. Water content reaches a minimum at 20–25 days postnatally (10).

P_{Na} also shows an increase. In contrast to hematocrit, the increase is abrupt and shows a critical period between 40 and 45 days of age (Fig. 1). Before this time, average P_{Na} is between 140 and 143 meq/liter, while after 45 days it is 146–147. P_K tends to change in the opposite direction, but the changes are relatively small and continuous (Fig. 1). A significant difference ($P < 0.05$) exists between the youngest age group (20–24 days) and the over-60-day-old age groups. No critical period is evident.

Clearance studies. Renal functional changes are shown in Fig. 2. Glomerular filtration rate (GFR) does not show any marked age dependence in these studies when normalized per kilogram body weight, although we have previously observed a low GFR in our youngest animals (8, 11). Some differences do exist between individual groups (35–39 and 45–49 days old being lower than others). Since no trend can be seen, it would appear that these differences are spurious and probably result from preparative or other experimental errors. Marked changes are seen in $U_{Na}V$ and $U_K V$. $U_{Na}V$ is low until the 40- to 45-day critical period and then rises, while

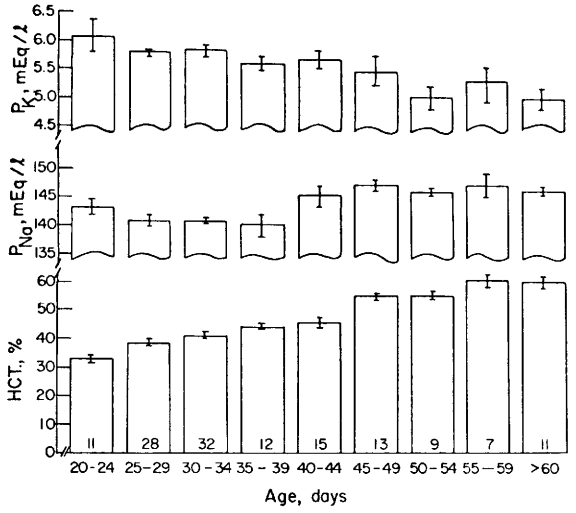

FIG. 1. Changes in plasma Na and K and hematocrit with development of rat pups. Numbers at bottom of columns indicate the number of rats in each group, here and in the following figures. Bars indicate SEM.

the opposite is true for U_KV. Because not all young groups show significant differences from all mature groups, we have analyzed the comparison of group means before 40 days of age as compared to after 45 days of age. The analysis is presented in Table I and indicates a highly significant difference in excretion of the two ions before and after the 40- to 45-day critical period.

Since both plasma concentrations and urinary excretions are changing during development, a better insight into the changes in renal function may be obtained by looking at fractional excretions of these ions. The individual age group data are shown in Fig. 3, and the group analysis is presented also in Table I. It is clear that a marked increase in FE_{Na} occurs at the 40- to 45-day critical period, while a decrease in FE_K takes place at the same time.

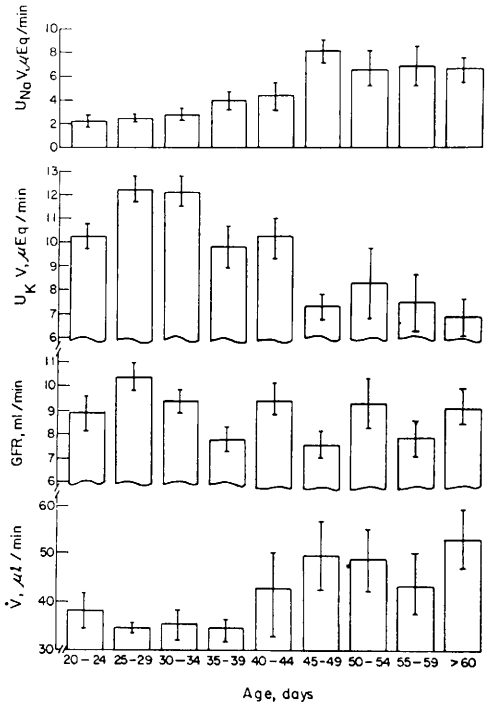

FIG. 2. Developmental pattern of changes in excretion of Na and K, and in $\dot{V}$, as well as changes in GFR. Bars show SEM. All data are normalized per kilogram of body weight.

Metabolic studies. Food intake, sodium, and potassium excretion are shown in Table II. As animals get older, food intake decreases when normalized for body weight. Water intake shows a parallel reduction. Renal excretion of both sodium and potassium decreases. The decrease, however, does not parallel the decrease in food intake. If the data are calculated as $U_{Na}V$ per gram of food ingested, there is an increase of about 20% between the youngest animals (27–30 days) and the animals 40 days of age (Table II). Thereafter, there is a second abrupt rise, with $U_{Na}V$ again doubling. A similar pattern is shown in excretion of potassium. The increase, however, in U_KV is less than half of the increase in $U_{Na}V$. Over the age range studied, U_KV always exceeds $U_{Na}V$. If, however, we examine the urinary Na/K ratio (Table II), we then find that up until 40 days postnatally it is constant, but then rises abruptly to a significantly higher level. These changes in Na/K are consistent with the changes in electrolyte excretion we observed in our clearance studies.

Discussion. These studies show that: (i) Both plasma sodium concentration and hematocrit increase as rat pups age; (ii) urinary electrolyte excretion during clearance studies also changes with age so that sodium excretion increases and potassium excretion decreases at the same "critical period," 40 days; (3) although absolute rates of Na and K excretion in metabolic studies are not like those in the clearance ratios, changes in Na/K excretion ratios

TABLE I. COMPARISON OF URINARY EXCRETION OF SODIUM AND POTASSIUM IN INFANT AND MATURE RATS.

	$U_{Na}V^a$	$FE_{Na}{}^b$	U_KV^a	$FE_K{}^b$
Infants	2.876 ± 0.398	0.249 ± 0.048	11.130 ± 0.637	22.2 ± 1.07
Mature	7.020 ± 0.354	0.655 ± 0.07	7.515 ± 0.296	17.0 ± 0.47
t	7.73	5.13	5.14	4.35
P	<0.001	<0.005	<0.005	<0.005

[a] $U_{Na}V$ and U_KV are expressed as milliequivalents per minute per kilogram body weight.
[b] FE_{Na} and FE_K are expressed as percentage of filtered load.

are consistent with the results obtained in clearance studies.

Although considerable work has been done on age-dependent changes in composition of body fluids postnatally, we are able to find but one report on developmental changes in plasma electrolytes in the rat. Yunibhand and Held found a P_{Na} of 142.8 meq/kg of H_2O in 1-day-old animals after 24 hr of water deprivation (12). P_{Na} then fell to 136 meq/kg of H_2O at 15 days, which was in turn succeeded by a rise which reached mature levels at 40 days. Comparable postnatal increases in P_{Na} have been described for the mouse (13) over the first 40 days of life. In the dog (14), there is a suggestion of a change in P_{Na} during the first postnatal week, while in man no postnatal age-dependent changes in P_{Na} are evident (15). A marked change in P_{Na} is found only in rodents, suggesting a species dependence. There are fewer differences between laboratories in reports about postnatal changes in P_K. In man (15), dog (14), and rat (12), there is a decrease in P_K after birth, although the time courses are different. In the dog, the change occurs during the first postnatal week, while in the rat it has been reported to continue until animals are 20 days of age (12). This latter finding is inconsistent with the data reported here, in that our youngest animals (20–25 days old) had the highest P_K and the trend was for a continuous decrease over the full age range for study. In the mouse, the postnatal change in P_K is equivocal (16).

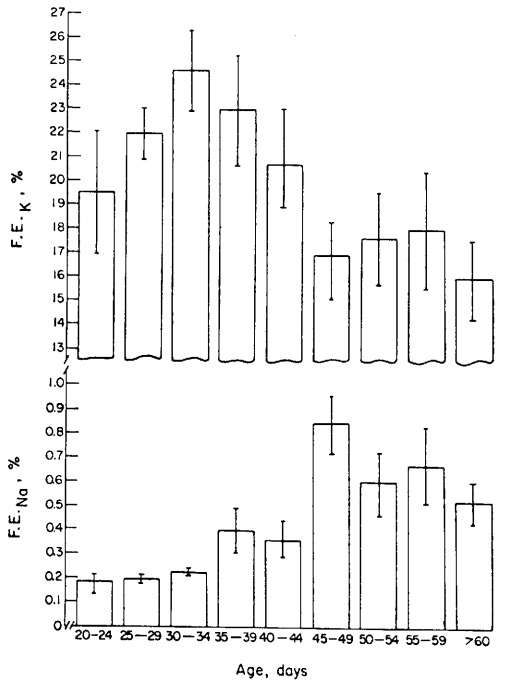

FIG. 3. Changes in fractional excretion of Na and K as rat pups grow older. Bars show SEM.

Kersten and Braünlich also found that sodium excretion increases with age or, as shown here (Fig. 2), in parallel with the change in control P_{Na}. In contrast to their studies, the excretion of K is reduced during development. In the study of Kersten and Braünlich (17), they found a minimum in K excretion at 15 days, with increasing rates of excretion as animals aged. The trend was not continuous, however, since 5-day-old rats had a high excretory rate. The results of these studies are in distinct contrast to ours. Since this study group also used Wistar rats, it is unlikely that this contradiction is a result of differences in rat strains. Our own metabolic results show changes in electrolyte excretion which are consistent with the excretion data obtained during clearance studies, so that one cannot account for the discrepancy on the basis that Kersten and Braünlich carried out their studies in metabolic cages.

TABLE II. FOOD INTAKE AND ELECTROLYTE EXCRETION OF CONTROL RATS DURING GROWTH.

Age (days)	N	Food intake (g/day/kg)	Water intake (ml/day/kg)	$U_{Na}V$ (meq/day/kg)	U_KV (meq/day/kg)	Na/K
<30	22	16.1 ± 0.28	31.2 ± 1.3	18.91 ± 1.03	24.48 ± 0.82	0.773 ± 0.030
31–35	26	14.1 ± 0.42	24.6 ± 0.97	18.42 ± 0.43	23.78 ± 0.49	0.776 ± 0.013
36–40	24	12.1 ± 0.18	21.8 ± 1.15	15.67 ± 0.35	20.12 ± 0.45	0.781 ± 0.012
41–45	26	11.0 ± 0.17	18.5 ± 0.40	15.30 ± 0.45	18.24 ± 0.43	0.840 ± 0.016
46–50	20	9.7 ± 0.19	17.3 ± 0.87	14.24 ± 0.31	16.92 ± 0.38	0.844 ± 0.016
51–55	10	9.2 ± 0.16	14.1 ± 0.36	14.15 ± 0.42	16.67 ± 0.56	0.850 ± 0.014

Other possibilities which could play a role are differences in route of fluid administration, strain, and food availability (18). Unless animals are grown under identical conditions, and are of the same strain and on the same diet, such differences as have been found may occur.

The question arises as to biological significance of the changes in electrolyte balance. If one examines the growth curves of rats in our laboratory, we get curves which are similar to those previously described by Widdowson and McCance (19). A striking feature of these curves is that there are two inflection points in the curve: one at about weaning, when there is an acceleration of absolute growth, and the other at about 40 days, when absolute growth rate reduces. Figure 4 shows the absolute growth rates for animals in our laboratory. Since food intake shows a continuous decline with age in our metabolic studies, one can hypothesize that one contributing factor is the need for sodium retention to accommodate the period of rapid absolute growth.

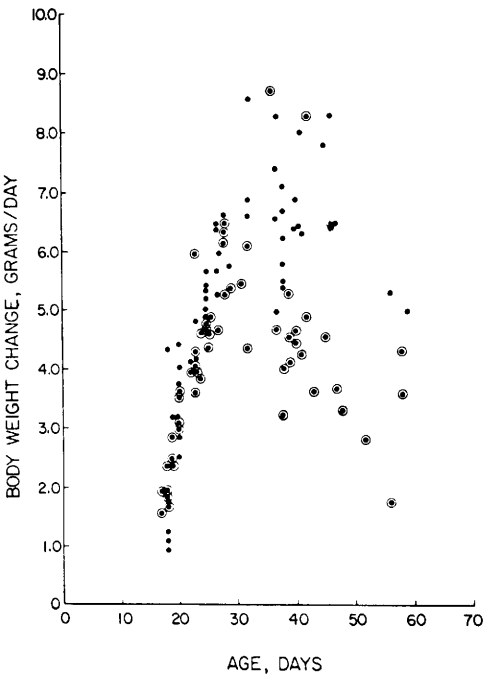

FIG. 4. Daily growth rates of rats as a function of age. Solid points show males; females are indicated by circled points.

Another factor which may be of significance is that tissue K relative to Na increases to 40 days of age, suggesting a maturation of the regulation of cellular to extracellular ionic ratios as has been found for muscle and brain (10, 20). Intracellular K increases in these tissues during this time period. No comparable studies are known for the kidney. Relatively more ingested K may be retained intracellularly in the mature to account for the decrease in K excretion.

One consequence of the relatively low plasma and $U_{Na}V$ is that the low plasma K may signal the kidney to conserve sodium. Such a signal may override the renal responses to blood volume expansion or saline loading and thereby be partially responsible for the inability of immature rats to respond appropriately to these stimuli (4, 8).

Summary. Rats show a critical period in electrolyte balance at around 40 days of age. P_{Na} and $U_{Na}V$ increase abruptly and U_KV falls at this time. P_K shows a continuous decrease over the age ranges studied. Hematocrit shows a hyperbolic increase. In metabolic studies, urinary Na/K shows a significant increase at the same critical period.

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