Renal Compensation for Body Water Loss during Dehydration in Neonatal Rats

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Abstract: The present study was designed to investigate whether the limited capacity for concentrating urine in neonatal rats is associated with an immature ability to regulate serum osmolality. During milk deprivation, the percent reduction in body weight per 10 h (mean±SE) was 4.3±0.2, 3.7±0.1, 4.8±0.2, and 6.0±0.1% in 4-, 7-, 10-, and 14-d-old rats, respectively (n=23–24, each age). The osmolality of urine increased to 718±12 (4 d), 741±28 (7 d), 792±20 (10 d), and 1,203±41 mosmol/kg H₂O (14 d). Free-water absorption (T₀H₂O) promptly increased after deprivation of milk: It significantly increased from 2.3±0.3 (0–4 h) to 3.4±0.1 (4–7 h) (4 d), from 3.1±0.3 to 4.1±0.3 (7 d), from 3.6±0.4 to 5.2±0.3 (10 d), and from 5.0±0.4 to 7.9±0.7 µl/min/100 g (14 d). The raised values were maintained at the later period of dehydration. Thus serum osmolality was unchanged throughout dehydration: 287±1.0 (7 d) and 292±0.9 mosmol/kg H₂O (14 d). On the other hand, the level of serum sodium concentration slightly but significantly increased (r=0.61) when the body weight reduction was higher than 5% of the control (14-d-old rats). These results indicate that neonatal rats of 4–14 d control their serum osmolality by reabsorbing free water in the kidney during the 10 or 12 h of milk deprivation. [Japanese Journal of Physiology, 48, 181–187, 1998]

Key words: neonatal rats, urine and serum osmolality, serum sodium concentration, osmoregulatory mechanism, free-water absorption.

Past studies on renal functional development during the early to late neonatal period indicated that urinary concentrating capacity is limited in neonates [1, 2]. The neonates concentrate their urine to 40–65% of the maximum level observed in elder children (16 years old) [3] and to 30–50% of that in adult rats [1, 4]. Most research on this topic has been developmental studies of the renal concentrating urine system. Overall, these studies identified four factors in the maturation of the renal concentrating mechanisms. The first is the countercurrent system [5–8]. Morphological changes of nephrons [1, 9–11] and capillaries [8, 10, 12] in the kidney medulla occur from 10 to 20 d of age. The second is urea recycling in the kidney medulla [1, 10]. Trimble [10] demonstrated that urea loading increased urinary osmolality in 20-d-old rats, but not in 10-d-old rats. Antidiuretic hormone (ADH) is the third factor. It has been shown that the level of plasma ADH concentration increases in response to prolonged dehydration (24 h) at 7 d of age [1]. Finally, Rane and Aperia [13] demonstrated that Na⁺/K⁺ ATPase activity of the kidney tubules (thick ascending limbs) developed from 16 to 20 d of age.

The research on these factors persuades us that the maturation of renal-concentrating ability in rats is completed within the first 2 or 3 weeks. However, the previous research studies did not refer to compensation for water loss during dehydration and may have overly emphasized that the osmoregulatory systems of the early neonates (4–10 d) are immature in function because of their low renal concentrating capacity.

Therefore this study intended to estimate whether, and to what degree, neonatal rats could maintain body fluid osmolality by concentrating their urine. It was also an important consideration in the design of this study that all animals used for collecting urine were living through the series of experiments (4–14 d).
MATERIALS AND METHODS

A total of 171 neonatal Wistar rats (male and female) were used. Each litter, usually 10–12 pups, was kept intact with their mother except on the days of experiments. The mothers were allowed water and laboratory food freely.

Experimental animals. To standardize data analysis in the development of renal concentrating ability, we used neonatal rats from a single litter for one complete series in the study. They were exposed to nearly identical dehydration at 4, 7, 10, and 14 d of age. To minimize variations in blood hormone concentrations because of circadian rhythm, we removed the neonates from their mother at about noon on the days of the experiments. They were numbered on the tail and placed singly into small plastic boxes lined with paper, which were kept at 30/31°C by circulating temperature-controlled water [14–16].

Collection of urine. Urine was collected from the external urethrae on a given time schedule of 0, 4, 7, 10, and 12 h after removal from the mother by probing and/or rubbing the suprapubic area with a cotton stick. The quantities thus obtained were so small (ranging approximately from 20 to 60 mg in the later period of dehydration) that it seemed preferable to weigh the urine rather than to rely on the determination of volume. Since the specific gravity of urine is not far from unity [4] and since its possible changes during dehydration are within 2% of the control [17], the weighed values (mg) of urine volume were used for analysis (μl) without modification throughout the present study. Feces passed during rubbing were less than a few mg and were discarded. Animals that passed urine between the periods of collection were excluded from the analysis.

Collection of blood. Some of the neonatal rats were lightly anesthetized with ether and decapitated. The blood (0.3–0.8 ml) was collected from the neck stump without heparin. Within 20 min it was centrifuged for 3 min at 6,000 r.p.m. The supernatant (serum) was stored in a refrigerator (4°C) until measurement.

Osmolality and Na⁺ concentration. The osmolality of urine and serum was determined by the method of freezing-point depression (Vogel OM801, Gießen, Germany, or Fiske One-10, Norwood, U.S.A.). Each sample was measured at least in duplicate. The concentration of serum Na⁺ was measured with an automated electrolyte (Na⁺, K⁺, Cl⁻) analyzer (Radiometer ABL505, Copenhagen).

Determination of extrarenal water loss. Body weight changes during dehydration were determined by weighing the neonatal rats on an electrical balance (precision 1 mg) before and after the collection of urine. Extrarenal water loss was determined as the difference between body weight loss and the sum of urine [4, 14, 15].

Free water absorption. Free-water absorption ($T_{H_2O}$) [18] can be given by the following equation.

$$T_{H_2O} = (U_{osm} - P_{osm}) / P_{osm} \times V$$

where $U_{osm}$ and $P_{osm}$ are the osmolality (mosmol/kg H₂O) of urine and plasma; $V$ is volume flow of urine (μl/min) voided in a given collection. In calculation, the values of serum osmolality were substituted for $P_{osm}$ in the present study.

Statistics. Values were expressed as means±SE and were normalized by body weight [2, 19]. Significance was assessed by either Student’s t-test or in multiple comparisons by analysis of variance (ANOVA). $p$<0.05 was accepted for statistical significance. Unless otherwise mentioned, n indicates the number of rats.

RESULTS

Body weight loss

On the four experimental days, body weight changes were determined by weighing neonatal rats before and after the collection of urine on a given time schedule of 0, 4, 7, 10, and 12 h after deprivation of milk. Percent reduction in body weight during the 10 h of milk deprivation was 4.3±0.2 (4 d), 3.7±0.1 (7 d), 4.8±0.2 (10 d), and 6.0±0.1% (14 d) ($n$=23 or 24, each age). Figure 1 illustrates time-dependent changes in the relative body weight before collecting urine at 30/31°C. A significantly larger decrease in body weight (represented by a steeper slope in the figure) was observed in the elder group (10- and 14-d-old rats), compared with the younger group (4- and 7-d-old rats) ($p$<0.001, ANOVA).

Milk serves essential as both food and water to the neonates. To eliminate the unexpected effects of deprivation of milk, such as starvation, the growth rate was examined through the series of experiments (4–14 d). We observed that the body weight in three litters increased linearly over this period: The mean values (±SE) were 12.1±0.3 (4 d), 17.3±0.5 (7 d), 23.6±0.6 (10 d), and 30.5±0.6 g (14 d) ($n$>20, each age). Furthermore, the growth rate of the neonates used for dehydration experiments (experimental, $n$=8) was similar to that of three neonates from the same litter, which had been nursed all the time by their mother (control, $n$=3). They probably restored their weight on the days between the experiments. More-
over, there was no significant difference in maximal urine osmolality between the two groups at 14d of age: 1,306±113 (control) and 1,203±41 mosmol/kg H₂O (experimental) ($p>0.3$). Thus we are convinced that the neonatal rats used in the present study developed normally.

**Urine volume**

Urine was collected at 0, 4, 7, 10, and 12 h after removal from the mother. Urine flow per body weight decreased from 3.1–5.6 (in the first period of water deprivation) to 1.7–3.0 μL/min/100 g (in the last period). Figure 2 illustrates decreasing and leveling off of the urine flow rate with the progress of dehydration. The urine output of 4-d-old rats showed a relatively greater fall between the 4th and 7th hour of collection than the elder rats did during the same period. It is speculated that in the elder group, the renal osmoregulatory mechanisms had already been switched on decreasing the level of urine flow in the first period (0–4 h). The steady-state rate of urine flow (1.7–3.0 μL/min/100 g) in the present study did not largely differ from the rate observed in newborn rats (3.7 μL/min/100 g) [15] and that in infant or adult rats (2.2 μL/min/100 g) [5].

**Extrarenal water loss**

Extrarenal water loss [4, 14, 15] was determined as the difference between body weight loss and sum of urine. This is approximately equal to the amount of water lost by insensible evaporation from skin and lungs. Figure 3 showed that the values per body weight were nearly unchanged during the period of dehydration within the same group, but were significantly different between ages. The rate of extrarenal water loss was much higher in 14-d-old rats (6.8 μL/min/100 g or 0.45% decrease per hour) than in 4-, 7-, and 10-d-old rats (3.6–4.8 μL/min/100 g or 0.21–0.28% decrease per hour) ($n=23$ or $24$, each age). Extrarenal water loss primarily depends on temperature and humidity in surroundings [20], but it is also dependent on biological factors such as exercise and body hair. These factors may account for the larger rate of extrarenal water loss in 14-d-old rats (Fig. 3). By 14 d of age, some rats have begun to grow body hair, to open their eyes, and to move around in their plastic boxes (18 cm in diameter) through the present experiments. We also found that the rate of 0.21–
0.28% was equivalent to those of adult rats kept at 20/21°C and of newborn rats kept at 33/34°C [15]. By using a standard temperature of 30/31°C, we could compare the data in the present paper with those obtained from animals of different ages.

**Urine osmolality**

Time-dependent changes in urine osmolality were examined during the period of dehydration. In 4-, 7-, and 10-old rats, the osmolality of urine was hypotonic (225–270 mosmol/kg H2O) at time 0. It increased in the first 7 h of dehydration and was nearly saturated in the later period (Fig. 4). The maximum osmolality of urine was 718±12 (4 d), 741±28 (7 d), and 792±20 mosmol/kg H2O (10 d) (n=16–24). In contrast, in 14-old rats the urine osmolality was hypertonic (480±38 mosmol/kg H2O) at time 0. It increased and was not saturated even in the last period of dehydration (10–12 h) (Fig. 4). The maximum osmolality of the urine (1,203±41 mosmol/kg H2O) was therefore much higher than in the younger rats.

**Serum osmolality**

Changes in the osmolality of serum and urine were studied during the 10 h of dehydration in 7- and 14-old rats (2 litters at each day of age). In this and the next series of experiments, the neonates were sacrificed to collect the blood just after the collection of urine. We found that serum osmolality was unchanged during the 10 h of dehydration, although the urine osmolality increased progressively (Fig. 5). Mean values of the serum osmolality were 287±1.0 (7 d) and 292±0.9 (14 d) (n=24, each age). There was a small but significant difference in serum osmolality between the two groups (p<0.05).

**Serum Na⁺ concentration**

Figure 6 illustrates the relationship between the serum Na⁺ concentration and the body weight reduction (% of control) during the 10 h of dehydration in 7- and 14-old rats. We could not detect a change in serum Na⁺ concentration (r=0.25) when the reduction was within 3% of the control body weight (7-old rats, n=12). However, serum Na⁺ concentration significantly increased (r=0.61) when the reduction reached 5% of the control (14-old rats, n=12).

**Free-water absorption**

The kidney produces urine and maintains home-
Compensation Ability for Body Water Loss

Fig. 7. Time-dependent changes in free-water absorption for each age after milk-restriction (n=6-24, each point). *p<0.05 and **p<0.01 compared with the initial rate (0-4 h) within the same group. $p<0.001$ compared with the data at 7 d by ANOVA.

Fig. 8. Time courses of compensation for water loss for each age during dehydration. All plots indicate mean±SE (n=6-24, each point). †p<0.05 between the groups of different ages by ANOVA.

Ostasis of body fluid volume, electrolytes, and osmolality. Free-water absorption (T\textsuperscript{\textdegree}H\textsubscript{2}O) [18], which is functionally in proportion to NaCl transport at the medullary thick ascending limb of Henle [13], can be given by Eq. 1 (MATERIALS AND METHODS). In the present study, the values of serum osmolality of 287 mosmol/kg H\textsubscript{2}O (7 d) were substituted for P\textsubscript{osm} of 4- and 7-d-old rats, and those of 292 mosmol/kg H\textsubscript{2}O (14 d) were substituted for P\textsubscript{osm} of 4- and 7-d-old rats. Figure 7 illustrates the time-dependent changes in T\textsubscript{\textdegree}H\textsubscript{2}O during dehydration with the different ages. T\textsubscript{\textdegree}H\textsubscript{2}O of each age increased in the first 7 h of dehydration: It increased from 2.3±0.3 (0-4 h) to 3.4±0.1 (4-7 h) (4 d), from 3.1±0.3 to 4.1±0.3 (7 d), from 3.6±0.4 to 5.2±0.3, and from 5.0±0.4 to 7.9±0.7 μl/min/100 g (14 d) (n=16-24). T\textsubscript{\textdegree}H\textsubscript{2}O from 7 to 12 h in 10- and 14-d-old rats was significantly higher, compared with what it was in 4- and 7-d-old rats (n=6-8, each age).

Compensation for water

Extrarenal water loss during the short period of dehydration, determined as the difference between the body weight loss and the sum of urine, is approximately equal to the body water loss. Because serum osmolality was unchanged during dehydration (Fig. 5), the water loss must have been compensated for by absorbing free water in the kidneys. A ratio between the sum of free-water absorption and the extrarenal water loss may therefore be a good index of the compensation for water loss during dehydration. The ratio indicates that if the value is less than unity, the body fluid is dehydrated; if it is near unity, the fluid osmolality is well regulated by the kidneys. Figure 8 illustrates the time-dependent changes in the compensation index of each age. Mean values of the index were below unity (0.64–0.85) in the first 4 h of dehydration and increased gradually to and above unity during the later period. They were 0.97 (4 d), 1.00 (7 d), 1.09 (10 d), and 1.04 (14 d) in the last period studied.

DISCUSSION

This is the first research experiment to show the time-dependent changes in renal compensation ability for water loss during a short period of dehydration in neonatal rats without sacrificing them for urine collection.

Our findings led us to believe that neonatal rats can compensate for extrarenal water loss during the 10 or 12 h of dehydration. In the present study, we have postulated an index, a ratio of the free-water absorption over the extrarenal water loss, to evaluate the renal osmoregulatory ability. The index values for the first 4 h of milk deprivation were less than unity in all ages (Fig. 8), which indicates negative water balance of the body fluid. These values increased and reached unity in the later period of dehydration. It is interesting that although the time needed for the value of the index to reach unity was longer in 4-d-old rats than in the elder neonates, it did so in all ages. This supports the view that the osmoregulatory mechanism in neonatal rats functions even in a premature form. The maximum compensation capacity for water loss was 0.97–1.09 in the present study, whereas the maximum osmolality of urine was only one third to one half compared with that of adults [1]. The relatively higher urine flow rate (Fig. 2) and the lower body weight loss (Fig. 1) may contribute to the high compensation ability for water.
loss, especially at 4 d of age.

In the measurements of 4-, 7-, and 10-d-old rats, the urine osmolality at time 0 (just after removal from their mothers) was 225–270 mosmol/kg H₂O, which was below the range of serum osmolality of 285–299 mosmol/kg H₂O (Figs. 4, 5). This suggests that neonatal rats usually take the surplus amount of milk from their mothers to compensate for the sum of renal and extrarenal water loss [15]. As a consequence, water balance can be maintained in normal suckling rats without concentrating urine. Therefore the lower abil-

ity of renal concentration is not basically a great threat, at least in normal circumstances, but it must be completed by weaning at 2–3 weeks after birth [1, 2, 9]. It is quite reasonable that 14-d-old rats fed with milk excreted the concentrated urine even at time 0 because they may have accustomed themselves to weaning.

The maximum osmolality of the urine in the present study was 718±12 (4 d), 741±28 (7 d), 792±20 (10 d), and 1,203±41 mosmol/kg H₂O (14 d). When we consider the changes resulting from development, these values are comparable to those reported by other researchers with 10-d-old neonatal rats (800–900 mosmol/kg H₂O) and 20-d-old rats (1,200–1,400 mosmol/kg H₂O) [1, 10]. It should be noted that these values are still only 30–50% of those in adult rats (2,000 mosmol/kg H₂O) [1, 4]. In contrast, the urine osmolality of 14-d-old rats increased progressively during dehydration and was not saturated even in the last experimental period from 10 to 12 h (Fig. 4). This finding apparently differs from the earlier observation that 8 h dehydration was sufficient to produce a maximally concentrated urine in 10- and 20-d-old rats [10]. The reason for the inconsistent results may be due to the differences in length of dehydration or because different animals were used. Synergistic interactions between foot shocks (experimental stimulus) and hypovolemia on vasopressin secretion have been reported by others [21]. Similarly, it is possible that the periodical physical stimulation for collecting urine during dehydration experiments may have affected the renal concentrating ability of the neonates.

Extrarenal water loss during the first 4 h after depriva-
tion of milk was about 1% of the body weight (4- to 10-d-old rats). This indicates that plasma osmolality should increase maximally by 1.4% (4 mosmol/kg H₂O) when we assume that in neonatal rats, 70% of body weight is water and that the gradual water deficit is distributed evenly throughout the body. Since the urine osmolality during the first 4 h of dehydration was significantly higher than that of the control (Fig. 4), the reduction in body weight may be large enough to cause a neurohypophysial hormone release (arginine vasopressin, AVP) [22]. In contrast, it is also true that serum osmolality did not increase over the period of dehydration (Fig. 5). This finding suggests that the osmoregulatory system could be stimulated by a minute (undetectable) change in serum osmolality. Its calculated threshold, being less than 4 mosmol/kg H₂O of difference (1.4%), is surprisingly within the normal range of healthy human adults (0.2–1.7%) [22]. A very tiny increase in serum osmolality may be automatically regulated by free-water absorption in the kidney. This is in agreement with the result obtained from calves in the neonatal period [23]. Skrzypczak [23] reported that 2-week-old calves increased urine osmolality and maintained a constant serum osmolality. In contrast, the plasma osmolality definitely increased when animals were exposed to a hot environment for 6 h [24] or were defective in AVP (Brattleboro homozygotes (diabetes insipidus rats) [25]). It increased 8 and 6% in response to 11.4 [24] and 10.6% [25] body weight losses, respectively.

On the other hand, the change in serum Na⁺ con-
centration may be a more precise signal to the putative osmoreceptor in the hypothalamus [22]. The serum Na⁺ level significantly increased (r=0.61, Fig. 6) even when the relative body weight reduction was within 5% of the control (14-d-old rats). This result is comparable to that in human newborns: There was a significant increase in both the serum osmolality and sodium concentration when the reduction in body weight exceeded 10% of the birth weight [26]. In adult rats exposed to a hot environment, plasma Na⁺ concentration increased 12–15 meq/kg H₂O in response to 8–9% [27] and 11.4% [24] body weight losses, respectively. The changes in Na⁺ concentration in plasma and cerebrospinal fluid are believed to be an effective signal to release AVP in rats [22, 24].

In conclusion, the renal osmoregulatory mecha-
nisms of the body fluid in rats develop in a relatively mature form even in the early neonates, although the renal concentrating ability is still immature and is lower than in adults.

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186

186
Compensation Ability for Body Water Loss


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