

Development of Urinary Concentrating Capacity in Weaning Rats

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ABSTRACT. This study describes the development of the capacity to concentrate urine in 10- to 40-day-old rats that were normally weaned between 16 and 18 days, breast-fed until day 20, or abruptly weaned on day 16. Urine osmolality after 24 h dehydration was significantly lower in 10- than in 20- and in 20- than in 40-day-old rats. The most pronounced increase occurred between 16 and 20 days in the three groups. The increase in urine osmolality was paralleled by an increase of papillary Na and urea concentrations. The length of the papilla increased linearly from day 10 to 40. Serum arginine vasopressin levels were not significantly different in dehydrated 10- and 20-day-old rats. Serum corticosterone increased significantly between 10 and 20 days of age in both normally weaned and breast-fed rats. The rise was accelerated between days 16 and 18 and paralleled the rise in urinary concentrating capacity. Adrenalectomy on day 16 abolished the increases in urine osmolality and papillary Na and urea concentrations, but not the growth of the papilla between days 16 and 20. The urinary concentrating capacity could be precociously induced by treatment with betamethasone from day 10 to 17 but not from day 17 to 20. Our results indicate that the accelerated development of urine concentrating capacity at the time of weaning is independent of dietary composition and most likely modulated by glucocorticoid hormones. (*Pediatr Res* 19: 472-475, 1985)

Abbreviations

NW, normally weaned
PS, prolonged suckling
AW, abruptly weaned

The development of the capacity to concentrate urine mainly occurs postnatally. In newborn infants (1-3), rats (4), and dogs (5) dehydration and the administration of ADH generally result in a urine osmolality that is or only slightly hypertonic to plasma osmolality.

In the rat, the greatest change in urinary concentrating capacity (4, 6) is observed during the first 3½ wk of life. Weaning occurs during the 3rd wk of life in rats. It is well established that the development of the gastrointestinal tract is accelerated at the

time of weaning (7). These developmental changes are of importance for the dietary transition from milk to solid food. Since this transition also entails a decrease in the water turnover, one may expect developmental changes in the control of water metabolism during weaning.

It is the purpose of our study to describe the development of the urinary concentrating capacity in rats during the weaning period. In the small intestine the maturation of a series of enzymes and transport systems is accelerated at the time of weaning (7, 8). There is considerable evidence that the acceleration of maturation is induced not by the change in food composition but by the rapid increase in corticosterone production that occurs in weaning rats (8). In order to differentiate between the effect of a change in the food composition and the effect of intrinsic endocrine changes at the time of weaning we studied three groups of animals: rats that were NW, rats AW on day 16, and rats fed only breast-milk (PS) up to the age of 20 days. Serum corticosterone was determined in all groups of rats. Since we observed that the capacity to concentrate urine developed in parallel to a rise in serum corticosterone levels, we further investigated the role of glucocorticoid hormones for the development of the concentrating capacity by studying adrenalectomized and betamethasone-treated animals.

METHODS

The studies were performed on male Sprague-Dawley rats whose ages ranged from 10-20 and from 40-42 days. The younger rats were subdivided into three groups with regard to the weaning pattern.

NW rats. The pups were kept with their dam until day 19, but they also had free access to food, Anticimex R-3 rat food. The protein content of this food corresponds to 21% of the calories.

PS rats. The pups were kept with their dam until the day of the study. They did not have access to rat food. The dam was removed from the cage for 1 h twice daily for feeding. The weight gain in this group was significantly less than in group 1.

AW rats. The pups were separated from their dam on day 16 and were fed only Anticimex R-3 rat food.

One group of rats was adrenalectomized at the age of 16 days and studied at 20 days. Adrenalectomies were performed under light ether anaesthesia. Following a midline incision the adrenals were gently dissected free from the perirenal fat and the vascular pedicle was clamped. After adrenalectomy the rats were given normal saline to drink.

Three groups of rats were treated with betamethasone (Glaxo) 60 µg · 100 g⁻¹ intramuscularly. This dose has been shown to have a maximal inductive effect on renal cortical enzymes taken from infant rats (9). The rats in the three groups were treated from day 10-13, day 14-17, and day 17-20, respectively.

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Separate control groups were used for the adrenalectomized and betamethasone-treated rats. The size of the litters was reduced to four to six pups. The NW rats weighed 23 ± 0.5 g at 10 days, 42 ± 1 g (NW), 28 ± 0.3 g (PS), and 42.5 ± 2.1 g (AW) at 20 days and 172 ± 2 g at 40 days. An attempt was made to use as few litters as possible for each protocol.

To assess maximal urine concentrating capacity, the rats were deprived of food and fluid for 24 h. The suckling rats were removed from their dams. At the time of the study the animals were anaesthetized by an intraperitoneal injection of Inactin, (Byk-Gulden), $80 \text{ mg} \cdot 100 \text{ g}^{-1}$. Following a midline abdominal incision, the urinary tract was visualized, the bladder punctured, and urine samples were aspirated immediately from the bladder. The kidneys were then removed and a median slice, including the intact papilla, was excised from each kidney. Before removal of the papilla, its length was measured under a microscope. The measurements had an accuracy of ± 0.10 mm. The papilla was then removed from the rest of the medulla and rapidly frozen on dry ice. The entire procedure from opening the abdomen until freezing the tissue took approximately 1 min. All the animals survived the 24 h of dehydration and the brief anaesthesia afterward.

A cardiac puncture was performed to obtain blood samples in unanaesthetized dehydrated rats for arginine vasopressin determinations and in unanaesthetized normally hydrated rats for corticosterone determinations.

Analytical procedures. Urine and homogenized papillary tissue were analyzed for sodium, urea, and osmolality. Material from three to five rats was used for each determination. In a few rats in each age group the water content of the papilla was determined as the difference between the wet weight and the dry weight. The dry weight was obtained by drying the tissue at 100°C until a constant weight was achieved. The water content decreased from 93% at 10 days of age to 87% at 40 days of age. Sodium concentrations were determined using a flame photometer. Urea was determined using an enzymatic colorimetric method (Boehringer-Mannheim). Osmolality was determined with a freeze-point osmometer.

Corticosterone in $50\text{-}\mu\text{l}$ aliquots of serum was determined by radioimmunoassay, as described in detail previously (10). The lowest level of corticosterone that could be distinguished from zero concentration was 10 nmol/liter . The intra- and interassay coefficients of variation were 7 and 16%, respectively.

Arginine vasopressin concentration in serum was determined by means of radioimmunoassay, as described in detail previously (11). The serum was first extracted by the acetone-petroleum method (12).

Calculations. Values are given as the mean \pm SE, p values < 0.05 were considered significant. Analysis of variance was used to compare the results obtained from different age groups.

RESULTS

Development of concentrating capacity in NW rats. The developmental changes in urine osmolality and papillary tissue concentration of urea and sodium from day 10 to 20 are shown in Figure 1. Urine osmolality was significantly lower in 10- to 12-day-old rats than in 18- to 20-day-old rats ($p < 0.01$) and significantly lower in 18- to 20-day-old rats than in 40- to 42-day-old rats ($p < 0.05$). Tissue urea and sodium concentrations were significantly lower in 10- to 12-day-old rats than in the older rats. The greatest increase occurs from day 16 to 18.

The relationship between urine osmolality and age seems to fit a monoexponential expression ($r = 0.93$) better than a linear expression ($r = 0.81$).

The length of the papilla increased significantly from 10- to 12 days of age (1.4 ± 0.1 mm) to 40 days of age (3.5 ± 0.1 mm). The relationship between age and papillary length fitted an exponential expression with the same probability as a linear expression ($r = 0.94$).

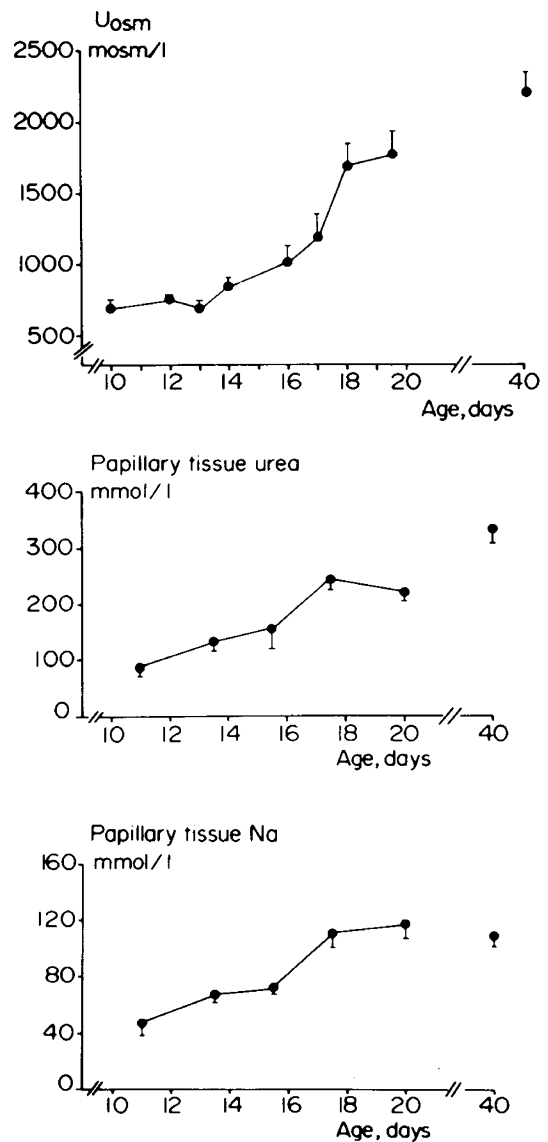


Fig. 1. Urine osmolality, papillary urea concentration, and papillary Na concentration following 24 h of food and fluid deprivation in NW 10- to 40-day-old rats. Values are the means of 3-11 determinations. The bars represent SE.

Development of urinary concentrating capacity during abnormal weaning patterns. Figure 2 illustrates the development of the urinary concentrating capacity in the PS group. An exponential increase in urine osmolality ($r = 0.90$) was found from 10 to 20 days of age. From 14-16 to 18-20 days there was a significant increase in the papillary tissue concentrations (mmol/liter) of both urea ($131 \pm 17 \rightarrow 196 \pm 20$) and sodium ($60 \pm 4 \rightarrow 93 \pm 8$) in the PS rats ($n =$ four to five in each group). A rapid increase in urine osmolality from day 16 to 20 also was observed in the rats that were AW at the age of 16 days (data not shown). Urine osmolality was not significantly different in 18- to 20-day-old NW, PS, and AW rats.

Hormone levels. Serum corticosterone increased significantly from 10 to 20 days of age in both the NW rats and the PS rats (Fig. 3, upper panel). The increase was steepest between 16 and 18 days of age. Serum arginine vasopressin was determined after 24 h of dehydration (Fig. 3, lower panel). Serum arginine vasopressin was not significantly different in 10- and 20-day-old rats and in 20- and 40-day-old rats. It was significantly higher in 40- than in 10-day-old rats.

Development of urinary concentrating capacity in betametha-

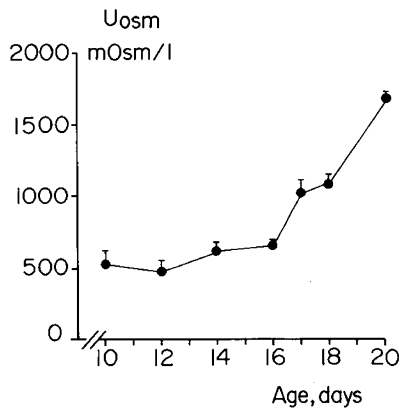


Fig. 2. Urinary osmolarity following 24 h of food and fluid deprivation in PS rats. Values are the means of four to six determinations. The bars represent SE.

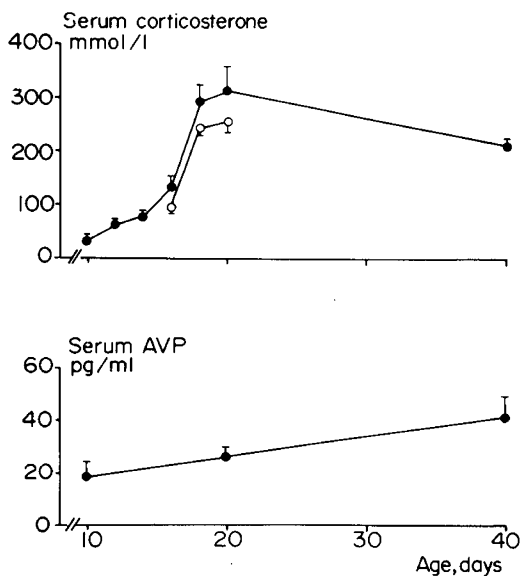


Fig. 3. Serum corticosterone was determined in normally hydrated rats that were NW (●) or PS (○). Values are the means of five determinations. The bars represent SE. Serum arginine vasopressin was determined in dehydrated NW rats. Serum from two to three rats was pooled. Values are the means of five determinations. The bars represent the SE.

sone-treated and adrenalectomized rats. Betamethasone significantly increased urinary concentrating capacity in rats aged 10–17 days, *i.e.* just before or during the period when there is a rapid increase in urinary concentrating capacity and serum corticosterone (Fig. 4). However, betamethasone did not further increase the urinary concentrating capacity when it had almost reached its maximum, *i.e.* in 17- to 20-day-old rats.

In the adrenalectomized 20-day-old rats urinary concentrating capacity and papillary tissue concentration of sodium and urea were the same as in 16-day-old control rats but significantly lower than in 20-day-old control rats (Table 1). The growth of the papilla was not inhibited by adrenalectomy. The length of the papilla was 3.1 ± 0.1 mm in the adrenalectomized 20-day-old rats and 1.93 ± 0.1 mm in the 16-day-old control rats.

DISCUSSION

This study shows that the development of urinary concentrating capacity in rats is accelerated at the time of weaning. The accelerated development of the concentrating mechanisms probably is due to accelerated renal maturation since the serum levels

of antidiuretic hormone were not significantly different in dehydrated 10- and 20-day-old rats.

It has been reported previously that the production of corticosterone increases at the time of weaning (8). The results of our study suggest that this is a biological event caused by intrinsic factors since it occurs in both the NW rats and rats that continue to be fed only breast-milk. Several observations in this study imply that maturation of the concentrating capacity at the time of weaning is modulated by glucocorticoid hormones. The increase in corticosterone production paralleled the development of the urinary concentrating capacity in both groups of rats; adrenalectomy at 16 days of age abolished the development of the concentrating capacity; and the capacity to concentrate urine was significantly increased by treatment with a synthetic glucocorticoid hormone from day 10 to 17 but not from day 17 to 20.

In accordance with previous reports (13) we found that the development of the capacity to concentrate urine at 10 to 20 days of age is accompanied by an increase in papillary sodium and urea concentrations. Adrenalectomy at day 16 not only prevented the increase in urine concentrating capacity but also the increase in papillary sodium and urea concentrations. High papillary sodium and urea concentrations depend on several factors, including active Na transport in the thick ascending limb of Henle and the action of ADH on the collecting ducts. Both Na-K-ATPase activity (5) and ADH-dependent cAMP production (14, 15) in the collecting duct cells could be targets for

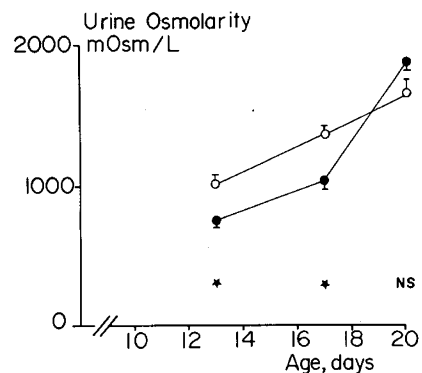


Fig. 4. Urine osmolarity following 24 h food and fluid deprivation in control rats (●) and rats treated with betamethasone $60 \mu\text{g} \cdot 100 \text{g}^{-1}$ for 3 days (○). There are four to six rats in each group. The bars represent SE. The asterisk denotes a significant difference between control rats and betamethasone-treated rats.

Table 1. Effect of adrenalectomy on the development of urinary concentrating development of urinary concentrating capacity during the weaning period (mean \pm SE)*

Age (days)	Urine osmolarity (mosmol/liter)	Papillary tissue urea (mmol/kg H ₂ O)	Papillary tissue Na (mmol/kg H ₂ O)
20 adx at 16 days	$804 \pm 36^\dagger$ <i>n</i> = 8	$153 \pm 17^\dagger$ <i>n</i> = 6	$61 \pm 4^\dagger$ <i>n</i> = 6
20 control	1926 ± 94 <i>n</i> = 8	235 ± 27 <i>n</i> = 7	109 ± 18 <i>n</i> = 7
16 control	987 ± 64 <i>n</i> = 8	139 ± 28 <i>n</i> = 5	64 ± 4 <i>n</i> = 5

* Urine osmolarity and papillary tissue concentrations of sodium and urea were recorded after 24 h of food and fluid deprivation.

† Significantly different from 20-day-old control rats. There was no significant difference between 20-day-old adx rats and 16-day-old control rats with regard to any of the parameters studied.

glucocorticoid. The activity of Na-K-ATPase, the enzyme mainly responsible for active sodium transport, has been shown to increase in the thick ascending limb of Henle from infancy to adulthood in rabbits (16). It is well established that Na-K-ATPase activity depends on the action of glucocorticoids (17). In preparations using proximal tubular cells it has been shown that Na-K-ATPase in immature cells can be prematurely induced by exogenous glucocorticoid administration (9). Immature collecting ducts (18, 19) have a lower sensitivity to ADH than mature collecting ducts. There is evidence that ADH-dependent cAMP can be induced by glucocorticoid hormones (14, 15). These data suggest that glucocorticoids induce one or more of the transport systems necessary for the creation of a hypertonic medullary interstitium.

It is generally agreed that increased protein intake and increased urea availability induced by urea infusion can enhance the capacity to concentrate urine. An increased protein intake has been shown to enhance the renal concentrating capacity in human infants aged 2–4 months (20). On the other hand, it has been shown that 10-day-old rats cannot utilize urea as well as 20-day-old rats to create a hypertonic papillary interstitium (21). Since the accelerated development of the urinary concentrating capacity was found in both the NW and the PS group, an alteration in protein intake is not a likely explanation of the accelerated development of the urinary concentrating capacity at the time of weaning.

It is well established from comparative species studies that the length of the loop of Henle is correlated to the capacity to develop a papillary osmotic gradient (22). We observed that the growth of the papilla, which may be taken as an index of the growth of the long loops of Henle, increased from 10–40 days of age. Edwards *et al.* (4) found that the most dramatic growth of the superficial loops of Henle in infant rats occurred between the ages of 10 and 16 days. In the present study adrenalectomy at day 16 inhibited the development of urinary concentrating capacity and the increase in papillary sodium concentration, but not the growth of the papilla. Thus it seems unlikely that growth of the loops is responsible for development of the concentrating capacity at the time of weaning.

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