Maturation of Renal Function in Infant Rats

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The physiological changes associated with the development of renal function require intensive investigation. It is apparent that newborn mammals of most species thus far studied are less able to compensate for water or salt excesses and deficits, and are more subject to changes in the volume and composition of body fluids (1-3). In general, urine excreted by newborn animals tends to be dilute and of relatively unvarying composition and volume (4, 5).

In the present investigation, the renal responses of infant rats to disturbances of osmotic and volume equilibrium were studied. The aim was to determine quantitatively to what extent renal function was altered during the postnatal period and to correlate changes in response to different stimuli. Such a study of maturation offers the possibility of identification of factors involved, by comparison of stages within a species relatively immature at birth. Ontogenetic changes in the alimentary tract, kidney, circulation and endocrine organs have been considered.

GENERAL METHODS

A stress, in the form of deprivation of food and water or of excess of substance to be excreted, was applied. Changes in urine volume and concentration were measured. The responses to specific experimental variables were always compared with responses by littermate control animals. Because of the deficiency in thermoregulation, experiments on infant rats were uniformly run when they were kept in air of 31°C.

Care and Selection of Experimental Animals. Healthy, young females of inbred Wistar and, occasionally, Holtzman strain were bred and maintained on checkers (Rockland rat diet) and water ad libitum. The offspring were kept with the mother except during the hours of an experiment. In order to insure adequacy of nutrition and hydration, no infant animal was used in an experiment unless it had gained at least 8% of its body weight over the preceding 24-hour period. Animals aged 0-24 hours were considered 1 day old, 24-48 hours 2 days old, and so on.

Warm distilled water or 0.5 M saline was administered by stomach through polyethylene tubing of ½ mm outside diameter. Usually 5% of the body weight of fluid was given.

Urine and Blood Collection. At half-hour or hour intervals urine was expressed from the bladder of the infant animals by applying suprapubic pressure and quantitatively collected in capillary pipettes. Since the specific gravity was low, the weight of urine was equated with volume. Bladder urine obtained immediately prior to an experiment was considered zero-hour urine. Urine flows were calculated as the percentage of body weight per hour.

Blood was obtained by decapitation with a sharp razor blade. Enough heparin powder to prevent clotting was added directly to the site of the cut. The blood was transferred for centrifugation by means of a capillary or long syringe needle to small melting point tubes with a flat bottom end made by sealing in a flame.

Analyses of Blood and Urine. For the determination of chloride in blood or urine, the micro-electro-metric titration method of Maurer (6) was adopted with some modifications. Titration was carried out in 0.08 N HNO₃. Samples were measured in a Linderström-Lang constriction pipette calibrated to deliver 6-10 µl.

To estimate total urinary solids, the refractive index of urine at 20°C was measured by means of a dipping refractometer. The results were usually expressed as the refractometer reading difference; that is, the scale reading on an unknown solution minus the reading for distilled water. Since the refractometer reading difference is an additive function of the concentration of each substance present, it is possible to estimate the total osmolar concentration. The refractometer was calibrated with sodium chloride and urea solutions of known concentrations. By the subtraction of that part of the refractive index due to sodium chloride alone, the concentration of other solutes was based on the assumption that they represent urea and other substances having the same refractive characteristics as urea. The total osmolar concentration was taken to be the sum of the "urea" concentration and twice the chloride concentration as determined by electrometric titration.

Statistical Analysis of Results. Small sample methods were used for the standard error of the mean. Fisher's t test was applied to determine the significance of differences (7). Differences were considered significant when they were below the 5% probability of identity.
Quantitative Aspects of Water and Electrolyte Excretion

Normal Development of Water Diuresis.

One of the characteristics of water diuresis in adult animals is the grading of excretion to water load. It was of interest to determine if the same generalization could be applied to infant animals. To this end, infant rats were subjected to moderate water excesses equal to 5% of the body weight given as one dose, or to higher water loads equal to 15% of the body weight, administered as three times 5% doses at half-hour intervals.

Urine flows in response to 5% of the body weight of water (fig. 1) show the absence of any significant rise in urine output in newborn rats upon hydration. A small, but significant, increase in urine flow was observed in 2-day old animals; maximum flow did not exceed \( 0.9 \pm 0.06 \%) \text{ of body weight per hour.} \) Urine flows increased progressively with age. An adult response obtained by 10–14 days of age.

In addition to lower urine flows of infant rats, another outstanding characteristic was a greater delay in the onset of diuresis. In the adult, urine flow rose above the initial value within 20–30 minutes after water was given, and reached a maximal value within 1 hour. In 2–3-day-old animals, increased urine flows did not begin until 30–60 minutes, reaching a maximal value only after 2–3 hours. Diuresis in 10–14-day-old rats had not only the same quantitative relations as adults, but also the same time relations. In animals of all ages, urine flow fell after reaching a maximum, although half or more of the water load still remained in the body. The rate of fall of urine flow in the descending portion of the diuresis curve was less in infant animals than in adult animals, commensurate with the higher water load remaining.

With increasing age and urine flows, there was a correspondingly increased dilution of urine. The time lag between water administration and the fall in urine concentration decreased as the animals aged. Maximal urine flows were associated with minimal urine concentrations. Hence, at this point maximal osmotic work in the elaboration of a hypotonic urine would be performed. In table 1 the theoretical work performed in the excretion of chloride has been calculated from the formula of Hill (8). The osmotic work per gram of kidney in the excretion of chloride by the newborn rat was one-fourtieth that of the adult. Nevertheless, it is significant that work was performed, and that the urine at birth was not an ultra-filtrate of the plasma. By 2 weeks of age the work per gram of kidney was approximately half the adult value. However, it can be shown that the osmotic work per
nephron was comparable to the adult, since at this age the number of fully formed nephra is one-half to two-thirds that in the adult (9, 10).

The administration of 15% of the body weight of water to newborn rats resulted in no significant increase in urine flow (fig. 2). This large excess of water proved lethal. Death occurred within an undetermined period in excess of 4 hours. Prior to death, the animals exhibited gross edema and cyanosis. Convulsions typical of water intoxication were not seen during the 4-hour period of the experiment. The probable cause of death was pulmonary edema.

In contrast to the newborn, 3-4 day old animals responded with marked increases in urine flow, reaching a maximum of 3.2 ± 0.4% of the body weight per hour. This is considerably less than that of the adult which equalled 6.8 ± 0.4% of the body weight with the same administered load (12). Although urine flows comparable to the adult on the basis of body weight after moderate water loads (5%) were observed by 10 days, age differences could be demonstrated with higher water loads. Urine flows of infant, but not newborn, rats were always greater after a 15% load than after a 5% load of water. In fact, urine flows of 3-day-old rats given 15% of the body weight of water equalled or exceeded those of adult rats which received 5% of water. Evidently when a water diuretic function appeared, the infant rat was able to attain some of the high urine flows which could be elicited from the adult, but required higher water loads as the stimulus.

Effects of Deprivation of Food and Water. How does an infant animal respond to dehydration, or more properly to deprivation of food and water, and how does this compare with the adult? Heller (5) has shown that newborn rats continued to excrete urine markedly hypotonic to plasma even after 24-hour deprivation, whereas adult rats produced urine markedly hypertonic to plasma. There was no demonstrable change in the blood of adults, but dehydration resulted in marked hemoconcentration and elevation of plasma urea in the newborn. The effect of 24-hour deprivation has been here investigated in 12-14-day-old rats and compared with the effect in adults.

Methods. Infant rats were denied access to the mother for 24 hours and were kept in an incubator at 30-31°C. Urine was collected during this period. In order to estimate excretion of water and solids of undeprived animals, urine was collected from littermates for 1 hour after removal from the mother. Excretion for 24 hours was calculated from the data for 1 hour. For comparison, adult rats weighing 225-300 gm were placed in a restraint cage (13, 14) in air of 22-24°C, and extra-renal water loss was calculated from total weight and urine and fecal losses during 24 hours. The 24-hour loss through the urinary channel of normal, undeprived adult rats was estimated from their excretion during the 1st hour after they had been placed on animal boards. Urine chloride was determined and the total osmolar concentration estimated from measurements of the refractive index and chloride concentration, as previously described.

Results. During the 24-hour deprivation period urine flows of newborn, 2-week and adult rats fell markedly (table 2). There was no difference (P > .2) in the urinary water losses per unit of body weight in any of the groups, infant or adult, when comparable methods of urine collections were used, contrary to the findings of Heller (5) of greater

### Table 1. Maximal theoretical osmotic work of the kidney in the excretion of chloride under conditions of moderate water loading

<table>
<thead>
<tr>
<th>Age, days</th>
<th>Max. Flow % Body Wt/hr.</th>
<th>Plasma Cl, mEq/l.</th>
<th>Urine Cl, mEq/l.</th>
<th>Kidney Wt. % Body Wt.</th>
<th>Osmotic work, cal/100 gm kid/hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(22)</td>
<td>0.6±0.1</td>
<td>67±9</td>
<td>0.96</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>9(16)</td>
<td>0.9±0.1</td>
<td>37±4</td>
<td>1.10</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>3-5(53)</td>
<td>2.5±0.1</td>
<td>30±4</td>
<td>1.42</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>6 (949)</td>
<td>2.0±0.1</td>
<td>20±1</td>
<td>1.52</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>10-14(17)</td>
<td>2.6±0.1</td>
<td>22±3</td>
<td>1.47</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>16-22(15)</td>
<td>2.8±0.1</td>
<td>15±3</td>
<td>1.36</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>Adult (4)</td>
<td>2.8±0.2</td>
<td>15±2</td>
<td>0.84</td>
<td>12.5</td>
<td></td>
</tr>
</tbody>
</table>

The number of animals is given in parentheses. Standard errors are indicated. Each group of plasma values represents the mean of 11-23 animals 1 hr. after hydration.

* Average chloride concentration at the time of maximal urine flow.
† Calculated from the tables of Donaldson (11).
TABLE 2. RESPONSES OF INFANT AND ADULT RATS TO 24-HR. DEPRIVATION OF FOOD AND WATER

<table>
<thead>
<tr>
<th>Age</th>
<th>Urine Flow, cc/100 gm/24 hr.</th>
<th>Extrarenal Loss, cc/100 gm/24 hr.</th>
<th>Cl. Conc. mEq/l.</th>
<th>Osmol. Conc., mOs/l.</th>
<th>Cl. Excr., mEq/100 gm/24 hr.</th>
<th>Osmol. Excr., mOs/100 gm/24 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn (1 day)</td>
<td>7.0 ± 0.9</td>
<td>93 ± 10</td>
<td>600 ± 50</td>
<td>0.6</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>Normal (16)</td>
<td>3.3 ± 0.25</td>
<td>4.0 ± 0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dehydrated* (14)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-14 day</td>
<td>7.4 ± 1.2</td>
<td>94 ± 7</td>
<td>760 ± 40</td>
<td>0.7</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>Normal (8)</td>
<td>2.9 ± 0.2</td>
<td>4.8 ± 0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dehydrated (12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-25 day</td>
<td>184 ± 43</td>
<td>1403 ± 125</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (7)</td>
<td>14.4 ± 2.9</td>
<td>204 ± 24</td>
<td>1930 ± 220</td>
<td>0.6</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>Dehydrated (6)</td>
<td>3.1 ± 0.4</td>
<td>5.5 ± 0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Data of Heller (5).

losses via the renal route in newborn rats. Since urine excretion was two times greater in adult rats allowed food and water ad libitum than in suckling rats, there was a relatively greater fall in the urine output of adult rats during deprivation. The extra-renal water loss of deprived infant rats at 30-32°C was somewhat lower than that of adult rats at room temperature.

Despite the decreased urine flow during deprivation, there was no significant change in urine concentration in newborn rats (Heller, (5)). Two-week infants in the experiments reported here were able to concentrate urine above the control levels. Total urine concentration rose from 760 ± 40 to 1030 ± 50 (P < .01). The average urine chloride remained unchanged (P > .6). The urine of these animals was still significantly less concentrated than the adult whose average urine concentration during the deprivation period reached 1930 ± 220 mOs/l. (P < .002). The ability to elaborate urine markedly hypertonic to plasma, absent at birth, was gradually acquired with age. Random sampling of urine of normal, undeprived suckling rats of 3 weeks showed concentrations well within the adult range.

Development of Osmotic Diuresis. What other evidence is there for an increased ability to concentrate urine at progressing ages? Hypertonic solutions, administered to animals cause a marked disturbance of the internal environment, which is compensated by an increased excretion of the administered solute consequent to an increased urinary concentration and volume. McCance and Wilkinson (4) have shown that newborn rats respond to hypertonic sodium chloride or urea solutions with only small increases in urine concentration of these substances and with negligible increases in urine flow. A similar inability rapidly to excrete administered solids was found in the human infant (3).

The response to hypertonic saline was studied in infant rats in order to determine at what age osmotic diuresis appeared and to correlate this with other tests of renal function.

Methods. Warm solutions of 0.5 sodium chloride were administered by stomach tube to the extent of 5% of the body weight. Urine was collected hourly and its chloride concentration determined. The effects on urine flow and chloride concentration in infant and adult rats are recorded in figure 3. In all groups there was increased urine flow, but maximal flows prior to 3 weeks of age were delayed compared to the adult. Urine chloride concentration rose rapidly in all groups, and was maintained at a high level for many hours. Urine chloride of 20-25-day-old animals was significantly higher than that of 4-8 day olds (358 ± 13 versus 225 ± 22 mEq/l., P < .001) and was comparable to adult concentration under similar conditions. Since urine flows and chloride concentrations rose more rapidly and to higher values in the older groups, there was a correspondingly faster elimination of chloride.

By 3 weeks of age, the infants, though not yet weaned, ate some solid food and drank...
water voluntarily. At that age an adult response to hypertonic saline by stomach and by intraperitoneal injection obtained (14). The ability to excrete a strongly hypertonic urine upon dehydration and the ability to excrete administered solute loads appeared to develop in parallelism.

Relationship of Specific Organ Systems to Water Diuresis

Absorption of Water From the Alimentary Tract. The possibility that changes in the absorption of water from the alimentary tract might be an important factor in the developmental response to water was investigated, since Heller (15) showed that absorption was appreciably slower in newborn than in adult rats.

Methods. Warm water equal to 5% of the body weight was given by stomach tube and the animals were decapitated at the end of 1 or 2 hours. The gastrointestinal tract was ligated and weighed, then dried in an oven at 100°C to determine the dry weight. The calculation of the amount of water remaining in the tract was based on the dilution generalization: \( C_1V_1 = C_2V_2 \) where \( C_1 \) is the percentage dry weight of the GI tract, \( V_1 \) the wet weight before the addition of water, \( C_2 \), the percentage dry weight and \( V_2 \) the wet weight after the addition of water. The amount of water remaining in the GI tract will be equal to: 

\[ V_2 - C_2V_2/C_1. \]

The value of \( C_1 \) was determined from littermate controls, \( V_2 \) and \( C_2 \) from the wet and dry weight of the alimentary tracts of animals which received water. Implicit in the method is the assumption that the solids of the GI tract in any given animal remain constant, at least relative to the movement of water during the short time period used.

The rate of water loss by all routes was determined simultaneously with the rate of water absorption. The total amount of water in the tissues could, therefore, be calculated.

Results. The rate of absorption of water
Table 3. Water Absorption from the Alimentary Tract

<table>
<thead>
<tr>
<th>Age, days</th>
<th>Dose in G.I. Tract. %</th>
<th>Net Tissue Load. % Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 hr.</td>
<td>2 hr.</td>
</tr>
<tr>
<td>1 *</td>
<td>75</td>
<td>46</td>
</tr>
<tr>
<td>3</td>
<td>53 ± 3</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>6-8</td>
<td>57 ± 5</td>
<td>5 ± 3</td>
</tr>
<tr>
<td>Adult *</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Adult †</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

* Estimated from the data of Heller (15). Adults in this series had milk in stomach prior to water administration.
† Data of Adolph and Northrop (16).

from the alimentary tract of 3-8-day-old animals lay intermediate between newborn and adult (table 3). The absorption rates of the 3-day and 6-8-day-old groups were not significantly different (P > .8). However, there was a significant difference in excretion between these two groups. Of the end of 2 hours the 3-day-old rats excreted renally 44.8% ± 1.3% of the dose, the 6-8-day animals 60.5 ± 3.7% (P < .01). As might be expected, the water load of the tissue, exclusive of the GI tract, would be greater in the younger group. It will be noted that the net tissue water excess built up slowly in the newborn (calculated from the data of Heller) because of the slow rate of absorption. It continued to rise even after 2 hours as a result of the failure to increase excretion. By the 6-8th day excretion was rapid enough to reduce the tissue load considerably at the end of 2 hours.

It is evident that the absorption rate exceeded the excretion rate at all ages, but that the divergence between the two was exaggerated in infant animals. The difference in the urinary response to water of 3-day-old and 8-day-old animals and possibly adults can be attributed to maturation of water excretion and not to differences in water absorption.

Renal Ontogenetic Alterations. Excretion has been shown to change during development. Maturation of the kidney as an end organ of excretion may underlie many, if not all, of the ontogenetic differences which have been observed. Considerable differentiation occurs in the rat kidney after birth, with a large increase in the number of functional units of the kidney (9, 10, 17). Experiments were designed to determine the effects on water diuresis of a contrary reduction in the number of nephra by means of unilateral nephrectomy. An attempt was also made to study renal changes by means of clearance techniques.

Effects of unilateral nephrectomy. Operations were performed in the unfasted state under cold narcosis. The left kidney was removed in all cases. Mock operations were performed in littermate control animals. In order to obviate the complications of compensatory hypertrophy, the animals were tested for their response to 5% of the body weight of water within 2 days and, in some cases, as early as 2-3 hours following operation.

No significant difference in urine flows was found in comparing a group of 3-4-day-old unilaterally nephrectomized animals and their littermate controls (eight observations in each group). Peak urine flows were 1.45 ± 0.11 in the partially nephrectomized group and 1.52 ± 0.12 of the body weight per hour in the control group (P > .6) at 13 hours following water administration. The average minimal urine chloride concentration of partially nephrectomized rats was 30 ± 4 and of controls 32 ± 7 mEq/l. (P > .9).

A group of animals, aged 7 days, was also tested 2-5 days following operation to see what effect, if any, the removal of one kidney would have on the maturation of the excretory response to water. Again no difference was found in the urine flows of six partially nephrectomized animals and their littermate controls (peak flows 1.96 ± 0.09 in the former group and 2.21 ± 0.19% of body weight per hour in the latter group, (P > .2)).

It has been shown that the low urine flows, compared to the adult, which follow the administration of 5% of the body weight of water do not represent maximal flows of the infant rat kidney since greater flows can be elicited by increasing the water load. By unilateral nephrectomy, the doubling of excretion by each kidney could be evoked without raising the administered water excess.

Insulin clearances. Methods. A method similar to that used by Dicker and Heller (18) on adult rats was adopted. 0.5 cc/100 gm
rat of a 10% solution of inulin in saline (Standard Products) at 37°C was injected subcutaneously. One-half hour later 5% of the body weight of water was given. Urine was collected in the usual manner at half-hour intervals. The clearance period of one-half hour duration began 1 hour after water administration. Heparinized blood was obtained by decapitation at the end of the clearance period.

The Hubbard and Loomis (19) method for the analysis of inulin in body fluids was modified, so as to be applicable to small samples. This method depends on the color reaction of fructose with resorcinol, after acid hydrolysis of inulin. As a preliminary step, 0.1 cc of undiluted plasma or diluted urine (1:30) was deproteinized according to Somogyi (20).

**COMPUTATION OF CLEARANCE.** Since in these animals it was impossible to obtain a mid-period blood collection without serious hemodynamic consequences which, of themselves, would alter the clearance rate, it was necessary to find a relationship of the terminal blood concentration to the mean concentration. At equilibrium, inulin may be evenly distributed in extracellular space and the change in plasma concentration ($P_1 - P_2$) in any given time be equal to the total amount of inulin excreted during that time, divided by the volume of distribution ($V_D$) of inulin. The mean plasma concentration ($P_m$) can be determined by graphical interpolation on semi-logarithmic paper by plotting log $P_1$ and log $P_2$ against time.

The $V_D$ of inulin was determined for 13-day old rats. The method of inulin injection and water administration previously described was used. One and one-half hours after inulin injection, the bladder was emptied and 0.3 cc of blood ($P_1$) was drawn from the heart in a heparinized syringe. The animals were unanesthetized. One-half hour later urine was collected and the terminal blood sample ($P_2$) taken by heart puncture or by decapitation. The average of three determinations of the $V_D$ of inulin was 28.6 ± 4.1%. This figure was used in subsequent determinations of renal clearance in suckling rats. Even if the $V_D$ were in error by 100%, the error in inulin clearance at low clearances and at the high plasma levels employed (50-130 mg %) would be less than 5%, and at high clearances less than 10%. However, failure to correct at all for changes of plasma concentration during the clearance period can lead to errors as large as 50%. The renal clearance of inulin was calculated as rate of inulin excretion divided by $P_m$.

**RESULTS.** The inulin clearance of 2-day old rats was low compared to that of adults (table 4). The mean was 7.1 ± 0.8 cc/100 gm/hr., compared to 38 cc/100 gm/hr., the average value reported for adult rats (18, 21-23). The inulin clearance of the newborn rat was, thus, less than one-fifth that of the adult per unit of body weight and less than one-twentieth per unit of surface area. Surface area was calculated from the formula of Lee (24). The values for the inulin clearances of 2 and 4-day-old rats were in close agreement with those of Dicker (25). The most marked rise in inulin clearance took place within the first 2 weeks after birth. Adult inulin clearances probably prevail by 30-40 days of age, although if results be compared on the basis of body surface area, adult values may not be equalled until some time later.

**Effects of Endocrine Secretions.** The possibility that immaturity of the endocrine systems or an altered renal response to their secretions, or both, might underlie some of the ontogenetic changes in response to water excesses was investigated. Attempts were made to obtain evidence of endocrine secretion and to study the effects of extracts of the neurohypophysis and of the adrenal cortex on water diuresis in infant rats. The effects of the adrenal medullary hormones, epinephrine and nor-epinephrine, were also tested, particularly as they might influence circulation.

**Neurohypophysis.** The generally hypotonic

<table>
<thead>
<tr>
<th>Age</th>
<th>Clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>days</td>
<td>cc/100 g/hr.</td>
</tr>
<tr>
<td>2 day (8)</td>
<td>7.1 ± 0.8</td>
</tr>
<tr>
<td>4 day (4)</td>
<td>13.8 ± 1.4</td>
</tr>
<tr>
<td>12-13 day (8)</td>
<td>21.7 ± 0.7</td>
</tr>
<tr>
<td>16-19 day (10)</td>
<td>27.9 ± 1.8</td>
</tr>
<tr>
<td>Adult*</td>
<td>38</td>
</tr>
</tbody>
</table>

* See text for references.
urine of newborn animals has suggested to some a possible analogy with diabetes insipidus (15). The lower antidiuretic content of the pars nervosa (26, 27) and the histologic immaturity of the gland and related structures (28, 29) are suggestive of possible hypofunction in the newborn. Moreover, there is evidence of renal hyposensitivity to posterior pituitary extract (30, 31).

Experiments were undertaken to determine the effects of Pitressin on water diuresis of infant rats and to ascertain at what age release of antidiuretic hormone of pituitary origin could be demonstrated.

**Effects of Pitressin.** Pitressin (Parke-Davis) 10 or 200 milliunits (mu)/100 gm rat, in physiological saline was injected subcutaneously one-half hour after 5% of the body weight of water had been administered by the gastric route. Control animals received water by stomach and were injected with saline alone. Urine was analyzed for chloride and its refractive index was determined.

Experiments on the injection of 10 mu of Pitressin/100 gm rat clearly indicated that water diuresis could be inhibited by 3 days of age. Two hours after water administration control animals had excreted 46.7 ± 4.8% of the test dose, while Pitressin-treated animals excreted 26.9 ± 2.5% of the dose (P < .01). The chloride concentration of control urine collected between the first and second hours averaged 37.0 ± 6.2 mEq/l., while that of Pitressin-injected animals averaged 92.0 ± 14.0 mEq/l. (P < .01). These results show that diuresis and antidiuresis become demonstrable at the same age.

The effects of 10 mu of Pitressin/100 gm and a much larger dose, 200 mu/100 gm, have been compared in a group of rats, aged 3–8 days (fig. 4). It will be noted that the minimal body weight of water had been administered urine flows were no different in these groups, although the larger dose exerted an inhibitory effect for a longer period of time. More marked effects on urine concentration were obtained with 200 mu/100 gm, with chloride rising to 149.0 ± 13.7 mEq/l., a value ap-

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**Fig. 5.** Effects of nicotine on water diuresis. A, Urine flow; B, Urine chloride concentration. *Closed circles,* animals which received nicotine at the time given by arrows; *open circles,* controls. Each curve the mean of 5–9 rats, aged 1–2, 3–5, 7–9, 17–22 days.

**Fig. 6.** Effects of adrenal cortical extract on excretion after water administration. A, Urine flow. B, Rate of chloride loss. *Closed circles,* rats which received adrenal cortical extract; *open circles,* controls. *Solid lines,* newborn rats; *broken lines,* 3–5-day-old rats. Each curve the mean of 5–9 animals.
proximately twice the resting value before water administration. The usual range of plasma chloride was 100–110 mEq/l. In these animals the urine chloride concentration was a more sensitive index of circulating Pitressin than the variation in excretion of a test dose of water conventionally used in the biological assay of antidiuretic substances (32, 33).

Urine osmolarity as reflected in the refractometer reading difference was raised to higher levels and maintained for a longer time by larger doses of Pitressin. However, in no instance did the urine refractive index rise to higher levels than those which existed prior to water administration.

Large doses of Pitressin, 200 µg/100 gm, had a marked chloruretic effect when compared with control animals receiving water alone. At the end of 3 hours following water, the former had excreted $279 \pm 41 \mu m/100$ gm rat, the latter $111 \pm 16$ ($P < .01$). There was no significant difference in the total chloride excretion ($128 \pm 13 \mu m/100$ gm) of rats given 10 µg Pitressin/100 gm and controls ($P > .4$). In adult rats as little as 0.3 µg/100 gm may be chloruretic (34).

The results reported here on the effects of Pitressin on water diuresis in infant rats contravene, to some extent, those of Heller (31) who was not able to show an inhibitory effect with 10 µg/100 gm until 4 weeks of age. In the present studies urine was collected at short intervals, while Heller measured the cumulative excretion over a period of hours. However, his results indicate that Pitressin antidiuresis in adult rats is more profound and longer lasting than in infant rats.

**Effects of Nicotine.** Since an antidiuretic action of Pitressin could be demonstrated in 3-day-old rats, it was of interest to determine if endogenous antidiuretic hormone was released in infant rats. Among the stimuli related to neurohypophysial secretion which induce inhibition of water diuresis are: electrical stimulation of the neurohypophysis (35), acetylcholine (36) and nicotine (37).

Nicotine hydrochloride in physiological saline was injected one-half hour after water administration (5% of body weight). The amount of nicotine injected equalled 0.25 mg of base/100 gm rat. In the case of animals younger than 3 days, nicotine was injected 1 hour after water ingestion to allow for longer urine collection periods. Symptoms of nicotine overdosage were slight and when they did occur, lasted only for the first 5 minutes.

Nicotine (fig. 5) produced no change in the urine flows of animals, aged 1–2 days. However, nicotine increased urine chloride concentration instead of decreasing it as in controls which received water alone. There was a significant inhibition of urine flow by nicotine from 3 days on. The pattern of inhibition was similar in all groups; inhibition lasted 45 minutes, followed by rapid recovery. The relative degree of inhibition of urine flow was progressively greater with aging. In the 17–22 day old group the response was almost identical that of adult rats similarly treated (37). Changes in urine chloride concentration inversely paralleled changes in urine flow.

The experiments indicate that the posterior pituitary, on which the effect of nicotine probably depends, is capable of releasing antidiuretic hormone by 2–3 days of age. Less inhibition is produced by nicotine than by injection of 10 µg of Pitressin/100 gm. On the basis of a rough estimate, it seems possible that about 1–5 µg/100 gm rat of antidiuretic material is released in the infant. A similar amount appears to be released by nicotine in the adult, as judged by the degree of inhibition produced by Pitressin, according to the Burn assay (32). This estimate is but a fraction of the amount of antidiuretic ma-

![Fig. 7. Effects of epinephrine and nor-epinephrine on urine flow of hydrated infant rats. Solid circles, animals which received either drug; open circles, controls. Each curve the mean of 5–9 animals.](http://ajplegacy.physiology.org/10.2203/334917.png)
TABLE 5. EFFECT OF EPINEPHRINE AND NOR-EPINEPHRINE ON INULIN CLEARANCE IN 2-DAY-OLD RATS

<table>
<thead>
<tr>
<th>Animals</th>
<th>Urine Flow, cc/100 gm/hr.</th>
<th>Inulin Clearance, cc/100 gm/hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated (4)</td>
<td>2.8 ± 0.4</td>
<td>9.3 ± 0.4</td>
</tr>
<tr>
<td>Controls (6)</td>
<td>0.7 ± 0.2</td>
<td>7.1 ± 0.8</td>
</tr>
<tr>
<td>P</td>
<td>&lt;.01</td>
<td>&lt;.05</td>
</tr>
</tbody>
</table>

Hence, adrenal cortical extract increases the rate of excretion of water at all ages without, however, decreasing the latent period of maximal flows characteristic of infant animals. The response of newborn rats to water was not converted to an adult one, nor even to a response of an infant but a few days older.

Adrenal cortex. It has been suggested that the underdeveloped water diuretic response of newborn rats might result from a relative adrenal cortical insufficiency at birth (38). Reports on the effects of adrenal cortical extract on the urine flows of newborn rats after water administration are conflicting (39, 40). The possible role of the adrenal cortex in water regulation during neonatal development was investigated.

A nearly salt-free extract was made from the original saline preparation (Upjohn) and administered simultaneously with water in a 1:1 ratio via the stomach. The total amount of fluid given equalled 5% of the body weight. Control animals received 5% of the body weight of a 0.01 M sodium chloride solution containing 2% ethanol.

Adrenal cortical extract resulted in a greater excretion of urine in newborn animals, but its enhancing effects were seen only after 3 and 4 hours following water administration (fig. 6A). An increase of urine flow in 3-5-day-old treated rats was also observed. Only maximal rates of output were affected. The characteristic delay between the time of water ingestion and maximal diuresis was unchanged by adrenal cortical extract. In no instance did the observed urine flows of the infant rats equal that of the untreated adult. Equally noteworthy was the fact that the elimination of water by treated newborns remained slower than by untreated older infants. The diuretic effect of the extract relative to controls was similar in infants to that reported for adult rats (41).

There was no significant difference in the concentration of urinary solids or of chloride in infant animals given extract and controls, even at the time when there were significant effects on urine flow. It follows, then, that the salt loss of the former was greater (fig. 6B).

Adrenal medulla. Among the most efficacious stimulants of blood pressure in appropriate doses are the hormones of the adrenal medulla. Arterial pressures are extremely low in the newborn of several species (42, 43) and might limit diuresis. Epinephrine stimulation (44-46) and depression (47, 48) of urine flow have been reported, although there is no correlation between arterial pressure and urine flow (49).

Thirty micrograms per 100 gm rat of l-epinephrine bitartrate or l-arterenol bitartrate monohydrate (Sterling-Winthrop) in peanut oil was injected subcutaneously immediately after 5% of the body weight of water had been given by stomach tube. Control animals received only water and peanut oil. Drug suspensions were stored at 4°C and freshly prepared every 2 weeks.

Effects on urine flows. Nor epinephrine and epinephrine were without effect on the urine flows of hydrated newborn rats (fig. 7). Neither treated nor untreated animals showed a diuretic response to water. The cutaneous vessels of the newborns were also unresponsive, since no superficial vasoconstriction was observed after injection, although the same preparation would produce intense vasoconstriction lasting nearly 2 hours in 2-3-day-old rats.

Epinephrine and nor-epinephrine had dramatic effects on urine flows of hydrated 2-3-day-old rats. The results with the two drugs have been pooled. There was an immediate and sharp rise in urine output reaching a maximum of 2.5 ± 0.3%/hr. at the end of 1 hour. This value is not significantly different from the urine flows of normal hydrated adult rats (P > .35). Both sympathetic amines were powerful stimulants of water diuresis also in an older group of infants, aged 7-9 days. This response, however, was not significantly different from that of 2-3-day olds (P > .6), but significantly dif-

Material contained in the pars nervosa of either newborn or adult (26).

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ferent from that of their respective littermate controls (P < .05).

Under the influence of epinephrine or nor-epinephrine, the major characteristics of adult water diuresis were imparted to infant rats: a prompt rise in urine flows, high rates of flow and a more prompt dilution of urine. The question arises of the mechanism of epinephrine and nor-epinephrine action during water diuresis. Is the effect directly on the renal circulation, the renal tubules, or is it mediated through some other organ?

**Effects on Inulin Clearances.** Inulin clearances of 2-day old rats were determined by the method described. One group received 30 μg/100 gm rat of either l-epinephrine or l-nor-epinephrine injected subcutaneously after water-administration, while the littermate control group received only water. The clearance period of one-half hour began 1 hour after water had been given.

The inulin clearances of 2-day-old animals given the hormones averaged 9.3 ± 0.4 cc/100 gm/hr, against 7.1 ± 0.8 for controls (table 5). This was a significant increase (P < .05). However, the inulin clearance remains low compared to the adult, although urine flows were comparable. Moreover, the relative increase in clearance of 30% above that of control infant animals in treated infants is to be compared with the 300% difference in urine flows of the two groups (table 5). These findings suggest the possibility that the adrenal medullary hormones may influence both glomerular filtration and reabsorption.

**Epinephrine and the Neurohypophysis.** O'Connor and Verney (50) have shown that water diuresis can be inhibited in dogs by emotional stress and that the inhibition is probably dependent on the release of antidiuretic hormone.

Epinephrine in small doses did not abolish Pitressin inhibition, but when injected before stress prevented the 'emotional release' of antidiuretic hormone. Nicotine inhibits water diuresis only in the presence of an intact hypophysis (37). If epinephrine, in the dosages used in the experiments reported here, acts via the posterior pituitary to prevent the release of its secretions, there might be little or no inhibition when nicotine is also present.

Epinephrine, in the same dosage as in the preceding section, was injected into 7-day-old rats at the time of water administration. One-half hour later 0.25 mg/100 gm rat of nicotine in Ringer was injected subcutaneously. The results indicated that inhibition of urine flow occurred, despite the presence of epinephrine (table 6). There was also significant elevation of urine concentration, a typical nicotine effect (see section on the posterior pituitary). It might be suggested, therefore, that epinephrine in the dosage at which it causes marked diuretic responses to water does not act via the neurohypophysis in infant rats, but may be antagonistic to Pitressin at the renal level as Gaunt et al. (44) found.

**Epinephrine and the Adrenal Cortex.** Epinephrine is known to stimulate adrenal cortical secretion, probably mainly through the hypophysis (51). It is conceivable, therefore, that the diuretic effect might be mediated through the adrenal cortex. Other evidence argues against this hypothesis. Epinephrine increases urine flow of adrenalectomized adult rats (44). It causes no significant fall in the ascorbic acid content of the adrenals (which serves as an index of increased adrenal cortical activity) in infant rats younger than 8 days (52); yet it has marked effects on water diuresis of younger rats. The response to adrenal cortical extract differs from that of epinephrine or nor-epinephrine, both in magnitude and time sequence. For these reasons, it seems likely that epinephrine has primarily as its site of action an organ system other than the adrenal-pituitary. Results reported here would

**Table 6. Antagonistic effects of nicotine and epinephrine in 7-day-old rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Before Nicotine*</th>
<th>After Nicotine†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose, %</td>
<td>Urine Cl</td>
</tr>
<tr>
<td>Epinephrine-nicotine (3)</td>
<td>10±2</td>
<td>10±1</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>&gt;.15</td>
<td>&gt;.8</td>
</tr>
</tbody>
</table>

* A control period of one-half hour before injection of nicotine.
† Urine collected for an hour period after nicotine injection. Dose, % refers to the amount excreted during each collection period. Urine chloride in m Eq/l.; R.I., the refractometer reading difference.

EFFECTS ON INULIN CLEARANCES.
be consistent with an hypothesis of a direct renal action.

**DISCUSSION**

At birth the kidney assumes the role of main end organs of excretion. The kidneys act not in isolation but in reciprocal relationship with other organ systems controlling general metabolic, circulatory, nervous and hormonal activities. The net status of the interactions can be tested by subjecting animals to such stresses as water excesses, water deficits or solute excesses. The excretory changes in response to these conditions are less marked in the newborn than in the adults of most species studied.

As in the adults, the water turnover of newborn rats was reduced when the animals were deprived of food and water. The inability of the newborn to elaborate a highly concentrated urine then led to relative retention of crystalloids, with consequent hemoconcentration and elevation of plasma urea. Another mode of testing urinary responses to induced alterations in the volume and composition of body fluids is through administration of a solute, such that its plasma concentration is elevated. An increased excretion of the solute ensues; its osmotic activity may retard water reabsorption and lead to elevated urine flows. In the newborn rat osmotic diuresis is virtually absent. It has been reported that concentrated urea solutions administered to newborn rats were lethal, while similar solutions given adult rats were well tolerated as a result of accelerated excretion. What may be termed an osmotic diuresis appeared by days of age in response to hypertonic saline, but the characteristics of the response differed from those in adults, for the diuresis was considerably delayed and below adult urine flows, and urine chloride concentration failed to reach adult levels.

There appears to be high correlation among maximal urine chloride, urea and total osmotic concentrations among species. The correlation would seem to apply to infant animals as well. The ability to elaborate urine of high osmolarity in hydropenic states developed in parallel with the ability to perform osmotic work after solute loading. By weeks infant rats excreted urine as concentrated as the adult.

What factors underlie these changes in function during ontogeny? The rat kidney undergoes considerable cellular differentiation and growth for some time after birth. The number of nephra is approximately doubled within the first weeks and trebled within the first 4 weeks of postnatal life. It has been estimated that the tubule length increases after birth about fold. Histological and functional maturity of the proximal tubules is not complete until 4 weeks of age. Renal changes may account for a large measure of the development of regulatory functions in the neonatal period.

It would be of interest to know to what extent the inability to excrete a concentrated urine at birth is conditioned by the state of maturation of the renal tubules and to what extent by extrarenal factors. The neurohypophysis has been thought to be involved in the elaboration of a hypertonic urine. Although lower in content of antidiuretic hormone, the newborn rat's pars nervosa probably contains sufficient material to prevent a state of diabetes insipidus. Release of endogenous antidiuretic hormone can be demonstrated by 3 days of age, but it is not known whether such release occurs in the infant under hydropenic states. While the infant rat is responsive to exogenous Pitressin, it appears to be less sensitive than the adult. On the other hand, fluid deprivation produces a more concentrated urine than posterior pituitary extracts do, suggestive of the involvement of additional factors.

The ability to produce highly dilute urine develops in response to excess water sooner than the ability to produce highly concentrated urine in response to dehydration or solute loading. When the stimulus of water excess is applied, the responses of infant, but not newborn rats, have all the characteristics of what may appropriately be termed water diuresis. Water excretion is then accompanied by loss of salt, minimized, however, by urinary dilution. Within limits, there is grading of excretion to load. The maturation of a diuretic response to water might be thought of as an 'inherent adaptation,' in contrast to the adaptation which can be ob-
tained by repeated subjection of animals to large water excesses, which might be termed an 'acquired adaptation.' Both have in common the features of a more rapid excretion of water, with earlier attainment of high urine flows and with greater maximal flows as well.

The results reported here support the conclusion that 'inherent adaptation' to water represents maturation of excretory processes rather than of changes in the rate of water absorption from the alimentary tract. Experiments with unilaterally nephrectomized infants suggest that maturation involves increased excretion per nephron rather than solely an increase in the number of functional units. Yet, the delayed excretion of water by infant animals can not be attributed to low capacity at the renal level, for it has been shown that the lower urine flows elicited with moderate hydration do not represent the maximal flows or osmotic work of which the infant rat kidney is capable, since these quantities can be increased by partial nephrectomy or by further increase in hydration. These results are suggestive of a relative deficiency in the ability to detect the presence of a water excess or to evoke activity which leads to rapid increase in urine output.

Of the endocrine substances tested, both adrenal cortical extract and adrenal medullary hormones increased excretion of body water excesses, the latter being of greater effectiveness. However, the pattern of water diuresis remained infantile in adrenal cortical treated animals, whereas the pattern of response was adult in animals given epinephrine or nor-epinephrine. Are the adrenal medullary hormones merely physiological curiosities in their effects on water excretion or do they have significance for the normal response to water? There was a correlation between the appearance of a diuretic response to water and of responsiveness to the sympathetic amines. The drugs produced little vasoconstriction and were without effect on the urine flows of hydrated newborn rats. The insensitivity of fetal and newborn rats to the action of epinephrine has been noted by other investigators; Burlingame et al. (58) reported that epinephrine produced only a bare rise in blood pressure of fetal rats near term, and Corey (59) noted that fetal and newborn heart rates were unaffected by epinephrine. By 2 days of age, when the adrenal medullary hormones had profound effects on water diuresis, cutaneous vasoconstriction was apparent. Some renal circulatory influences of the drugs may have been reflected in the inulin clearance which was 30% greater than controls; however, urine flow was 300% greater.

The diuretic action of epinephrine obtains only in doses which may be considered unphysiological, small doses being ineffective (44). Even if the phenomenon be no more than a curiosity, a knowledge of the mechanism of the effects of the adrenal medullary hormones may supply clues as to the normal mode of diuresis and the development of this function.

SUMMARY

Maturation of the renal responses of infant rats to water excesses, to water deprivation and to hypertonic solutions was studied. A diuretic response to water, absent at birth, developed gradually. Deprived of food and water, infant rats had comparable water losses, but urine was not concentrated to the same degree as in adults. Urine, markedly hypertonic to plasma was excreted by 3-week-old rats. The ability to excrete urine of high osmolarity during hydropenia was correlated with the ability to concentrate urine with respect to the particular administered solute.

Possible factors in the maturation of the response to water of infant rats here considered were: alimentary absorption, renal changes and endocrine secretions. Water absorption from the alimentary tract in infant rats was significantly slower than in adults, but at all ages absorption was faster than excretion. The lower urine flows of infants could not be attributed to the lesser amount of functional renal tissue, since further reduction by unilateral nephrectomy did not alter their normal responses to hydration. Inulin clearances were low at birth, less than one-fifth the adult on a body-weight basis, or one-twentieth on a body-surface basis, and rose most rapidly during the first 2 weeks of life.

At 3 days of age the response to water became subject to inhibition by Pitressin and
by nicotine, the latter probably acting via the neurohypophysial system. The degree of inhibition with Pitressin was less than in adult rats with comparable doses. Adrenal cortical extract increased the rate of elimination of water, but the character of water diuresis remained infantile. The adrenal medullary hormones imparted to the water flow than of inulin clearance. Epinephrine and nicotine had antagonistic effects on water diuresis. It was suggested that the sympathetic amines directly affected the kidney.

The author wishes to thank Dr. E. F. Adolph for his guidance, stimulation and encouragement in this work.

REFERENCES