Nonuremic Hyperosmolality Produced by Sodium Depletion

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FALK, J. L. AND M. TANG. Nonuremic hyperosmolality produced by sodium depletion. PHYSIOL. BEHAV. 10(4) 793–799, 1973.—Rats were rapidly sodium depleted by a 4-hr intraperitoneal dialysis against isotonic glucose, and various serum and urine measures were taken at 4, 12 and 20 hr from the start of dialysis for dialyzed and control groups. No food or water was allowed during this postdialysis period. At 4 hr, dialyzed animals were hypotonic, hypovolemic, hyposmolal and oliguric. This was followed by a general picture of renal sodium and chloride saving and a return of relatively normal urine volume. Plasma volume and electrolyte concentration became approximately normal as did urine glucose and urea clearances. However, serum osmolality progressively increased relative to control groups, and at 20 hr was 22.5 mOsm greater than controls. Of this difference, 15.1 mOsm was not accounted for by the osmotic equation calculated from serum sodium, glucose and blood urea nitrogen. These body water distortions in concentration and locus are discussed in the light of increased water and NaCl intakes observed in our previous research.

Hyponatremia Sodium depletion Hyperosmolality Uremia Hypovolemia Dialysis

METHOD

Animals

Eighty male, albino, Holtzman rats were used, with a weight range of 296–450 g (mean = 365 g) and ages ranging from 90–110 days at the start of the experiment.

Apparatus

Animals were housed individually in either standard or metabolism cages (Acme Research Products) under temperature-controlled, light-dark cycle conditions (lights off 9 p.m.–8 a.m.).

Procedure

Maintenance conditions. From 9 a.m.—12 noon daily the animals were given free access to food (Purina Laboratory chow, pelleted) and tap water. At noon they were weighed and returned to their cages from which food had been removed. Consequently, food was available for 3 hr per day, whereas water was always available. They remained on this schedule for 9 days. Experimental design. On the tenth day the animals were divided into two major groups: dialyzed and control. Both of these major groups were composed of three subgroups which were sacrificed at different times after a dialysis or a control procedure. The dialyzed animals were sacrificed at

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Whenever possible double determinations were obtained for all the above measures. Instrument calibration and validity of the determination methods were checked with pairs of clinical standard solutions: Versatol, Versatol A, Calibrate 1, 2 or 3. (General Diagnostics, Warner-Lambert Pharmaceutical Co.). The osmometer was calibrated and checked with vials of Fiske certified standards.

Calculations. Estimations of plasma volume changes resulting from dialysis relative to the appropriate control groups were made using both hematocrit and plasma protein measures. The plasma volume of a dialyzed group (PV,) may be expressed as a per cent of its control group value (PV,) by the following formulae:

\[
\frac{PV_2}{PV_1} = \frac{Hct_1}{Hct_2} \times \frac{1 - Hct_2}{1 - Hct_1} \times 100
\]

\[
\frac{PV_2}{PV_1} = \frac{Protein_1}{Protein_2} \times \frac{1 - Protein_1}{1 - Protein_2} \times 100
\]

Since plasma protein level changed with dialysis, electrolyte concentrations were expressed as mEq/l of serum water. Conversion from mEq/l of serum was accomplished by use of the conversion tables supplied with the American Optical refractometer instruction manual.

Since the dialysis procedure led to elevated serum glucose and urea values at specific times, the observed serum sodium concentrations were depressed by the water osmotically retained by these substances. This is a dilutional effect and does not reflect a true sodium loss. Therefore, corrected values of serum sodium will be indicated in the results in addition to the uncorrected values. The corrections were calculated by subtracting the appropriate control group glucose or urea nitrogen value from the dialyzed group value, dividing by the molecular weight of glucose or urea nitrogen, and multiplying the result by 10 to convert to mM/L. This value was then divided by 2 since sodium salt ionizes into two particles.

Corrected serum sodium concentration in mEq/L = observed Na mEq/l + (10)(IPD glucose mg% - control glucose mg%) + (10)(IPD BUN mg% - control BUN mg%) + 180

The observed serum osmolalities were compared with the osmolalities calculated with the following formula [36]:

Calc mOsm/kg = 1.86 (observed Na) + \frac{glucose}{18} + \frac{BUN}{2.8}

Glucose and urea nitrogen clearances were calculated as follows:

clearance (ml/min) = \frac{urine conc}{serum conc} \times \text{urine vol}

Osmolal clearance was calculated by:

C_{osm} = \frac{\text{urine osm}}{\text{serum osm}} \times \text{urine vol}

Free water reabsorption was calculated by:

T_{C H_2 O} = C_{osm} - \text{urine vol}
**TABLE 1**

**COMPOSITION OF DIALYSATE (4 HR)**

<table>
<thead>
<tr>
<th>Na</th>
<th>K</th>
<th>Cl</th>
<th>mOsm/kg</th>
<th>Glucose mg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>97.5</td>
<td>4.3</td>
<td>75.3</td>
<td>287.3</td>
</tr>
<tr>
<td>S.E.</td>
<td>0.8</td>
<td>0.1</td>
<td>1.2</td>
<td>5.5</td>
</tr>
</tbody>
</table>

**RESULTS**

Table 1 shows the composition of the dialysate upon withdrawal at 4 hr after the injection of the dialyzing solution. These data reveal that considerable body electrolyte became sequestered in the peritoneal cavity, while glucose underwent partial absorption. The mean amount of sodium removed per animal was 3.87 ± 0.08 mEq (9.70 mEq/kg body weight) which is approximately one-quarter of exchangeable body sodium for rats in this body weight range [40].

![Graphs showing changes in hematocrit, plasma protein, serum osmolality, serum chloride, and serum sodium over time](image)

**TABLE 2**

**PER CENT PLASMA VOLUME DECREMENT OF DIALYZED ANIMALS COMPARED TO CORRESPONDING CONTROL GROUPS**

<table>
<thead>
<tr>
<th>Time since start of dialysis</th>
<th>Calc. from hematocrit</th>
<th>Calc. from plasma protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 hr</td>
<td>49.2%</td>
<td>26.4%</td>
</tr>
<tr>
<td>12 hr</td>
<td>29.2%</td>
<td>23.0%</td>
</tr>
<tr>
<td>20 hr</td>
<td>6.2%</td>
<td>4.4%</td>
</tr>
</tbody>
</table>

By 20 hr from the start of dialysis both hematocrit and plasma protein values were elevated markedly compared to the 4-hr control group. In the 12 and 20-hr groups the differences became progressively less, although they still differ at the 0.05 level (t-test) at 20 hr for plasma protein. The plasma volume decrements (see Table 2) produced by dialysis were estimated using Equations (1) and (2). Equation (1) assumes that red cell volume does not change differentially in dialysis and mock dialysis procedures. However, at 4 hr dialyzed animals were hyposmotic with the presumed red cell expansion producing an overestimation of the plasma volume decrement.

![Graphs showing changes in serum osmolality and sodium](image)

**Figure 1** shows that 4 hr from the start of dialysis both hematocrit and plasma protein values were elevated markedly compared to the 4-hr control group. In the 12 and 20-hr groups the differences became progressively less, although they still differ at the 0.05 level (t-test) at 20 hr for plasma protein. The plasma volume decrements (see Table 2) produced by dialysis were estimated using Equations (1) and (2). Equation (1) assumes that red cell volume does not change differentially in dialysis and mock dialysis procedures. However, at 4 hr dialyzed animals were hyposmotic with the presumed red cell expansion producing an overestimation of the plasma volume decrement.

At 4 hr from the start of dialysis hyponatremia was evident (Fig. 1), even when serum sodiums were corrected for the osmotic contributions of serum glucose and urea (see Equation 3). Serum sodium was still significantly lower than controls at 12 hr ($p<0.01$), but the corrected sodium levels were significantly higher than the corresponding control groups at 12 and 20 hr ($p<0.01$). Dialysis produced a marked reduction in both urine sodium and chloride concentrations which persisted throughout the 20 hr (Fig. 2). Relative oliguria in dialyzed animals was followed by a diuretic phase measured at 12 hr, with the 20-hr volume returning to a level not significantly different from the control group.

Serum osmolality at 4 hr (Fig. 1) was significantly lower than the control group ($p<0.001$). However, it increased progressively over time and was significantly higher than the control values at 20 hr ($p<0.001$). The actual serum osmolality measured may be compared with the serum osmolalities calculated from Equation (4) which takes into account the contributions to osmolality of the measured sodium, glucose and urea nitrogen values. This equation normally underestimates the serum osmolality by an amount presumed to be determined by the osmotic contribution of small amounts of substances present in the serum, but not included in Equation (4). Table 3 shows the observed osmolalities, the terms composing the calculated osmolalities with their sums, and the differences between the observed and calculated values. The difference between the calculated osmolalities of the 20-hr groups (7.4 mOsm) was contribu-
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FIG. 2. Urine values for animals as per Fig. 1.

FIG. 3. Blood and urine glucose and urea nitrogen values for animals as per Fig. 1.

TABLE 3

<table>
<thead>
<tr>
<th>Group</th>
<th>serum mOsm/kg observed</th>
<th>serum mOsm/kg calculated</th>
<th>mOsm/kg obser.-calc.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na</td>
<td>BUN</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4 hr 315.4 284.0 8.2</td>
<td>12 hr 308.6 278.3 6.5</td>
<td>14.9</td>
</tr>
<tr>
<td></td>
<td>20 hr 305.8 280.7 6.8</td>
<td>294.8 11.0</td>
<td></td>
</tr>
<tr>
<td>Dialyzed</td>
<td>4 hr 305.4 254.6 17.0</td>
<td>12 hr 314.4 268.0 7.9</td>
<td>16.1</td>
</tr>
<tr>
<td></td>
<td>20 hr 328.3 278.1 7.0</td>
<td>298.3 16.1</td>
<td></td>
</tr>
</tbody>
</table>

uted largely by the BUN component (see Fig. 3 as well). However, this difference cannot account for the large difference between the observed osmolalities of these groups (22.5 mOsm). The remaining 15.1 mOsm which are unaccounted for by Equation (4) obviously made up the major portion of the marked hyperosmolality of the 20-hr dialyzed group.

It is clear from an inspection of Fig. 4 that two parameters known to be important determinants of fluid intake were changing over the measured 20-hr dialysis period. While plasma volume progressively expanded from its initial low value at 4 hr to approximate that of the control group, serum osmolality increased from initial hyposmolality to patent hyperosmolality. Thus, while any stimulus to drinking produced by hypovolemia would be decreasing, a concurrent increase in hyperosmotic stimulation would be developing.

The moderate, progressive increase in the control group...
Fig. 4. Per cent changes in plasma volume decrement (plasma protein and hematocrit estimates) and in serum osmolality increments. Animals as per Fig. 1.

Fig. 5. Glucose and urea nitrogen renal clearances and urine volume rates for animals as per Fig. 1.

Fig. 6. Renal osmolal clearance and free water reabsorption for animals as per Fig. 1.

Hematocrits (Fig. 1) might be a function of water deprivation, although this fails to be reflected by the plasma protein values.

Figures 3 and 5 show that the initial hyperglycemia was compensated for between the 4 and 12-hr points. The lack of renal failure is also indicated by the partial compensation by 20 hr of the dialysis-induced uremic state (Figs. 3 and 5). In addition, acute renal failure would be indicated by positive free water clearance, however, as shown in Fig. 6, free water reabsorption occurred in all control and dialyzed groups.
DISCUSSION

While there is often a short period of behavioral depression following intraperitoneal dialysis against glucose, especially in the dog [12], most studies agree that water intake increases following this manipulation [8, 17, 18, 32, 43]. The present study attempted to elucidate the stimuli which may account for the increased intake by following body water changes after dialysis when no postdialysis intake was allowed. The short-term (4-hr) state confirmed previous findings which indicated a general picture of hyponatremia, hypovolemia, oliguria and renal sodium conservation [2, 11, 12, 15, 24, 25, 32, 44, 49]. Our findings of a subsequent progressive correction of serum electrolyte concentrations and plasma volume in the absence of exogenous input contrast with procedures which allow water intake during the postdialysis period. When water intake was permitted for 1–2 days postdialysis, rats remained severely hyponatremic [4, 14, 38]. Apparently, access to water conspires with osmolar water exchange in the dialyzed animal to maintain the hyponatremic state.

The precise stimuli which might maintain the elevated water intake in the dialyzed animal are of considerable theoretical interest. There was no depletion of water in the dialysis performed: isotonic glucose was injected and the same volume of an approximately isotonic dialysate was subsequently removed. Along with the short-term hyponatremia and hypovolemia, a state of cellular overhydration occurs as water passes into the cells owing to the osmotic differential [11, 40]. Also, free water reabsorption occurred over the entire 20-hr period rather than free water clearance. The hypovolemia produced by this pure salt depletion may be compared with the hypovolemia produced by the intraperitoneal injection of hyperoncotic colloid dissolved in isotonic saline [10, 22] or the subcutaneous injection of formalin or polyethylene glycol [45]. These latter manipulations result in a state of "mixed depletion" ([48], p. 94–5) in which both body water and electrolytes are sequestered for a time making them unavailable for bodily functions. Therefore, in pure salt depletion the cellular overhydration, together with the free water reabsorption, ensures that observed increases in water intake are not attributable to a total body water depletion.

The putative stimuli for water intake comprise those that increase the effective osmotic pressure of the extracellular fluid and those that decrease extracellular volume, especially intravascular volume [16, 23]. In the classic studies of dialysis-induced hyponatremia, it was assumed that since a large amount of extracellular electrolyte was rapidly removed that therefore serum osmolality was below normal. It would presumably remain hyposmotic until the electrolytes were restored or reconcentration occurred with the excretion of free water. However, in the absence of both electrolyte input and free water output osmolality progressively increased to the severe toxicity shown in Fig. 1. The decline in osmolality shown in the control groups was probably a function of the relative postprandial hypertonicity of the 4-hr groups: dialysis or mock dialysis was begun immediately following the 3-hr daily feeding period. The portion of the observed osmolality difference in the 20-hr groups which cannot be accounted for by Equation (4) (15.1 mOsm, see Results) was contributed by unknown substances. Whether these substances penetrate cellular space or remain extracellular contributing to an increased effective osmotic pressure is likewise unknown. These largely unidentified substances are known to accumulate in various disease states and shock and produce large discrepancies between the measured and calculated serum osmolalities [6]. Similar discrepancies have also been noted specifically in dilutional hyponatremia and pure salt depletion [41].

The elevated blood urea level of the dialyzed group measured at 20 hr contributed 9.8 mOsm more to the serum osmolality than the urea level of the 20-hr control group. The difference between the groups measured at 12 hr was even greater (see Table 3). Classically, the increased blood urea levels found in sodium depletion states were thought to reflect a reduction in renal function. While there is evidence that this may be a contributing factor, Hamberger et al. ([29], p. 434) report that BUN rises more rapidly and to a greater height in water-loaded anuric animals than in unloaded animals. Likewise, hyponatremia increases the production of urea in nephrectomized rats, and this effect is enhanced by cellular hydration produced by water loads [35]. In the present experiment, the initial hyponatremia and cellular overhydration undoubtedly contributed to the elevated BUN values. Clinically, urea administration has found use in the reduction of cerebral edema and cerebrospinal fluid pressure [33] owing to its low rate in penetrating the blood-brain and blood-cerebrospinal fluid barriers [9, 34, 42]. The resulting osmotic difference between blood and brain may account for the prevention of brain cellular edema by water in dialysis against glucose [49]. This capacity of urea to reduce brain water content may contribute to its modest, but by no means insignificant, dipsogenic properties [1, 17, 21, 31].

While serum osmolality showed a progressive increase postdialysis, plasma volume was largely reconstituted by 20 hr (see Fig. 4). Previously observed postdialysis increased water intake may be explicable by either one or both of the distortions in body fluids implied by these measures. Likewise, the various measured values presented are congruent with a sodium depletion picture, but provide no certain basis for explaining the extreme and prolonged postdialysis intake of highly hypertonic NaCl solution [17, 18, 20].

Sodium depletion by dialysis in the rat [28] and dog [50] produces a marked increase in renin release, as does volume depletion by nonhypotensive slow hemorrhage [7] or mercurial diuresis [47]. Activity of the renin-angiotensin system is an important determiner of water intake [23]. Thus, renin release occasioned by the sodium depletion, hypovolemia or decreased systemic blood pressure produced by dialysis may be implicated in the increased water intake. Whether the renin-angiotensin system plays a role in the determination of postdialysis NaCl intake increase has not been determined.

REFERENCES


