NORMAL URINE CONCENTRATING ABILITY IN MAGNESIUM DEPLETION

S. M. SUH AND J. SELLORS

Department of Physiology, University of Toronto, and
The Research Institute, The Hospital for Sick Children, Toronto, Canada

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SUMMARY

1. We studied urine concentrating ability in ten magnesium-depleted puppies and compared the results with those of match-fed, littermate controls.
2. The experimental puppies became hypomagnesaemic and hypocalcaemic without evidence of potassium depletion. After 24 h of food and water deprivation, urine osmolality increased to 1350 ± 340 mosm/kg of water. This value did not differ from that of control animals.
3. We also studied urine concentrating ability in a child with primary hypomagnesaemia and secondary hypocalcaemia. He could achieve a urine concentration of 1080 mosm/kg of water after 20 h of water deprivation when he was hypomagnesaemic and hypocalcaemic.
4. We conclude that urine concentrating ability is normal in magnesium depletion uncomplicated by hypercalcaemia or severe potassium depletion.

Key words: urine concentration, magnesium depletion.

Magnesium depletion in rats and humans has been reported to impair (Smith, Baxter, Lindner & Ginn, 1962; Gitelman, Graham & Welt, 1966) and not to impair (Manitius & Epstein, 1963; Dunn & Walser, 1966) the ability of the kidney to concentrate urine. In rats magnesium depletion is often associated with hypercalcaemia and potassium depletion (Walser, 1967), both of which are known to impair urine concentrating ability. In the present study we investigated the effect of magnesium depletion on urine concentrating ability in magnesium-depleted puppies and in a patient with primary hypomagnesaemia.

METHODS AND MATERIALS

Studies in animals

Urine concentrating ability was studied in ten littermate pairs of 9-week-old, pure-bred.

Correspondence: Dr S. M. Suh, The Research Institute, The Hospital for Sick Children, Toronto, Ont., Canada.
female beagle puppies, which had been prepared for another investigation (Suh, Csima & Fraser, 1971, Study 2). One animal of each pair consumed a magnesium-deficient semi-synthetic diet ad libitum for $5\frac{1}{2}$ weeks. A littermate, pair-fed a magnesium-supplemented diet, served as control. Magnesium-deficient and control diets were prepared as described previously (Suh et al., 1971). The mineral contents, determined by analysis, were: calcium, 600 mg/100 g of diet, phosphorus, 730 mg/100 g, and magnesium, 0.3 mg/100 g (deficient) and 73 mg/100 g (control). The puppies were kept in individual metabolic cages with a constant ambient temperature of 23°C.

After 4 weeks on the experimental diet, an episiotomy was performed to permit easy catheterization of the bladder. One week later, immediately before studies of urine concentrating ability were begun, the glomerular filtration rate was measured in four pairs of puppies by determining exogenous creatinine clearance during four consecutive 30-min periods (Smith, 1956). In the remaining six pairs of puppies, blood was drawn twice daily at 09.00 and 16.00 hours for 4 days and voided urine was collected continuously from 16.00 to 09.00 hours and 09.00 to 16.00 hours during the same period. The bladder was catheterized at the end of each collection to ensure complete emptying. Plasma concentrations of magnesium, calcium, inorganic phosphate, sodium and potassium and urine concentrations of magnesium, calcium, phosphate and blood pH were determined.

Urine concentration tests were then carried out in ten pairs of puppies. On day I (16.00–16.00 hours) of the test, the puppies received food according to their established pair-feeding schedule and ad libitum intake of water. On day II (16.00–16.00 hours), they received no food or water. Body weight was recorded twice daily. Osmolalities were determined on plasma and urine samples, collected as described above.

On completion of the concentration test, six pairs of animals were exsanguinated. Specimens of kidney (cortex + medulla, 2 g) were trimmed free of visible fat and connective tissue and analysed for electrolytes after ashing at 450°C for 24 h and extracting with dilute nitric acid. Kidney tissue, fixed in 10% neutral formalin solution was examined histologically.

Studies in a patient with primary hypomagnesaemia

An 8-year-old boy with primary hypomagnesaemia was studied. The features of his disease in infancy and its clinical course until he was 3 years old have been reported (Paunier, Radde, Kooh, Conen & Fraser, 1968). On oral supplements of 36 mmol of magnesium per day, the patient was normomagnesaemic and normocalcaemic and was in a good physical condition, weighing 20 kg. However, the patient developed hypomagnesaemia and secondary hypocalcaemia promptly if oral supplements of magnesium were withheld.

The assessment of his urine concentrating ability was conducted in the course of an investigation into the pathogenesis of hypocalcaemia in the hypomagnesaemic state (Suh, Tashjian, Parkinson & Fraser, 1972). The entire study protocol was approved by the Committee on Human Experimentation of the University of Toronto, and the implications were discussed in detail with the parents.

Hypomagnesaemia was induced by withholding magnesium supplements for 1 week and urine concentrating ability was assessed in this state after 20 h of water deprivation. During this study the patient was closely observed at the Clinical Investigation Unit of the hospital. No fits or other ill-effects were noted.
Laboratory methods

Plasma and urine osmolalities were determined cryoscopically. (Advanced Osmometer, Model 64-31. Advanced Instruments, Inc., Newton Highlands 61, Mass., U.S.A.) Plasma, urine, tissue, and dietary electrolytes were determined by methods described previously (Paunier et al., 1968; Suh et al., 1971). Student’s t-test was performed for paired observations by standard formulae.

RESULTS

Studies in animals

Urine concentrating ability. Fig. 1 indicates urine volumes and osmolalities for ten pairs of control and magnesium-depleted puppies. On day I, when animals received food according to their established pair-feeding schedule and water ad libitum, urine volume and osmolalities were not significantly different in the two groups of animals \((P>0.1)\). On day II, when food and water were withheld, urine volume decreased significantly and osmolality increased significantly in both groups of animals \((P<0.01)\). The osmolalities of the urine after 24 h of

![Graph](image)

Fig. 1. Urine osmolality and urine volume in control and magnesium-depleted puppies. There were ten animals in each group. Vertical lines indicate \(\pm 1\) SD. Day I: while ingesting diet (pair-fed) and distilled water \((ad\ libitum)\). Day II: no food or water.
food and water deprivation were 1180 ± 340 mosm/kg of water (mean ± SD) in control animals and 1350 ± 340 mosm/kg of water in magnesium-depleted animals, and these values were not statistically different (P > 0.1).

In addition, plasma osmolality was constant throughout the study, body weight decreased but to an equal degree in both magnesium-depleted and control animals, and the water intake (day 1) did not differ significantly in the two groups (P > 0.1).

*Electrolytes in plasma and urine and creatinine clearance.* Plasma concentrations of magnesium, total and ionized calcium, inorganic phosphate, sodium and potassium and the blood pH were measured.

In magnesium-depleted animals, plasma magnesium and total and ionized calcium concentrations were decreased and inorganic phosphate was increased, whereas plasma sodium and potassium concentrations and the blood pH remained in the normal range, as reported previously (Suh et al., 1971).

The average urinary excretion rates of magnesium, calcium and phosphate were 2.1, 0.9 and 443 μg/min respectively in control animals and 0.1, 0.4 and 390 μg/min in magnesium-depleted animals; the decrease in the rates of magnesium and calcium excretion in the magnesium-depleted puppies was significant (P < 0.01).

Exogenous creatinine clearances averaged 44.2 ml/min in control and 47.3 ml/min in magnesium-depleted animals and these values were not significantly different (P > 0.5).

Concentrations of electrolytes in kidney tissue. Concentrations of magnesium, calcium, sodium and potassium in kidney tissue of control animals averaged 3.08, 1.86, 39.9 and 31.6 mmol/100 g dry weight and of magnesium-depleted animals 3.04, 1.91, 40.9 and 31.9 mmol/100 g dry weight respectively. Values in the two groups of animals did not differ significantly (P > 0.5).

**Table 1.** Effect of water deprivation on urine concentration in a patient with primary hypomagnesaemia

<table>
<thead>
<tr>
<th>Time</th>
<th>Duration of water deprivation (h)</th>
<th>Body weight (kg)</th>
<th>Serum osmolality (mosm/kg of water)</th>
<th>Volume (ml)</th>
<th>Sp. gr.</th>
<th>Osmolality (mosm/kg of water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>06.30</td>
<td>11</td>
<td>20.2</td>
<td>—</td>
<td>130</td>
<td>1.020</td>
<td>850</td>
</tr>
<tr>
<td>13.30</td>
<td>18</td>
<td>—</td>
<td>290</td>
<td>96</td>
<td>1.023</td>
<td>960</td>
</tr>
<tr>
<td>15.30</td>
<td>20</td>
<td>20.0</td>
<td>288</td>
<td>46</td>
<td>1.029</td>
<td>1080</td>
</tr>
</tbody>
</table>

*Histological findings in kidney sections.* Histological examination was carried out on kidney tissue from six pairs of animals. In the magnesium-depleted animals generalized deposits of calcium were found in the tubules, Bowman's spaces and blood vessels.

*Studies in the patient with primary hypomagnesaemia* At the time of study, magnesium supplements were withheld for 1 week and the plasma concentration of magnesium decreased from 0.66 to 0.47 mM, the concentration of calcium from 2.76 to 2.44 mM and the ionized calcium decreased to 0.84 mM (normal value 0.95–1.20 mM). Concentrations of other electrolytes in venous plasma were: sodium 139 mM, potassium 4.0 mM, chloride 106 mM, pH 7.41 and Pco₂ 35 mmHg.
Normal urine concentration in magnesium depletion

Table 1 shows the results obtained in the patient when water was withheld for 20 h. Body weight decreased slightly. Serum osmolality remained normal. Urine volume decreased greatly, the specific gravity increased to 1.029, and the osmolality of the urine increased to 1080 mosm/kg of water. A maximum urine osmolality ± SD (after overnight dehydration) in normal children 2–16 years of age is reported to be 1089 ± 110 mosm/kg of water (Edelmann, Barnett, Stark, Boichis & Soriano, 1967).

**DISCUSSION**

Our investigation shows that the urine concentrating ability was normal in magnesium-depleted puppies, and in a patient with primary hypomagnesaemia. This finding is contrary to the observations of Smith et al. (1962) in magnesium-depleted rats and Gitelman et al. (1966) in a hypomagnesaemic human, but is in agreement with those of Manitius & Epstein (1963) in rats and of Dunn & Walser (1966) in humans.

Several factors can affect the urine concentrating mechanism. Among these, potassium depletion (Epstein, 1966; Schwartz & Relman, 1967; Abbrecht, 1969; Bennett, 1970) and hypercalcaemia (Gill & Bartter, 1961; Epstein, 1966, 1968; Schwartz & Relman, 1967; Suki, Eknoyan, Rector & Seldin, 1969; Bennett, 1970) have been implicated.

Potassium depletion may accompany magnesium depletion in animals (Walser, 1967) and humans (Dunn & Walser, 1966; Gitelman et al., 1966; Shills, 1969). The study of Manitius & Epstein (1963) presents evidence regarding the influence of magnesium and potassium depletion on urine concentrating ability. Their magnesium-depleted animals developed a degree of potassium depletion but the urine concentrating ability was unimpaired and they concluded that magnesium depletion in some way prevented the deleterious effect of potassium depletion on urine concentrating ability. However, the protective effect of magnesium depletion was relative because when the rats were subjected to a diet deficient in both magnesium and potassium, normal urine concentrating ability was no longer preserved, presumably because the combined deficiency further depleted the potassium stores.

The magnesium-depleted rat commonly manifests hypercalcaemia (Walser, 1967); the animals of Smith et al. (1962) had hypercalcaemia at the time their defective concentrating capacity was demonstrated. In the magnesium-depleted rats of Manitius & Epstein (1963) plasma calcium concentrations were not significantly higher than those of controls, and their animals showed no defect in urine concentrating ability. In our magnesium-depleted puppies the plasma calcium concentration was low, calcium concentration in kidney was normal, and potassium concentrations in plasma and kidney remained normal. Thus, our model allowed us to study urine concentrating capacity in the state of magnesium depletion uncomplicated by hypercalcaemia or potassium depletion. Urine concentrating capacity was normal.

When our patient was made hypomagnesaemic by withholding magnesium supplements for 1 week, the urine concentrating capacity after a 20 h water deprivation was 1080 mosm/kg of water, a value similar to that reported in normal children (Edelmann et al., 1967). At the time of the study, plasma calcium and ionized calcium were slightly decreased and plasma potassium was normal. Dunn & Walser (1966) reported normal urine concentrating ability in two normal subjects who were depleted of magnesium by dietary restriction. They were hypomagnesaemic, normocalcaemic, and one subject was hypokalaemic. By contrast, the only other report bearing upon this question in the human concerns a patient with familial hypo-
magnesaemia and hypokalaemia who was receiving large doses of vitamin D and had hyper-
calcaemia at the time of study (Gitelman et al., 1966). In this patient, the urine concentration
after an overnight fast did not exceed 535 mosm. We suggest that perhaps the defect in
concentrating ability observed in this patient is attributable to concomitant hypercalcaemia
or severe potassium depletion or both.

Although the possibility has not been ruled out that other unidentified factors may be operative,
these observations suggest that hypercalcaemia and probably severe potassium depletion
are important modifying factors in determining the urine concentrating ability of magnesium-
depleted animals and humans. We conclude, however, that hypomagnesaemia as such does
not impair urine concentrating capacity.

Deposition of calcium in the kidney has been noted in the magnesium-depleted rat (Hess,
MacIntyre, Alcock & Pearse, 1959; Ko, Fellers & Craig, 1962; Schneeberger & Morrison,
1965; Whang, Oliver, Welt & MacDowell, 1969). Calcium deposits were noted in our patient
with primary hypomagnesaemia (Paunier et al., 1968), and in the kidneys of our magnesium-
depleted puppies. In spite of the histological evidence of calcification, there was no significant
increase in the concentration of calcium in the kidneys of our magnesium-depleted animals.
Possibly the samples submitted for analysis of calcium may not have been representative of
the kidney as a whole or the histologically visible deposits of calcium may not have caused a
sufficient increase in calcium concentration to be detected by the analytical method employed.
Both in the animals and the patient, despite histological evidence of calcification, urine con-
centrating ability and glomerular filtration rate were unimpaired.

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Research Institute, The Hospital for Sick Children, when this study was carried out, and
J.S., a medical student at McMaster University, Hamilton, Ontario, Canada, was doing
elective studies at The Hospital for Sick Children.

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Normal urine concentration in magnesium depletion


