Renal function in the hibernating, and hypothermic hamster Mesocricetus auratus

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Tempel, George E., and X. J. Musacchia. Renal function in the hibernating and hypothermic hamster Mesocricetus auratus. Am. J. Physiol. 228(2): 602-607. 1975.—Plasma and urine concentrations of Na⁺, K⁺, and urea were examined in hibernating, hypothermic, and normothermic hamsters. Plasma Na⁺ and K⁺ appear unaffected by 48 h of hypothermia (Tₑ 7°C); however, plasma Na⁺ increased (P < 0.05) from control values of 125.8 ± 10.2 to 173 ± 9.2 meq/liter in hibernators. Plasma K⁺ of the hibernator increased to 9.6 ± 3.2 meq/liter from control values of 5.5 ± 0.8 meq/liter (P < 0.05). Plasma urea concentrations were increased (P < 0.05) in both metabolically depressed groups from a control value of 0.5 ± 0.5 to 8.2 ± 1.6 and 7.2 ± 2.8 mM in hypothermic and hibernating groups, respectively. Urine concentrations of solute for the hypothermic animals showed no detectable change from control values for Na⁺ and a decrease for both K⁺ and urea. Concentrations from hibernators showed a decrease from control values for both Na⁺ and K⁺ with no detectable change in urea. Renal tissue slice analysis demonstrated a marked corticomedullary solute gradient for Na⁺ and urea in normothermic control animals which is eliminated in hibernators hypothermic for 48 h and reduced in animals hypothermic for 15 min. Rewarming animals did not show a return of the solute gradient at Tₑ 18°C. However, animals that had rewarmed to Tₑ 37°C demonstrated a complete return with no difference (P > 0.05) from control values. Hibernators showed a slight (P < 0.05) gradient for Na⁺ and no gradient for urea. Animals in all instances demonstrated a decrease in K⁺ concentration from cortex to papilla. A greater concentration of K⁺ was found in the renal cortex of animals hypothermic for 15 min and in hibernators (P < 0.05).

Recent investigations have demonstrated the depression of a variety of renal functions in hibernating ground squirrels (8, 13, 14) and marmots (25). Additional studies have likewise shown a depression of kidney function in experimentally hypothermic dogs (5, 9, 15, 19), rabbits (1), and man (12, 15). In these latter studies core temperatures were between 20 and 27°C, considerably higher than the temperature of a hibernator.

Our interest in depressed metabolism has centered around investigations of the physiologic state of the hypothermic hamster. These studies have involved efforts to prolong survival time as well as to further characterize the physiologic state of the metabolically depressed animal. Our motivation has been that further characterization might enable use of the helium-cold hypothermic hamster as a model of natural hibernation, a model that would require neither time-consuming preexposure to cold nor dependence on the season.

Since hibernation is a response not only to lowered ambient temperatures, but also to water deprivation (10), and since fluid intake by the hypothermic animal is also absent, it is likely that water conservation and electrolyte balance are of considerable importance in both metabolically depressed states. The present study was undertaken to examine renal function in an artificially hypothermic animal whose core temperature more closely approximated that of a hibernator, and to further characterize the physiologic state of the helium-cold hypothermic hamster. A preliminary report of these results has appeared (22).

Methods

Animal Protocol

Male and female golden hamsters from our closed colony weighing 100-130 g were employed in these investigations. They were maintained on a diet of Wayne Lab-Blox supplemented with fresh lettuce and water ad libitum. These animals were divided into eight groups: hamsters from group 1 were taken directly from the animal quarters with no prior treatment; group 2 animals were deprived of water for 72 h prior to sacrifice; group 3 animals were given free access to food and water and maintained in helium and oxygen 80:20 for 48 h prior to sacrifice. The hamsters from the experimental groups were made hypothermic with a rectal temperature (Tₑ) of 7°C by exposure to gas mixtures containing 90% helium, 10% oxygen at an ambient temperature (Tₐ) of 7°C, a modification of the technique described by Musacchia (16). After reaching Tₑ 7°C, the animals were kept at Tₑ 7°C in room air. Group 4 hamsters were sacrificed after approximately 15 min at Tₑ 7°C; group 5 animals were maintained at Tₑ 7°C for 48 h prior to sacrifice; group 6 and 7 animals, likewise hypothermic for 48 h, were allowed to reawarm to Tₑ 18°C (group 6) and Tₑ 37°C (group 7) by placing the animal in a 22°C environment. Group 7 animals were normothermic for a 2-h period prior to sacrifice; group 8 animals were induced to hibernate by exposure to Tₑ 7°C environment for a period of several weeks. The hibernating hamsters were sacrificed after approximately 48 h in hibernating torpor with Tₑ ≈ 7°C.

The hamsters were sacrificed by cervical transection, and the thorax and abdomen were entered by means of a continuous midline incision. An average of 1 ml of blood was collected from the heating heart into a heparinized syringe using a 22-gauge needle and transferred to a centrifuge tube.
After centrifugation at 7°C for 20 min, the plasma was collected and stored frozen at -20°C until analyses were performed. A urine sample, collected by direct puncture into the urinary bladder, was likewise frozen. A small section of the rectus abdominis (approximately 1 cm square) and the kidneys were removed. Blood and urine collection and the preparation of the renal tissue were carried out in less than 5 min following cervical fracture.

Renal Tissue Preparation and Analysis

The renal capsule was removed and the kidney sectioned as described by Moy (13). Upper and lower poles were removed with a scalpel or razor blade, and cortical tissue of the anterior and posterior surfaces likewise was removed to leave a midsagittal section which was immediately frozen in Dry Ice and acetone. The renal tissue and the section of the rectus abdominis were frozen and stored at -76°C in a Revco ultra-low refrigerator until subsequent analyses of tissue electrolyte and urea concentrations were performed.

The tissues were removed from storage and prepared for analysis in the following manner: the midsagittal section was sliced frozen into three readily distinguishable divisions: cortex, medulla, and papilla (Fig. 1). No distinction was made between the left and the right kidney. The tissue slices were then weighed to the nearest 0.2 mg on a Roller Smith precision balance. Three-milliliter sample cups were placed on a Sartorius Balances analytical balance, and approximately 200 μl of ammonia-free distilled water were added to those cups that were to receive the cortical and medullary slices. To those cups that were to receive the smaller papilla, approximately 100 μl were added. The sample cups were then reweighed and the frozen tissue slices added. After being tightly sealed, the cups were heated at 80°C for 5-10 min to denature the tissue enzymes. The samples were again weighed to make certain no evaporative water loss had occurred. The samples were then stored at 7°C for 24 h before analysis to maximize the diffusion of solutes from the tissue residues into the solvent.

The dilution factor to correct for the addition of water was determined by the following formula:

\[
\text{dilution factor} = \frac{\text{wt of tissue water} + \text{diluent}}{\text{wt of tissue water}}
\]

Tissue water was estimated at 80% of the total tissue weights (18).

Solute Analysis

Tissue, plasma, and urine sodium and potassium were analyzed by flame photometry. Urea determinations were made spectrophotometrically by a modified Bertholet reaction (Sigma Chemical Co.), and standards were run with each group of unknowns.

Intergroup comparisons of plasma, and urine solutes, and corticomedullary gradients were made using the nonparametric Mann-Whitney test (11).

RESULTS

Plasma and urine sodium, potassium, and urca levels were compared in normothermic, hypothermic, and hibernating hamsters. The data for plasma and urine electrolyte and urea concentrations are summarized in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Na⁺, meq/liter</th>
<th>K⁺, meq/liter</th>
<th>Urea, mM</th>
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<tbody>
<tr>
<td>Plasma</td>
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<tr>
<td>Normothermic water ad libitum</td>
<td>117.4 ± 7.0 (6)</td>
<td>6.5 ± 0.8 (6)</td>
<td>0.5 ± 0.05 (6)</td>
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<tr>
<td>Normothermic dehydrated 72 h</td>
<td>125.8 ± 10.2 (6)</td>
<td>5.5 ± 0.8 (6)</td>
<td>0.5 ± 0.05 (6)</td>
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<tr>
<td>Hypothermic Tₑₑ = 7°C for 40 h</td>
<td>110.3 ± 12.6 (9)</td>
<td>9.6 ± 0.7 (9)</td>
<td>7.2 ± 0.10 (7)</td>
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<tr>
<td>Hibernator Tₑₑ = 1°C for ≥ 48 h</td>
<td>113.2 ± 9.2 (8)</td>
<td>3.2 ± 3.2 (5)</td>
<td>2.8 ± 2.8 (8)</td>
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<tr>
<th>Urine</th>
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<tr>
<td>Normothermic water ad libitum</td>
<td>99.4 ± 43.2 (10)</td>
<td>209.2 ± 90.8 (10)</td>
<td>96.1 ± 16.5 (7)</td>
</tr>
<tr>
<td>Normothermic dehydrated 72 h</td>
<td>115.7 ± 61.8 (7)</td>
<td>206.6 ± 33.9 (7)</td>
<td>97.3 ± 17.7 (7)</td>
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<tr>
<td>Hypothermic Tₑₑ = 7°C for 48 h</td>
<td>80.4 ± 37.7 (9)</td>
<td>78.3 ± 45.9 (9)</td>
<td>1.8 ± 1.2 (7)</td>
</tr>
<tr>
<td>Hibernator Tₑₑ = 1°C for ≥ 48 h</td>
<td>24.3 ± 8.6 (8)</td>
<td>100.3 ± 38.0 (8)</td>
<td>114.7 ± 20.4 (8)</td>
</tr>
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</table>

Values are means ± SD. Numbers in parentheses are numbers of observations.
Plasma

Plasma concentrations of electrolytes were compared in two groups of normothermic controls with two experimental groups with animals at reduced core temperatures. Sodium and potassium were 117.4 ± 7.0 and 6.5 ± 0.8 meq/liter in the normothermic animals allowed access to water ad libitum. Seventy-two hours of water deprivation produced no significant changes (P > 0.05) with values of 125.8 ± 10.2 meq/liter for Na⁺ and 5.5 ± 0.8 meq/liter for K⁺. Forty-eight hours of hypothermia produced no significant change (P > 0.05) from control values in either Na⁺ or K⁺ which were 110.3 ± 12.6 and 5.5 ± 0.7 meq/liter, respectively. Unlike the hypothermic and control animals, in the hibernating hamster plasma sodium increased significantly (P < 0.05) to 173.2 ± 9.2 meq/liter. Plasma potassium concentrations, comparable in normothermic water-deprived, water ad libitum, and hypothermic hamsters, increased in the hibernator to 9.6 ± 3.2 meq/liter, and the difference is significant at the .05 level.

Plasma urea concentrations demonstrated no effect relatable to water deprivation in the normothermic control group with means of 0.5 ± 0.05 mM in both the water ad libitum and water-deprived hamsters. By contrast, plasma urea concentration rose significantly (P < 0.05) above control values in metabolically depressed animals. In the helium-cold hypothermic hamster, plasma urea concentration increased slightly to 0.8 ± 0.10 mM while that of the hibernators increased markedly to 7.2 ± 2.8 mM.

Urine

Urine samples from the normothermic hamsters provided water ad libitum showed a mean sodium concentration of 99.4 ± 45.4 meq/liter. Hamsters deprived of water for 72 h and hamsters hypothermic for 48 h showed no significant differences in urine sodium concentration (115.7 ± 61.0 and 80.4 ± 37.7 meq/liter, respectively) from control values. In contrast, urine sodium concentration, 24.3 ± 8.6 meq/liter, of the hibernating hamster showed a significant decrease (P < 0.05) from normothermic control values.

Urinary potassium concentrations of normothermic hamsters likewise showed little change due to water deprivation. The concentration for deprived animals was 206.6 ± 33.9 meq/liter while the value for hamsters given water ad libitum was 299.2 ± 90.8 meq/liter. Urinary potassium concentrations of both hypothermic and hibernating hamsters, 78.3 ± 45.9 meq/liter and 110.3 ± 38.0 meq/liter, showed a significant decline (P < 0.05) from control values.

Urine concentrations of urea show no significant difference between normothermic hamsters provided water ad libitum, water-deprived, and hibernating hamsters. However, in the hypothermic hamster, the urine urea concentrations were 1.8 ± 1.2 mM, a 50-fold decrease from control levels.

Renal Tissue Slices

Sodium concentrations. The sodium concentration observed in the renal tissue slices from normothermic, water-deprived hamsters and controls from hypothermic hamsters in the scatter diagram (Fig. 2A). In order to quantitate the gradient for each tissue section as given in Figs. 2 and 3, a mean and standard deviation were calculated. Sodium concentrations in kidneys from normothermic water-deprived hamsters were 64.4 ± 6.0, 94.1 ± 17.2, and 136.6 ± 33.9 meq/liter in cortex, medulla, and papilla slices, respec-
tively. The sodium concentration gradient of the normothermic control hamsters given water ad libitum did not differ significantly ($P > 0.05$) from either the water-deprived animals or the helium-oxygen group (Fig. 2, A, B, C). The gradient for sodium is, however, essentially eliminated in hamsters hypothermic for 72 h. The mean sodium concentrations were 55.4 ± 3.2, 64.0 ± 11.3, and 55.7 ± 9.8 meq/liter in the cortex, medulla, and papilla, respectively. Hamsters sacrificed immediately upon reaching $T_{re} 7^\circ C$ showed a slight, although not statistically significant ($P = 0.18$), decrease in the renal corticomedullary sodium gradient (Fig. 3A). Rewarming animals did not demonstrate a gradient at $T_{re} 18^\circ C$ (e.g., values were 61.8 ± 9.0, 62.4 ± 7.2, and 64.7 ± 11.7 meq/liter for cortex, medulla, and papilla, respectively). By contrast, hamsters that had rewarmed to $T_{re} 37^\circ C$ showed a definite gradient after 2 h: 64.2 ± 5.7, 101.1 ± 17.9, and 177.0 ± 17.4 meq/liter for cortex, medulla, and papilla, respectively. This represents a notable return to levels comparable to control values (Fig. 3C).

Tissue fluid (TF)-to-muscle (M) ratios of sodium were determined for cortex, medulla, and papilla in the normothermic, hypothermic, and hibernating hamsters. The data (Table 2) indicate a pronounced sodium gradient in the normothermic hamster, which is eliminated in the hypothermic hamster and reduced in the hibernator.

Potassium concentrations. Potassium decreased in concentration from the cortex to the papilla (Figs. 2 and 3) in all groups examined. The slopes of the lines are the same for the normothermic groups and for the 48-h hypothermic and rewarming groups. There is, however, a significant ($P < 0.05$) increase in the negative slope in the animals sacrificed immediately after reaching a temperature of $T_{re} 7^\circ C$ and in the hibernating animals.

Urea concentrations. Urea gradients reflect the trends observed in the sodium data. Urea concentrations in kidneys of normothermic water-deprived hamsters increased from 25.6 ± 5.1 to 140.5 ± 88.1 mM, from the cortex to papilla, respectively. This gradient does not exist in kidneys from hamsters hypothermic for 48 h, with values of 16.2 ± 2.8, 16.1 ± 1, and 12.9 ± 1.1 mM for cortex, medulla, and papilla, respectively (Fig. 2A). The other groups of normothermic hamsters, i.e., those given water ad libitum and the helium-oxygen group, are comparable statistically (Fig. 2, B, C).

Hamsters sacrificed upon reaching $T_{re} 7^\circ C$ demonstrated a gradient from cortex to papilla with values of 19.0 ± 2.9 and 55.7 ± 13.2 mM, respectively; these values are significantly less ($P < 0.05$) than control values. Hamsters hypothermic for 48 h that had rewarmed to $T_{re} 18^\circ C$ showed no gradient; 16.1 ± 1.6, 14.5 ± 2.2, and 13.8 ± 2.3 mM for cortex, medulla, and papilla, respectively. Animals that rewarmed to $T_{re} 37^\circ C$, however, showed a return to control levels. Hibernating hamsters, like the helium-cold hypothermic hamster, demonstrated the absence of a gradient.

![Fig. 3. Solute concentrations from renal tissue slices from hypothermic hamsters. A: hamsters that had just reached a rectal temperature of 7°C (open triangles). B: hamsters hypothermic for 18 h which had rewarmed to 37°C (diamond) and 18°C for 2 h (solid circles). C: hibernating hamsters (half-shaded circles).](http://ajplegacy.physiology.org/)
with values of $10.8 \pm 3.9$, $9.4 \pm 3.1$, and $8.5 \pm 2.3$ mM for cortex, medulla, and papilla, respectively.

TF-to-plasma (P) ratios of urea determined for all sections of the kidney likewise demonstrate a pronounced gradient in the normothermic animal which is absent in both the helium-cold, hypothermic hamster, and the hibernating hamster.

**Discussion**

The effect of hypothermia on the kidney may be viewed in terms of interactions of physical factors affecting filtration pressure, such as blood pressure and renal vascular resistance, and active transport processes, such as a sodium pump mechanism. In both hypothermia and hibernation there occurs a decrease in systemic blood pressure which results in a decreased filtered load presented to renal tubules and, in turn, a depression in their ability to transport filtered elements. Thus, at very low temperatures filtration and hence excretion might be expected to be eliminated. Indeed, the loss of fluids, electrolyte, and other plasma constituents, which might occur due to depressed tubular transport capacity at low temperatures, is minimized by a concomitant reduction in filtration and is of definite adaptive advantage (9).

The mechanisms responsible for the corticomedullary concentration gradient are hypothesized to involve a countercurrent system consisting of the vasa recta and juxtamedullary nephrons. Available evidence suggests that a countercurrent multiplier system consisting of the ascending and descending limbs of the loop of Henle establishes this gradient. Countercurrent flow in the vasa recta prevents the dissipation of this gradient once established. This increase in solute concentration from cortex to medulla could be altered by such factors as the redistribution of blood flow to the nephrons, by increased blood flow in the vasa recta, by a redirection in the reabsorption of solutes in the ascending thick limbs of the tubules, or by a reduction in the secretion of antidiuretic hormone. Although the alterations noted above are possible, the reduced concentration of urea in the urine of hypothermic animals would suggest a reduction or elimination of filtration greater than the reduction in tubular reabsorption. The reduction in nervous activity, which occurs in hypothermia and hibernation, would argue against blood flow changes while the hemococoncentration that occurs would suggest increased, not decreased levels of antidiuretic hormone. It is also inherent in the countercurrent hypothesis that in order for the gradient to exist, glomerular filtration is present, and that in the absence of filtration, equilibration between the renal interstitial fluid and plasma occurs (27).

The depression of factors involving filtration pressure and tubular transport mechanisms (23) coupled with observations of morphologic changes (26) would also suggest the absence of glomerular filtration in animals profoundly hypothermic. Our results, which demonstrate the absence of a solute gradient in the hypothermic hamster and a marked depression in the hibernating hamster, suggest the absence of filtration in the hypothermic hamster and minimal, if any, in the hibernator. This study also demonstrates the absence of a corticomedullary solute gradient in animals rewarmed to $T_r$, 37–38°C. We also reasoned that urine samples obtained from metabolically damped hamsters are, therefore, not a reflection of renal activity during either hypothermia or hibernation. They are rather likely a reflection of the time course of events, hypothermia being induced more rapidly than entry into hibernation.

**Urine**

Urine values of sodium in the hypothermic hamster demonstrated no significant change from control values, which suggests a parallel reduction in filtration and tubular reabsorptive mechanisms. By contrast, the sodium concentration in the urine of hibernating hamsters showed approximately a fourfold decrease from control values. This would suggest a more marked reduction in filtered load than in tubular transport prior to complete cessation of function. It is also possible that reduction in urine sodium concentration reflects the response of the renin-angiotensin-aldosterone system. A decrease in systemic arterial pressure, through increased renin release, results in increased angiotensin (AII) levels. Such an increase in AII stimulates aldosterone secretion, which results in greater Na+ retention and lower urine Na+ concentration.

Filtered potassium is largely or completely reabsorbed (2), and the potassium that appears in the urine is due to secretion. The depression in urine concentrations of potassium in both hypothermia (approximately threefold) and hibernation (over twofold) might well be explained in terms of a general metabolic depression of active tubular processes (7, 19).

Urine urea concentrations, although demonstrating no change in the hibernating hamster, decreased approximately 50-fold in hypothermic hamsters. An explanation for this enhanced reabsorption prior to hypothermia with cessation of renal function may reside in a lower urea concentration in medullary tissue. This would increase the urea diffusion gradient and result in a decrease in excreted urea. That this does not occur in the hibernator may be a reflection of a reduction in the filtered load before the elimination of the gradient.

The Na+ and K+ values determined for normothermic animals compare favorably with values reported in the literature (17). However, reports for values of urine urea concentrations were lacking.

**Plasma**

Plasma concentrations of sodium and potassium were comparable in the control and hypothermic animals. The hibernating animals, however, demonstrated approximately 40 and 60% increases for Na+ and K+, respectively. Experiments using other laboratory species, generally conducted at higher temperatures, suggest a sodium decrease in hypothermia (2, 12, 20), while others report no change (6, 21). Potassium data from the literature suggest little alteration in plasma K+ from hibernating animals (24), although experiments on animals at more elevated temperatures suggest a decreased plasma K+ due to hypothermia (9). In our study, plasma hemoglobin was not assessed. We recognize that hemolysis can contribute to a rise in plasma K+; however, care exercised to prevent lysis of the cells and visual
inspection of the plasma argue against such an event. In our opinion the plasma $K^+$ values reflect a true increase. Several tissues (erythrocytes, brain, kidney, diaphragm) are known to possess adaptations that minimize the loss of intracellular potassium at low temperatures in vitro (24). Although, perhaps functional in the hibernator, the extent to which intracellular $K^+$ loss may be prevented is questionable in that plasma $K^+$ rose markedly in our animals. The loss of this important electrolyte in the urine may, however, to possess adaptations that minimize the loss of intracellular tissues (erythrocytes, brain, kidney, diaphragm) are known potassium at low temperatures in vitro (24). Although, opinion the plasma $K^+$ values reflect a true increase. Several may be regarded as a reflection of the extent of amino acid metabolism and liver function. Although ongoing in both the relative extent of metabolic activity in hypothermia in com-

**REFERENCES**


