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Urine Solute Composition of Rats Exposed to Chronic Centrifugation* (33525)

HOWARD H. BENGELE[1,2] AND CHARLES C. WUNDER
Department of Physiology and Biophysics, College of Medicine, University of Iowa, Iowa City, Iowa 52240

Chronic exposure of rats to simulated high gravity can cause an increased urine output (1). At 1.7*g* and 3.0*g*, the urine volumes were found to increase in 4–6 days by 200–300% and then drop back towards control levels. Furthermore, while an initial decrease in water consumption was followed by an increase and probable overshoot, such increases did not match those measured for the urine volumes. The present paper is part of a continuing attempt to understand the influence of the inertial field environment upon fluid balance. Since there are two general mechanisms whereby urine flow can be increased (2), namely, osmotic diuresis and water diuresis, the information on urine solute composition described here was obtained in order to gain some insight into the etiology of the observed centrifugation polyuria.

Methods and Materials. Throughout the preexposure, experimental and control conditions, male Simonsen rats (250–350 g) were housed two to a metabolism cage fitted with a drinking device and an internal feeder containing ground Purina lab chow. The general procedures relating to animal maintenance and to 24-hr measurements for body weight, food and water intakes, and urine output for both the baseline and exposure periods were essentially the same as those previously described (1). After at least 1 week of preconditioning to handling and to the room environment and after baseline measurements, a total of 36 unrestrained rats, constituting two separate studies, were transferred to a 205-cm radius centrifuge (3) and exposed to a resultant inertial field of either 3.0*g* or 1.7*g* at 36.1 rpm. The centrifuge was run continuously except for daily stoppages of approximately 15 min for measurements and for cage maintenance. Thirty-two control animals were housed in cages postioned along the periphery of the centrifuge enclosure where environmental conditions, with the exception of the inertial field, were similar to those for the experimental animals.

Urine osmolalities (mosmols/kg of water) for all animals were measured without dilution on the day of collection by a freezing point depression method in which sodium chloride solutions of known osmotic concentration were employed as the standards of comparison. The total, urinary, osmotically-effective, excretion load (mosmols/day; hereafter referred to as total solute excretion) was calculated as the urine volume multiplied by the urine osmolality.

PART I (3.0*g*). Six rats were centrifuged for 17 days to study the effects of exposure to 3.0*g* upon urine osmolality and calculated total solute excretion. These results were compared with those from 4 *ad libitum* fed control animals (subsequently to be referred to as *ad libitum* control animals).

PART II (1.7*g*). In order to determine the

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TABLE I. Food Consumption (g/day).[a]

Days	3.0*g*	1.0*g* *Ad libitum* controls	1.7*g*	1.0*g* Pair-fed controls
0	26 ± 0 (6)	28 ± 1 (4)	24 ± 0 (30)	23 ± 1 (28)
1	3 ± 1 (6)	28 ± 0 (4)	9 ± 1 (30)	9 ± 0 (28)
2	9 ± 1 (6)	30 ± 2 (4)	13 ± 1 (30)	12 ± 0 (28)
3	12 ± 1 (6)	29 ± 2 (4)	15 ± 1 (30)	15 ± 0 (28)
4	13 ± 1 (6)	29 ± 1 (4)	16 ± 1 (30)	16 ± 0 (28)
5	16 ± 0 (6)	28 ± 4 (4)	17 ± 1 (30)	18 ± 1 (28)
8	18 ± 1 (6)	28 ± 2 (4)	18 ± 1 (30)	17 ± 1 (28)
10	20 ± 1 (6)	27 ± 3 (4)	19 ± 1 (18)	20 ± 1 (16)
12	21 ± 1 (6)	26 ± 2 (4)	21 ± 1 (8)	21 ± 1 (6)
17	25 ± 1 (6)	25 ± 1 (4)	22 ± 1 (8)	22 ± 1 (6)

[a] All values are means ± SE; numbers in parentheses are sample sizes.

effects of exposure to centrifugation at 1.7*g* upon the urine osmolality and calculated total solute excretion, as well as the sodium and potassium excretions of chronically exposed rats, a series of 2 separate but similar experiments was run, and the appropriate data were combined for presentation.

Series A. Eight experimental animals were exposed to 1.7*g* for 17 days. Six control animals (referred to hereafter as pair-fed control animals) were maintained on caloric intakes (see Table I) matched to those of these 1.7*g* rats.

Series B. Twenty-two 1.7*g* rats were centrifuged for a period of 9 (12 animals) or 10 (10 animals) days and were compared to the same number of pair-fed control animals. In addition to the osmotic studies, sodium and potassium concentrations in 24-hr urine samples were also determined by flame photometry. The appropriate 24-hr urinary electrolyte excretions (meq/day) were calculated as urine volume multiplied by urine electrolyte concentration.

The number of observations for statistical analyses and graphical presentations is one-half the total number of animals, since the rats were placed two to a metabolism cage. The Student *t* test, or, when appropriate, a modified *t* test (4), was used to test the significance of differences between means. The null hypothesis was rejected at the $p<0.05$ level.

All data summarized in the figures were normalized to appropriate, individual, average precentrifuged values and are expressed as mean percentage of average baseline values ± standard errors. A complete report of the data will be available elsewhere[2]. Curves shown in the figures were fitted by sight.

Results. Urine osmolality. The data displayed in Fig. 1 show the urine osmolalities relative to baseline values for the 3.0*g* experi-

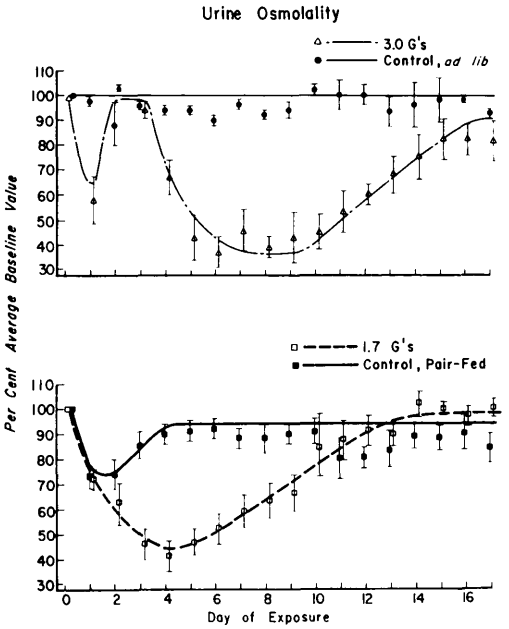

FIG. 1. Centrifugation-induced alterations in urine osmolality of 3.0*g*, (△); and 1.7*g*, (□); rats in comparison to those of the *ad libitum* fed, (●); and pair-fed, (■); control animals. The 100% values are: 2046, 2390, 2088, and 2436 mosmols/kg of water, respectively.

mental and *ad libitum* control animals, as well as for the 1.7*g* rats and their pair-fed control counterparts.

During the initial 24-hr exposure period, the urine osmolalities of the 3.0*g* and 1.7*g* centrifuged rats, as well as the pair-fed control animals, dropped significantly below baseline levels ($p<0.01$, $p<0.001$, and $p<0.001$, respectively). Although they were not significantly different from each other, they were different ($p<0.05$) from those of the *ad libitum* control rats, which demonstrated little change.

For the 3.0*g* rats, an initial trend towards a return to baseline osmolality occurred on the second and third days of exposure. The urine osmolalities for these animals again dropped to a significantly low level ($1.01<p<0.05$) throughout the fourth to the thirteenth days of exposure. Subsequently, a second though slower return to normal levels was apparent. No such polyphasic response could be noted for the 1.7*g* osmolality, which remained significantly below both the baseline and, after the second day, the pair-fed control values ($0.001<p<0.01$), until the ninth day of the experiment, by which time a slow return towards the control levels was apparent. The urine osmolality of the pair-fed control animals appeared to parallel the drop in urine osmolality exhibited by the 1.7*g* animals for only 2 days ($0.001<p<0.01$) and did not demonstrate measurably low values after the third ($p<0.05$) day.

Total solute excretion. The calculated, total, urinary, osmotically-effective, solute excretions for the centrifuged and control animals are summarized in Fig. 2. Similar to the initial decreases in urine osmolalities observed for the 3.0*g* and 1.7*g* experimental animals and the pair-fed control rats, the total solute for these groups demonstrated parallel drops below the 100% baseline level during the same period ($p<0.01$, $p<0.01$, and $p<0.001$, respectively). Both the 3.0*g* and 1.7*g* centrifuged rats' urine osmotic loads were not significantly different from that of the baseline by the second day of the experiments, nor were they significantly different from those of the *ad libitum* control animals,

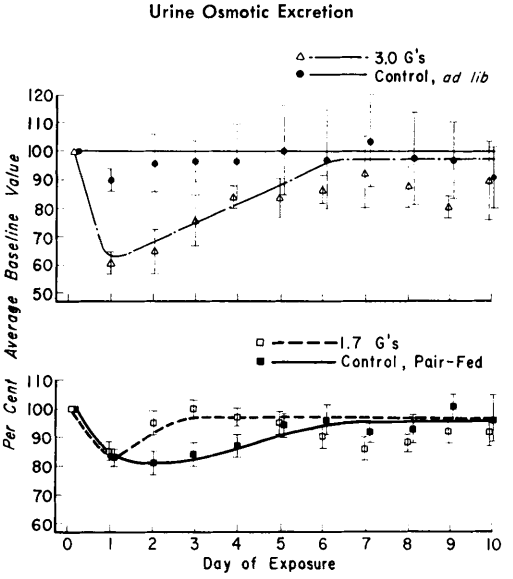

FIG. 2. Urine osmotic loads of the 3.0*g* and 1.7*g* centrifuged rats in comparison to those of the *ad libitum* fed and the pair-fed control animals. The animals used and notation are the same as for Fig. 1. The 100% values are: 30.9, 25.4, 33.1, and 24.1 mosmols/day, respectively.

which again showed little change from the baseline throughout the experimental period. In contrast, the total solute excretions of the pair-fed control rats remained below baseline values through the fourth day ($0.001<p<0.01$) of the experiment. The 3.0*g* urine total solute excretions and that of the *ad libitum* control animals were significantly different from each other only on day 1 ($p<0.02$), while the total solute excretions of the 1.7*g* animals were significantly different from those of their pair-fed control counterparts on days 2 ($p<0.02$) and 3 ($p<0.01$).

Urinary electrolyte excretion. Figure 3 displays the effects of 1.7*g* exposures upon the sodium and potassium excretions of centrifuged rats in comparison to those of the control animals maintained on similar caloric intakes. From the first through the eighth days of exposure, there was a trend towards lower than baseline sodium excretions for both the 1.7*g* experimental ($0.001<p<0.05$ for days 1–6; and $p<0.01$ for day 8) and the pair-fed control animals ($0.001<p<0.02$ for days 2–8). The sodium excretions for these

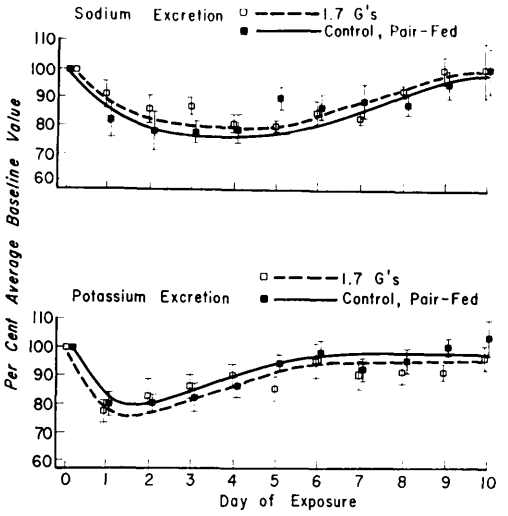

FIG. 3. Urine electrolyte excretions for the 1.7*g* experimental animals and their pair-fed control counterparts as a function of time. Notation is the same as for Fig. 1. The 100% values are: 2.12 and 2.04 meq/day for sodium and 3.13 and 2.76 meq/day for potassium for the 1.7*g* animals and the pair-fed control rats, respectively.

animals were not significently different from each other at any time except on day 5 ($p = 0.05$). The potassium excretions for these same groups displayed similar trends towards levels lower than the baseline from the first through the ninth day of the experiment ($0.001 < p < 0.01$ for days 1–4; and $0.001 < p < 0.05$ for days 1–5 and $0.02 < p < 0.05$ for days 8 and 9, for the centrifuged and pair-fed control animals, respectively). The potassium excretions for the experimental and pair-fed control animals were not significantly different from each other throughout the experimental period.

Discussion. A comparison between the 1.7*g* animals and their pair-fed control counterparts demonstrates that dietary factors are involved in the osmolality drop that occurred during the first 2 days of exposure. It is evident from the disappearance of the dilute* urine osmolalities measured for the pair-fed control rats beyond the second day (see Fig. 1) that, for the 1.7*g* animals, factors other than caloric restriction are included in the overall response. At this stage in our research program, we are not prepared to explain the early transient return towards the baseline osmolality displayed by the urine from the 3.0*g* rats.

The reduced urine osmolality, in the presence of an increased urine output, observed in these studies could reflect a dilution either by osmotically unobligated water (water diuresis) or as a result of an increased output of solute (osmotic diuresis), which increases not only the absolute output of water but also the amount relative to the solute so that the osmolality becomes more dilute (2). Information pertaining to the total, osmotically-effective, solute excretion therefore becomes an important means for distinguishing between the two general mechanisms whereby the urine flow can be increased and the urine osmolality reduced.

Since the total solute excretion has been reported to vary approximately linearly with food intake (5), the comparable low excretions demonstrated by both the 1.7*g* rats and their pair-fed control counterparts during the first 24 hr indicates that this early response can be attributed to centrifugation-induced food restriction. The failure of the pair-fed control animals to demonstrate a return to normal total solute excretions as soon as the experimental rats indicates alterations induced by other factors in addition to food restriction. The possibility that changes in sodium and potassium excretions may account for this difference seems remote, since the urinary excretions of these electrolytes for both the 1.7*g* centrifuged and the pair-fed control animals remained at low levels until the sixth and ninth days, respectively. It is to the excretion of other solutes, therefore, that we must look to account for this discrepancy between the 1.7*g* experimental and the pair-fed control animals. It has been reported (6) that centrifuged rats excrete a decreased quantity of nitrogen during the first 24 hr of exposure followed on the second day by an increase above the precentrifugation level. The results of preliminary studies[2] performed in our laboratory tend to confirm that this effect can be demonstrated for both total nitrogen and nonprotein nitrogen excretions in rats ex-

* Dilute relative to base-line urine osmolality.

posed to 1.7*g*. Since over 50% of the total, urinary, osmotically-effective, solute excretion is comprised of nonprotein nitrogen compounds (urea, uric acid, creatinine, and creatine), the greater than baseline excretions for this class of solutes could account for the difference observed between the 1.7*g* animals and their pair-fed control counterparts. Additional studies are necessary to obtain a complete picture of the effects of centrifugation exposure upon urinary solute excretion patterns.

Nevertheless, the observation that there is no significant increase above precentrifugation values for the total urinary solute excretion for centrifuged rats, despite an almost fourfold increase in urine volume, suggests that the attendant dilute urine osmolality results from a decreased reabsorption of water in the distal renal tubules and collecting ducts. This, coupled with a drop in the antidiuretic-like activity in the blood[2], indicates that the antidiuretic hormone (ADH) is involved at least in part in the observed centrifugation polyuria.

Summary. The present experiments show that the urine osmolality of chronically centrifuged rats is reduced below that of either the *ad libitum* fed or the pair-fed control animals and that this dilution is not accompanied by an increased excretion in the quantity of osmotically-effective solutes. These alterations accompanied by an increased urine output, indicate an enhanced free water excretion thus implying the involvement of the antidiuretic hormone (ADH) in the observed centrifugation polyuria.

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