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# Effects of Fasting on Plasma Volume and Fluid and Sodium Exchanges in Male Rabbits (39658)[1]

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The polydipsia (1-3) and increased adrenal mineralocorticoid secretion (4, 5) in the adult fasted male rabbit have been attributed to a presumed decrease in plasma volume (PV) (1, 6), a well-known stimulus to thirst (7) and adrenal mineralocorticoid secretion (8); this presumption is based upon the marked natriuresis which accompanies fasting in the male rabbit (1-3). The validity of this presumption is supported by the classic study of Gamble, Ross, and Tisdall (9) which indicated that the extracellular fluid volume decreased in the fasting human, and by the work of Bloom and Mitchell (10) which demonstrated that the volume reduction was clearly related to the urinary sodium loss. The fasting-induced reduction in extracellular fluid volume was subsequently confirmed by direct determination of plasma volume in the human (11-13).

The objectives of these studies using adult male rabbits were: (i) to determine the sequential changes in PV and relevant plasma constituents during total fasting, (ii) to determine if the time of onset of the natriuresis coincided with the change in PV, (iii) to determine if the natriuresis was independent of the increased urine volume by studying $Na^+$ excretion during food and water deprivation, and (iv) to provide quantitative data on the PV changes which are needed for proper evaluation of recently reported changes in plasma concentrations of the renin-angiotensin components in fasted rabbits (6).

*Methods.* Thirteen adult male New Zealand rabbits weighing 3.2 ± 0.1 kg were used. Methods for the determination of electrolyte content of the diet and for the daily measurements of body weight, water intake, and urinary volume and electrolytes have been reported (3, 5, 14). The $Na^+$ content of the diet (Rockland Rabbit Ration) was 0.01 mequiv/g dry weight. All volumes for fluid intake and urine output were corrected for evaporative losses. Blood samples were obtained during the control period (fed), after 1, 2, 3, and 4 days of total food deprivation, and after 1 day of food restoration; water was available *ad libitum* throughout. Each rabbit was cycled through this regimen two or three times following appropriate rest periods to ensure a return to normal electrolyte balance and hydration. Each rabbit was used twice for blood sampling in each cycle, except when a third sample was taken for the recovery period; samplings were always separated by at least a 3-day interval. After each control sample, the rabbits were fed for at least 3 additional days before beginning the starvation period. Pilot experiments using normal control (fed) animals indicated that the blood loss due to the sampling procedure did not alter the PV when consecutive determinations were separated by 24 hr but did reduce the hematocrit; thus, to minimize the effect of sampling volume, all PV determinations were separated by at least 3 days, and the hematocrits were not used as indices of plasma volume changes. The PV was measured by the volume distribution method using $^{131}I$-labeled albumin (Albumotrope, Squibb). In addition to the control sample, four 2.0-ml serial blood samples were taken at 10-min intervals following the injection of Albumotrope. Duplicate samples were counted for gamma radiation with a Packard Model 3003 counter. Plasma protein concentration and osmolality were determined with the method of Lowry *et al.* (15) and the freezing point depression method (Fiske Osmometer), respectively. Results were statistically evaluated by means of Student's *t*-test of significance.

The daily urinary $Na^+$ excretion and volume were measured in an additional three adult male New Zealand rabbits when fed

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and when deprived of food and water for 2 days. The $Na^+$ content of the diet (Teklad, Rockland) was 0.02 mequiv/g dry weight. Because of the expected low urine volume when fluid intake is zero, the bladder was catheterized, according to our previously reported procedures (14), and washed three times with 2.0 ml of tepid distilled water at the time of food and water withdrawal and at the end of each day of deprivation to ensure quantitative recovery of the excreted $Na^+$ within the bladder.

An additional eight New Zealand adult male rabbits were used to determine the effect of fasting on plasma glucose; four were used for blood sampling on Days 1 and 4 of deprivation, and the remaining four for sampling on Days 2 and 6 of deprivation. Plasma glucose was determined by the glucose oxidase method (GLUCOSTAT, Worthington Biochemical Corp.).

*Results.* The PV was significantly ($P < 0.005$) reduced from 33.4 ± 0.6 to 29.3 ± 2.1 ml/kg body weight after 1 day of food deprivation and remained at this reduced level during the remaining 3 days of deprivation ($P < 0.005$, Table I). The PV reduction was approximately 12% of the control value.

The daily urinary $Na^+$ excretion and volume were significantly ($P < 0.005$) increased after 1 day of food deprivation and continued to increase to reach maximal values after 3 days of deprivation ($P < 0.005$, Table I). The urinary $Na^+$ excretion and volume increased 12- and about 3-fold over the control value, respectively, by Day 4. Water intake also increased significantly but not until the second day of food withdrawal; water intake became maximal on the third day ($P < 0.005$, Table I). The water intake doubled by Day 4 compared with the control value.

Plasma osmolality was significantly ($P < 0.05$) decreased from the control value of 286 ± 1 to 283 ± 2 mOs*M*/liter after one day of deprivation; it progressively decreased to 276 ± 8 mOs*M*/liter after 4 days of zero food intake ($P < 0.05$, Table I).

The plasma protein $Na^+$ and $K^+$ concentrations were not significantly changed by total food deprivation for 4 days when compared with their respective control values (Table I).

There were no statistically significant changes in the grouped absolute body weights (Table I), because the initial weights were quite different (range: 2.5–4.1 kg), and the body weight of each rabbit did not contribute to the mean of every day of deprivation. Furthermore, the daily body weight measurement is greatly influenced by the balance between recent large fluid intake and amount of urine remaining in the bladder. Each animal lost weight each day of deprivation, and the average loss by Day 4 was 248 ± 25 g.

All parameters returned to the normal control range after 1 day of *ad libitum* food intake with the exception of plasma osmolality which remained significantly reduced (Table I).

When the rabbits were deprived of food and water, a marked natriuresis was induced ($P < 0.005$) despite the expected reduction ($P < 0.05$) in urine volume (Table II). The

TABLE I. SEQUENTIAL CHANGES FOLLOWING CALORIC DEPRIVATION IN 13 MALE ADULT RABBITS.

| Parameters | Control (Fed) (17)[b] | Caloric deprivation[a] Day 1 (5) | Day 2 (5) | Day 3 (3) | Day 4 (5) | Recovery (Fed) (7) |
|---|---|---|---|---|---|---|
| Plasma volume (ml/kg) | 33.4 ± 0.6 | 29.3 ± 2.1[c] | 28.4 ± 1.3[c] | 29.0 ± 2[c] | 29.3 ± 1[c] | 34.6 ± 1 |
| Plasma protein (g%) | 7.3 ± 0.2 | 7.3 ± 0.1 | 8.2 ± 0.7 | 7.2 ± 0.2 | 7.0 ± 0.2 | 7.0 ± 0.3 |
| Plasma $Na^+$ (mequiv/liter) | 143 ± 1 | 145 ± 1 | 143 ± 1 | 143 ± 2 | 144 ± 1 | 145 ± 1 |
| Plasma $K^+$ (mequiv/liter) | 4.5 ± 0 | 4.4 ± 0 | 4.1 ± 0 | 4.2 ± 0.1 | 4.7 ± 0.1 | 4.8 ± 0.1 |
| Plasma osmolality (mOs*M*/liter) | 286 ± 1 | 283 ± 2[d] | 281 ± 4[d] | 280 ± 5[d] | 276 ± 8[d] | 281 ± 3[d] |
| Water intake (ml/kg/day) | 122 ± 7 | 146 ± 42 | 173 ± 29[d] | 292 ± 50[c] | 251 ± 39[c] | 105 ± 12 |
| Urine volume (ml/kg/day) | 44 ± 6 | 114 ± 32[c] | 101 ± 27[c] | 213 ± 35[c] | 166 ± 32[c] | 36 ± 7 |
| Urinary $Na^+$ (mEq/kg/day) | 0.01 ± 0 | 0.05 ± 0.01[c] | 0.06 ± 0.01[c] | 0.14 ± 0.04[c] | 0.13 ± 0.05[c] | 0.01 ± 0 |
| Body weight (kg) | 3.2 ± 0.1 | 3.1 ± 0.3 | 3.1 ± 0.2 | 2.6 ± 0.2 | 3.2 ± 0.2 | 3.2 ± 0.2 |

[a] Each value is the mean ± SEM.
[b] Numbers of measurements in parentheses.
[c] $P < 0.005$.
[d] $P < 0.05$.

TABLE II. NATRIURESIS IN FOOD- AND WATER-DEPRIVED ADULT MALE RABBITS.[a]

| Subject | $n^b$ | Body weight (kg) | Urinary volume (ml/kg/day) | Urinary $Na^+$ (mequiv/kg/day) |
|---|---|---|---|---|
| Control: food + water | 3 | 3.6 ± 0.3 | 112 ± 17 | 0.02 ± 0.004 |
| Deprived: no food and water | | | | |
| Day 1 | 3 | 3.4 ± 0.4 | 38 ± 13[d] | 0.62 ± 0.01[c] |
| Day 2 | 3 | 3.3 ± 0.4 | 14 ± 1[c] | 0.82 ± 0.13[c] |
| Recovery: food + water | 3 | 3.4 ± 0.3 | 40 ± 10[d] | 0.03 ± 0.01 |

[a] Each value is the mean ± SEM.
[b] $n$, Number of rabbits.
[c] $P < 0.005$.
[d] $P < 0.05$.

control (fed) urinary $Na^+$ excretion and volume were greater than usual (see Table I vs Table II); this is a reflection of the greater $Na^+$ content of the diet used for this set of experiments.

Plasma glucose concentration was significantly reduced 24 hr after food deprivation (115 ± 4 mg%, $P < 0.02$) when compared with the control value (131 ± 3 mg%), but did not differ from the control value on Days 2, 4, and 6 following food withdrawal.

*Discussion.* Consonant with our previous reports (1–3), total food deprivation in the adult male rabbit induced natriuresis, polyuria, and polydipsia. Significant increases in urinary $Na^+$ excretion and volume occurred after 24 hr of deprivation, and a significant increase in drinking took place after 2 days; this has been the typical pattern noted previously and suggests that the polydipsia is secondary to the $Na^+$ and consequent PV loss. The occurrence of the natriuresis when both food and water intakes were totally restricted and with the polyuria consequently absent, provides direct evidence that the $Na^+$ loss in fasting animals is independent of the attendant increased urine volume and polydipsia. The polydipsia is clearly secondary to the reduced plasma volume, and it (the polydipsia), then further enhances the increased formation of a large volume of dilute urine. Adjunct support for this interpretation is provided by the observation that estrogens abolish the polyuric–polydipsic characteristics by inhibiting water ingestion but do not abolish the natriuresis (14). The primary event appears to be the increased renal loss of $Na^+$ when the caloric and salt deprivation of total fasting occur.

The significant changes in urinary $Na^+$ and volume are coincident with the PV change at the earliest time period studied, 24 hr, and this is consistent with the hypothesis of their causal role in the reduction of the PV. Thus, the presumption that a reduced PV accompanies the urinary $Na^+$ loss in food deprivation in the male rabbit is substantiated and is in agreement with the findings in fasting humans (9–13). The decrease in PV is much reduced or abolished when dietary sodium supplements are given to the fasting human (16, 17), which supports the view that the reduced PV of total fasting appears to be dependent upon the presence of sodium depletion (10). It is known that sodium excretion during total fasting significantly exceeds that found during low-salt plus adequate caloric intake (10, 18, 19). Similarly, the sodium-sparing action of adequate caloric intake is illustrated by the observation that feeding adult male rabbits a salt-free diet does not induce the triad of natriuresis, polyuria, and polydipsia, but caloric deprivation in the absence of a salt intake does (unpublished data); replacement of salt to the drinking water of food-deprived male rabbits abolishes the polyuric–polydipsic characteristics [(1), upublished data].

The observed decrease in PV (33.4 to 29.3 ml/kg) after 1 day of food deprivation was maintained during the entire deprivation period and was approximately 4 ml/kg or a total loss of 12–13 ml. This significant reduction in PV represents an adequate stimulus to thirst (7) and adrenal mineralocorticoid secretion (8), and it probably represents the stimulus which accounts for the enhanced fluid intake (1–3) and adrenal mineralocorticoid secretion (4, 5) reported for the food-deprived male rabbit. The increased mineralocorticoid secretion mini-

mizes the $Na^+$ loss in calorically deprived male rabbits (4, 5), but mineralocorticoid effectiveness under these conditions is apparently not sufficient to prevent the continued natriuresis; a similar decreased responsiveness to the $Na^+$-retaining action of exogenous mineralocorticoid during starvation has been noted by others [see (5, 22) for discussion and review].

The PV was maintained at a reduced value despite the continued daily natriuresis and urinary fluid loss. Since the plasma $Na^+$, $K^+$, and protein concentrations remained within the normal control range and cellular water (%) was not altered in food-deprived male rabbits (1), any movement of electrolytes out of cells was most likely accompanied by water. The maintained normal plasma [$Na^+$] along with the stabilized PV, albeit reduced, means that $Na^+$ must be moving into the plasma compartment from cells and/or the large amount of gut contents of this herbivore in order to compensate for the continued urinary $Na^+$ loss. The data do not provide a basis for understanding the unchanged plasma protein concentration. Failure of food deprivation to alter plasma $Na^+$, $K^+$, and $Cl^-$ concentrations has been reported previously for the rabbit (1, 20). An inspection of Huang's data, the first report of fasting-induced polyuria–polydipsia in the rabbit, indicates that the plasma $Na^+$ and $Cl^-$ concentrations did not change for approximately the first 10 days of total fasting in rabbits and, then, only gradually declined with more prolonged fasting (20).

The data do not provide an explanation for the reduced plasma osmolality and the accompanying normal $Na^+$, $Cl^-$, and protein concentrations. The decreased osmolality cannot be due to hypoglycemia, for the plasma glucose concentration was only temporarily lowered and returned to a normal level by Day 2 of food deprivation; these changes noted in glucose concentration confirm those previously reported by others (21) for the fasting rabbit. The acidosis of fasting is accompanied by an expected reduction in extracellular [$HCO_3^-$] (9), and replacement of the $HCO_3^-$ by osmotically less active metabolites may contribute to the reduced osmolality; a complete plasma cation–anion analysis is required to test this possibility. In addition, the fasting-induced reduction in [$HCO_3^-$] may account for the renal $Na^+$ loss, as suggested by Chinn and co-workers (22), since an adequate interstitial [$HCO_3^-$] is required for renal $Na^+$ reabsorption (23, 24). The reduced osmolality could account for the diminished secretion of vasopressin during total fasting in the rabbit (25, 26) and apparently does not alter the ability of the contracted extracellular fluid volume to stimulate drinking (27). The reduced vasopressin secretion and the stimulation of thirst which occur in the food-deprived rabbit greatly enhance the urine flow beyond that due to the initial $Na^+$ loss, producing the marked polyuria.

The 12% reduction in PV can account for only a small fraction of the reported 280% and >1000% increases in plasma concentrations of angiotensinogen and angiotensin II, respectively (6). Thus, the concentration changes in the various components of the renin–angiotensin system in the fasted male rabbit are true changes and not simply the consequence of a reduced plasma volume.

It appears that the natriuresis is the initial event which triggers the cascade of events which include: a reduced PV, increased mineralocorticoid secretion, increased thirst, reduced plasma osmolality, reduced ADH secretion, and polyuria. The mechanism of the natriuresis remains unknown. The evidence cited here indicates that the reduced extracellular [$HCO_3^-$] and the markedly altered components of the renin–angiotensin system may possibly contribute to an intrarenal mechanism accounting for the $Na^+$ loss; these hypotheses should be tested experimentally.

*Summary.* Daily determinations of fluid exchanges, urinary $Na^+$ excretion, and plasma volume, $Na^+$, $K^+$, protein, and osmolality were made using 13 adult male rabbits during periods of *ad libitum* feeding, food deprivation for 4 days (water available), and 1 day of refeeding. Significant increases in urinary $Na^+$ excretion and volume occurred after 24 hr of deprivation, and a significant increase in drinking took place after 2 days; maximal values were obtained by Day 3 for all three parameters. The plasma volume was significantly reduced from 33.4 to 29.3 ml/kg, coincident with the changes in urinary volume and $Na^+$ excretion after 1 day of deprivation, and re-

mained at this reduced level. Plasma osmolality was decreased after 1 day of deprivation and continued to decline. Plasma concentrations of $Na^+$, $K^+$, and protein did not change. In a second group of rabbits deprived of food and water, a marked natriuresis was induced despite the expected reduction in urine volume. In a third group of rabbits, plasma glucose concentration was decreased after 24 hr of fasting but returned to the control level by Day 2 of fasting.

The data substantiate the assumption that the fasting-induced natriuresis and increased urine flow in the rabbit are accompanied by a reduction in plasma volume. The diuresis and natriuresis persist in fasting male rabbits with no further change in plasma volume after the first day of fasting. The natriuresis is clearly independent of the increased urine flow and fluid intake. The polydipsia is secondary to the reduction in plasma volume and enhances the increased urine flow to produce a marked polyuria.

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