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SODIUM METABOLISM DURING ACCLIMATION TO WATER RESTRICTION BY WILD MICE, MUS MUSCULUS

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THOMAS MICHAEL MCKENNA

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SODIUM METABOLISM DURING ACCLIMATION TO WATER RESTRICTION

BY WILD MICE, MUS MUSCULUS

APPROVED BY

Howard Haines

Mary Whitmore

A Kurt Weiss

DISSERTATION COMMITTEE

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SODIUM METABOLISM DURING ACCLIMATION TO WATER RESTRICTION BY WILD MICE, MUS MUSCULUS

ABSTRACT

Changes in sodium and potassium balance and urinary aldosterone excretion (free plus 'acid labile') were surveyed in house mice acclimating to a progressive and step-wise water restriction. Sodium-22 and $^{3}H_2O$ distribution and exchange were also measured. Each water restriction caused negative sodium and potassium balance, followed by conservation of both ions. At the first water restriction, negative balance was produced by natriuresis and kaliuresis, at successive water restriction, a negative balance was due to a decrease in food consumption. Aldosterone excretion doubles at the first water restriction, and then remains slightly elevated thereafter. Intestinal absorption of both ions shows a persistent increase after the third day of water restriction. The Na:K ratios in urine and feces decline as mice respond to each water restriction, and return to control levels in acclimated animals. Mice maintain their weight-relative hydration when acclimated to water restriction, since distribution volumes of ^{22}Na and $^{3}H_2O$ in acclimated mice approximate control values. The biological half-life ($t_{\frac{1}{2}}$) of $^{3}H_2O$ increases with each water restriction, and remains elevated in acclimated animals. The $t_{\frac{1}{2}}$ of ^{22}Na increases

at each water restriction, but decreases in acclimated animals. Discussion centers on possible interplay between hormonal and hemodynamic inputs to acclimation responses.

natriuresis; kaliuresis; aldosterone; acclimation; house mice

INTRODUCTION

During acclimation to water restriction, wild house mice exhibit an initial constriction and, later, a restoration and defense of extracellular fluid, particularly plasma (32). Other parameters which change during acclimation include the development of hypernatremia (vide infra), a decrease in food consumption, and a general weight loss (31). Upon complete acclimation, food consumption returns to normal and body weight stabilizes at a value less than that of a non-stressed animal. Alterations in body fluid compartments during the acclimation process should be reflected by changes in sodium and potassium balance, and perhaps in the activity of the renin-angiotensin-aldosterone system. Plasma renin and aldosterone concentrations have been examined in sheep (5) and camels (27) undergoing dehydration by combined thermal and water deprivation stresses, with mixed results. Both species exhibited a significant elevation of plasma renin activity after 5 to 10 days of dehydration, while plasma aldosterone concentrations increased significantly in the camels, and dropped in the sheep.

The present study was designed to survey sodium and potassium balance throughout the acclimation process. In addition, simultaneous measurements of urinary aldosterone were performed. The urinary aldosterone excretion rate has been demonstrated to be a reliable index of adrenal function in rats (36,40). The experimental protocol

minimized stress by being non-invasive and eliminating all handling of the mice. Finally, sodium and water distribution and exchange were determined in a separate, but equivalent, group of mice.

MATERIALS AND METHODS

Reagents

The following reagents were purchased and used with no further purification: lysozyme (3 times crystallized), and methylene chloride (ketosteroid quality), from Sigma Chemical Co.; Fluoralloy liquid scintillation fluor from Beckman Instrument Co.; aldosterone standard for radioimmunoassay (500 ng/0.5 ml absolute ethanol), and Merit liquid scintillation fluid, from Isolab Inc.; ethylene glycol (chromatoquality), and isooctane (spectrophotometric grade), from Matheson, Coleman, and Bell; ethylacetate (reagent grade), from Mallinckrodt; toluene (scintillation grade), from J. T. Baker Co.; and Aquasol liquid scintillation fluid from New England Nuclear Corp.

Sodium-22 chloride (99% radiochemical and radionuclidic purity) and tritiated water were purchased from New England Nuclear Corp. Tritiated aldosterone (1,2,6,7 - ^{3}H)-aldosterone, sp. act. 82 Ci/mM, purchased from New England Nuclear Corp. was donated by Dr. John Higgins, University of Oklahoma Health Sciences Center, and stored in absolute ethanol at -20 ^{o}C. Aliquots of the solution were purified on Celite microcolumns (47) prior to use in the radioimmunoassay.

Antiserum to aldosterone 18,21-dihemisuccinate (lot #088) was donated by the National Institute of Arthritis, Metabolism and Digestive Diseases, Pituitary Hormone Distribution Program. This antiserum exhibits a high titer and less than 1% cross reactivity with other corticosteroids (35).

Housing and Maintenance

Five young male and five young female adult mice were used in each of two studies: 1) ^{22}Na and $^{3}H_2O$ distribution and exchange, and 2) sodium and potassium balances and aldosterone excretion. Mice were F_1 or F_2 offspring of wild parents captured locally.

The mice were individually housed in matabolism cages. Cages were fabricated from 1 lb coffee cans, opened at the bottom and closed at the top with clear Lucite lids. The cages rested on grids of tightly stretched stainless steel wire (0.28 mm in diameter) strung at 1 cm intervals from welded steel frames. These assemblies rested on 29 X 14 X 4 cm enameled trays so that urine and feces fell into the trays. Cage and grid assemblies were tared to a common weight to allow weighing the mice without disturbance. Urine and feces were collected under paraffin oil (Saybolt viscosity 27 - 38 ^{o}C in 15 X 2 cm Petri dishes lined with thick polyethylene film. The Petri dishes were positioned in the tray 4 cm under the grid.

Mice were kept in an environmental chamber at 30 $\pm$ 2 ^{o}C, which is within their thermal neutral zone (in prep.); 30% relative humidity; and a 12 h photoperiod. Weighed volumes of distilled water were provided from drinking tubes formed from a glass tube (9 mm O.D. X 14 cm) which had one end closed and the other open (2 mm). The tube was fastened through a hole in the cage lid. Mice were fed a pelleted high carbohydrate test diet (Teklad Mills, modified TD-69363), containing essentially twice as much potassium (60.4 µEq/g) as sodium (30 µEq/g). Food was given within a feeder fashioned from a 4 cm long X 2.8 cm wide (O.D.) glass cylinder glued to a glass rod. The rod extended through the cage lid, and prevented the feeder from being tipped by the mouse.

One end of a food pellet was inserted into a rubber washer, which in turn was forced into the bottom of the feeder. One pellet contained at least twice the daily ad libitum food consumption, which was taken as the weight difference of the feeder over one day.

Acclimation Sequence and Sampling Protocol

Mice were sequentially restricted to one-half and then one-fourth of their daily ad libitum (control) water intake (Fig. 1). For details of the acclimation procedure see 32. In brief, for the sodium and potassium balance and aldosterone excretion study, measures of body weight, food and water consumption, and collection of the urine and fecal output of non-acclimated (control) mice were performed daily (mean interval between collections throughout the study was 24.0 $\pm$ 2.1 (SD) hours) for four consecutive days. The daily water ration was then reduced to 1.2 g/day, which is one-half of the average ad libitum consumption (32), and sampling continued for an additional four days. Imposition of water restriction to ½ ad libitum induced an immediate daily loss of body weight (termed a nonsteady state condition, ½ NSS) which became progressively less as the mice acclimated to that level of water restriction and attained the succeeding steady state (½ SS) condition. Mice were deemed to be at steady state condition when body weight varied by no more than 3% over several days. After 13 days of ½ ad libitum water rations, the same sampling protocol was used to collect four days of ½ SS samples. Water intake was then restricted to 0.6 g/day (¼ ad libitum ration) and four days of ¼ NSS samples collected. Mice acclimated to the ¼ ad libitum water supply over 16 days, after which four days of ¼ SS samples were collected. Henceforth, the term

treatment refers to the water ration that the mice were given (i.e. control, ½, or ¼ ad libitum) while condition refers to the physiological state of the mice (steady state or nonsteady state).

Sodium and potassium balances for each day within each condition were calculated from the sodium and potassium content of the food consumed and the urinary and fecal outputs. In addition, aldosterone content of the urine samples from three contiguous SS and NSS days in each treatment was determined.

The distribution and exchange of ^{22}Na and $^{3}H_2O$ was measured in another group of mice as they underwent the same acclimation procedure. The sampling protocol is detailed in a subsequent section of this paper.

Sodium and Potassium Balance

Urine was aspirated from the oil with finely drawn transfer pipets. Since it was difficult to withdraw only urine by this technique, the aspirated sample was emptied into another disposable pipet in which the tip had been pre-loaded with oil and then sealed. A distinct oil-urine-oil banding pattern formed, and remained intact as the contents were slowly emptied after cutting off the tip of the pipet. The urine portion was placed in a pre-weighed polyethylene snap-cap vial (1.5 ml capacity), the vial weighed again, and stored frozen until analysis. Specific gravity of the urine averaged 1.08 ± 0.025 (SD) g/ml during all conditions (N = 50). Urine weights were converted to volumes with this factor.

Feces were removed from the oil with forceps and placed in 15 X 45 mm shell vials. Residual oil was removed by adding 3 ml of petroleum ether to the vials, stoppering them, and shaking gently for

one minute. After discarding excess petroleum ether, the feces were dried under vacuum at 100 °C for a minimum of three days. The feces were then weighed to 0.1 mg and stored in capped test tubes until analysis.

Sodium and potassium were measured by flame photometry (Radiometer, Model FLM 2). Feces were prepared for analysis by wet ashing with 100 µl of concentrated nitric acid. After 24 h, two ml of distilled water were added. After an additional 24 h, indigestible residues were removed by filtering the extract through Whatman No. 1 filter paper. One-hundred microliter aliquots of the extract were analyzed with standard solutions and blanks containing the same final concentration of nitric acid. Pellets of food were processed in the same manner as fecal samples. Ten microliter samples of urine were analyzed.

Assay for Urinary Aldosterone

The radioimmunoassay (RIA) for free plus 'acid labile' urinary aldosterone was an adaptation of published techniques. The RIA buffer was 0.05 M Tris-HCl, pH 8.0 with 1 g/l lysozyme added to prevent non-specific binding (23). A 0.2 M KCl-HCl buffer with a pH of 1.0 was used to hydrolyze urine samples (10).

Purification of urine samples. Kley et al. (40) demonstrated urinary free aldosterone was not degraded either by exposure to room temperature for 24 h, nor by repeated freeze-thaw cycles. After thawing and mixing, 50 to 200 µl aliquots of mouse urine, depending on the sample volume available, human urine standards, and 200 µl of distilled water for blanks were pipetted into 13 X 100 mm screw cap

culture tubes and hydrolyzed by the addition of three volumes of hydrolysis buffer followed by incubation 24 h at room temperature in the dark. Mouse urine has more buffer capacity than human urine, so it was usually necessary to add small amounts of 3 N HCl to the samples to attain a pH of 1.0 $\pm$ 0.1. Malvano et al. (46) determined that free aldosterone is not appreciabely damaged by this treatment. After hydrolysis, the pH was adjusted to 7.0 $\pm$ 0.1 by the addition of saturated Na_2CO_3, and 50 µl of ethanol containing 500 cpm of chromatographically purified ^{3}H-aldosterone were added to each urine sample for recovery estimations. After 30 min incubation, the steroids were extracted by adding five volumes of ice cold methylene chloride to the vials and vortexing the mixture for 30 sec. The aqueous and organic phases were separated by centrifugation, the aqueous phase aspirated and discarded, and the methylene chloride was evaporated with a stream of dried and filtered air. The dried extracts were stored, under vacuum, in the dark.

Aldosterone was separated from potentially cross-reacting steroids with discontinuous partition chromatography on Celite microcolumns. Increasing percentages of ethylacetate in isooctane was the mobile phase and a mixture of ethylene glycol and water (80:20) was the stationary phase. Details of the methodology are in references 1, 10, and 47. The microcolumns were repacked after three uses. Solvents were pre-equilibrated prior to each use and, after each run, the microcolumns were eluted with 5 ml ethylacetate followed by 5 ml isooctane (57).

The final microcolumn eluates (50% ethylacetate) were collected in 17 X 60 mm screw cap vials. The solvents were evaporated with dried and filtered air, and the residue was dissolved in 1 ml of RIA buffer.

One-half ml of the samples were emulsified in 10 ml Aquasol and counted to 1% accuracy with a liquid scintillation spectrometer (Beckman Model LS-133). These counts provided recovery estimations. The remaining portion of the samples was stored at 4 °C.

Radioimmunoassay. Fifty microliter aliquots of the column eluates or unlabeled aldosterone standards (0, 5, 10, 20, 40, 80, 160 pg) in RIA buffer were pipetted in duplicate or triplicate into 12 X 75 mm disposable culture tubes. One hundred microliters of combined antiserum (1:75,000 dilution/50 µl) and ^{3}H-aldosterone (2500 cpm/50 µl) (21), both diluted in RIA buffer, were added, the tubes vortexed for 3 sec, warmed to 37 °C for 5 min, and incubated overnight at 4 °C. Fifty microliters of ^{3}H-aldosterone plus 100 µl of buffer were pipetted and processed in the same manner to provide "total counts" tubes.

After incubation, free hormone was separated from the antibody-bound hormone with ammonium sulfate, which stabilizes the antigen-antibody complex, followed by extraction of the free aldosterone into a toluene-based scintillation fluid (23,41). All reagents and tubes were kept on ice, and tubes were processed in groups of 10-20, with all of the first members of a pair being processed first, and then all of the second members. Saturated ammonium sulfate (150 µl) was pipetted into the tubes, the tubes were vortexed briefly, and then 2.8 ml of scintillation fluid (8.5 g Fluoralloy/1 toluene) was added. The tubes were capped and secured in pre-chilled screw cap vials which in turn were placed in a test tube rack. When a group of tubes accumulated, they were shaken vigorously by hand for 1 min. The tubes were then removed from the vials and placed in an ice bath 2 h to equilibrate. After equilibration, 2.5 ml of scintillation fluid was pipetted from

the tubes into mini-scintillation vials (New England Nuclear Corp.) and counted to 1% accuracy.

The data were transformed into logit B/T* (B = cpm bound, T* = cpm bound with no unlabeled hormone), and graphed versus the log pg unlabeled aldosterone (50). Inspection of the graphs indicated a slight but persistent deflection from linearity between 40 and 80 pg, hence the standard curves were truncated at 40 pg (less than 5% of the values were greater than 40 pg). The least squares slope and intercept were calculated, and the sample aldosterone content was determined from the regression. The daily output after correcting for the water blank was calculated by the method of Drewes et al. (21).

Sodium and Water Spaces and Biological Half-lives

Extracellular volume and total body water were measured with isotope dilution of $^{22}NaCl$ and tritiated water respectively. Mice in the control state were injected I.P. with 50 µl of 0.9% (w/v) saline solution containing 1.15 µCi $^{22}NaCl$ and 11.65 µCi $^{3}H_2O$. At each series of injections, two standards were made by pipetting 5 µl portions of the saline solution into 15 X 125 mm test tubes pre-loaded with 1 ml of water. After 45 min equilibration (the optimal time for maximal distribution of the isotopes, as determined by a pilot study) 5 to 20 µl blood samples were drawn from tail veins of the mice according to the technique of Lewis et al. (44). Additional blood samples were taken at days 1, 3 and 5 post-injection. The mice were processed in a similar manner after acclimation to ½ and ¼ ad libitum water rations, except that a blood sample was taken immediately before injection (to correct the dilution volume for the contribution of residual activity

from previous injections) and the sampling period extended to day 7.

Blood samples were centrifuged and a measured volume of plasma was transferred into test tubes prepared as above. The ^{22}Na in the samples was counted to 1.5% accuracy with a Nuclear Chicago well-type single channel gamma scintillation spectrometer. After ^{22}Na counting, $^{3}H_2O$ and ^{22}Na were separated with a water distillation technique. The test tubes were fitted with a stopper through which two 18 ga needles (with hubs removed) were inserted. The tubes were placed in a hot water bath, and flushed, via one needle, with dry nitrogen gas. The other needle was attached to 1 mm X 80 cm capillary tubing in which the water vapor condensed and was directed into small test tubes. One-half ml of the collected (tritiated) water was pipetted into mini-vials, 4.5 ml of Merit scintillation fluid added, and the samples counted to 1% accuracy. Fifty microliter aliquots of distilled standards (plus 450 µl distilled water for equivalent quenching) provided standard counts.

The counts for each isotope were corrected for background counts and any daily variation in the counters, reduced to cpm/µl plasma, and a semilog regression of the counts vs. days post-injection was used to determine the distribution volume and biological half-life of each. The sodium and tritiated water dilution volumes were multiplied by factors of 1.05 and 0.94 respectively, to correct for the Gibbs-Donnan distribution of Na^+ between plasma and interstitium (25) and the contribution of plasma protein to plasma volume (32).

Statistical Analysis

Tests for significant responses to water restriction were by sequential Wilcoxon signed-ranks comparisons (13) between the mean control value of each variable and the daily response during each treatment. The tests were run on a computer, utilizing a nonparametric statistics program (Biomedical Computer Programs, P-series; W. J. Dixon, Series Editor; Univ. of California, Los Angeles). Ratios (i.e. output/input) were converted to a fractional format (i.e. output/(output + input)) to increase symmetry about the median values. An arcsine transformation was applied to all fractional data before analysis. Reference to statistical significance denotes a probability of 0.05 or less. Linear regression and correlation values were calculated with a bivariate scatter plot and statistics program in the same statistics package.

RESULTS

Sodium and Potassium Balance

Food consumption (i.e. total ion input) decreased significantly during nonsteady state conditions, and approached or returned to control values during the succeeding steady state conditions (Fig. 1). The decrease in food consumption was both greater and more rapid in ¼ NSS than ½ NSS. Body weights decreased significantly during each NSS and remained at a lower weight at ¼ SS (Fig. 1). A previous study (in prep.) indicated that the body weight losses were composed entirely of water and fat, with the lean body mass remaining constant throughout the acclimation sequence. No differences between sexes were observed in any portion of the study.

Water restriction to ½ *ad libitum* rations elicited an immediate urinary natriuresis during the first day of restriction, followed by a significant conservation on days 3 and 4 (Fig. 2). Potassium was excreted in a similar pattern, with the exception that kaliuresis lasted two days, after which excretion declined to control levels during the next two days (Fig. 3). In contrast, sodium and potassium excretion did not exceed control levels when ½ SS animals were initially restricted to ¼ *ad libitum*, and both ions were significantly conserved by the second day (Figs. 2,3).

Urinary sodium and potassium excretion during ½ and ¼ SS approximated control levels, with scattered differences probably reflecting the slightly smaller food consumption and/or continuing small adjustments to the preceeding nonsteady states.

Excretion of both ions in the feces declined significantly by the third day of ½ ad libitum rations, and remained depressed throughout the acclimation sequence (Figs. 2,3). This decline was due to a progressively decreasing concentration of both ions in the feces (Fig. 4A). As a result, the fecal contribution to total ion output significantly lessened during water deprivation (Fig. 4B).

The relationship between the input and output of each ion is shown in Fig. 5A. The combined outputs (urinary plus fecal) of sodium and potassium were highly correlated throughout the acclimation sequence ($n = 200$, $r = 0.794$, $P<0.01$). Deviations from the overall correlation were apparent during the first day of ½ NSS, in which renal excretion of sodium was proportionally greater than potassium excretion, and during the latter portion of the nonsteady states, when renal sodium conservation exceeded that of potassium. Intestinal uptake of sodium was also greater than potassium uptake during ½ NSS (Fig. 5B).

The natriuresis and kaliuresis observed during the first two days of ½ NSS were reflected in the urine volume and ion concentrations. Urine output remained at or above control levels while the ions were maximally excreted (Fig. 6). The concentration of urinary sodium increased significantly, while potassium concentration approached a significant increase ($P = 0.07$). When the mice were further deprived to ¼ ad libitum, urine volume dropped rapidly and concentration of both

ions increased significantly. Urine volume and ion concentrations returned to control levels during ½ SS, while at ¼ SS the volume remained decreased and concentrations remained elevated.

Aldosterone Excretion

Reliability Criteria. Repeated determinations (n = 16) of the aldosterone content of the human urine standard (14.02 ± 1.44 (SD) ng/ml) yielded an interassay coefficient of variation (CV) of 9.8%, and a mean intraassay CV of 6.0% (3 quadruplicate determinations). Blanks averaged 3.3 ± 0.8 (SD) pg/tube, while the sensitivity, calculated at the 95% confidence level by the method of Ekins et al. (24), was 3.0 ± 1.2 (SD) pg/assay. Recoveries of 160 and 320 pg of unlabeled aldosterone added to 50 µl samples of the urine standard (n = 4 for each determination) were 94.7 ± 29.9 and 87.5 ± 18.8 (SD)% respectively. Recoveries of labeled aldosterone added to the samples averaged 59% (n = 144, SD = 7.6%). The human urine standard, from a normal individual, agrees well with values reported by Malvano et al. (46). Mean control excretion rates of aldosterone in mice approximated those of rats (mice excreted 86 versus about 22 and 60 pg per day per gram body weight in rats (36,40)). Only free urinary aldosterone was measured in the rats, but the additional increment released from conjugated aldosterone is less than 10% (36).

Responses to Water Restriction. Urinary aldosterone content was significantly increased throughout the ½ NSS condition (Fig. 7, $P<0.007$ during peak excretion on day 2). Aldosterone excretion remained slightly, and occasionally significantly, elevated during all subsequent conditions. The mice were quite variable in the magnitude and timing of their individual responses. This variation was particularly evident during nonsteady state conditions.

Distribution Volume and Turnover Rates of ^{22}Na and $^{3}H_2O$

Water restriction produced a significant reduction in total body water (TBW) and extracellular volume (ECV), in ½ SS mice. Total body water was significantly reduced at ¼ SS, while ECV approximated control amounts (Table 1). Extracellular volume expressed relative to body weight remained at control volumes throughout the study, while TBW per weight decreased at ½ SS and returned to control values at ¼ SS.

The biological half-lives ($t_{\frac{1}{2}}$) of both isotopes yielded linear plots at all conditions (worst-case $r = 0.98$, $P<0.01$). Half-lives for ^{22}Na and $^{3}H_2O$ showed distinctly different patterns of response which were attributable to differences of input of food and water respectively (8). Increased $t_{\frac{1}{2}}$ of ^{22}Na during NSS conditions resulted from decreased food consumption and excretion of sodium after the second day of NSS conditions. When mice attained the succeeding steady state, food consumption, urinary excretion of sodium, and the $t_{\frac{1}{2}}$ approached or returned to control levels (Figs. 1,2, Table 1). In contrast, the sequential reduction in water rations induced a stepped and progressively greater $t_{\frac{1}{2}}$ for $^{3}H_2O$, with no difference apparent between NSS and the next SS conditions. One mouse died during ¼ NSS, hence the tabular data is based on nine animals.

DISCUSSION

Responses of mice during the acclimation sequence are summarized in Table 2. The first two days of transition from normal water intake to ½ ad libitum was characterized by natriuresis and kaliuresis accompanied by a slight increase in urine volume, a doubling of aldosterone excretion, and a gradual reduction of food consumption. Urinary sodium concentration rose on day 1 but returned to normal on day 2. Antidiuresis and antinatriuresis occurred on days 3 and 4 of ½ NSS, kaliuresis ceased, and aldosterone excretion decreased but was still greater than normal. At this time elevated intestinal absorption of both ions was established, a feature that persisted throughout the ½ SS and on into the ¼ ad libitum transition.

The natriuresis and kaliuresis which occur on day 1, and to a lesser extent day 2, of ½ NSS (Figs. 2,3) are probably due to release of vasopressin (ADH) in response to both increased plasma osmolality or sodium concentration (Table 3) and decreased blood volume (22,56). Acute administration of ADH induces a natriuresis in hydrated sheep, pigs, dogs, and rats (45,48,52,53), due to a direct intrarenal effect of ADH (26,38). The effect is not due to suppression of mineralocorticoid secretion, since administration of ADH to adrenalectomized sheep increases electrolyte excretion (4), and a natriuresis occurs in mice despite an increasing aldosterone secretion (Figs. 2,7).

Hydropenic rats also display a saluresis (45), and hydropenic dogs undergo a natriuresis and diuresis (7,26,42). The body electrolyte stores of the subjects apparently determine which ion or combination of ions is excreted (38). The concurrent aldosterone release (Fig. 7) is presumably caused by activation of the renin-angiotensin system and a direct effect of plasma potassium on the adrenal gland. Dluhy et al. (19) suggest an additive relationship exists between the two inputs to aldosterone secretion. The control of renin release is complex (for review see 16), but hypovolemia is one factor which increases renin release and is in effect during the nonsteady states (30,49). We demonstrated previously (32), that plasma volume decreases 25% by day 4 of the nonsteady states. Small increments in plasma potassium concentration, less than occur during the nonsteady states (Table 3), are known to cause a release of aldosterone in the rat (6,14). It is unlikely that the stress of a partial water restriction was great enough to induce the release of large quantities of adrenocorticotrophic hormone (ACTH) which in turn causes an elevated secretion of aldosterone. Surgery and sodium depletion are two stressors implicated in ACTH induced aldosterone release (28), but mice were not subjected to such stresses. Furthermore, complete water restriction for a period of 8-10 days was not sufficient to elevate plasma cortisol or corticosterone levels in camels (27), and acute hypovolemia due to blood removal followed by three days of water deprivation significantly increased aldosterone production in the rat, while corticosterone synthesis remained unchanged (17). However, in the absence of simultaneous measurements of glucocorticoid excretion, an influence on aldosterone secretion by ACTH in house mice cannot be ruled out.

Attenuation of the natriuresis and kaliuresis in the latter part of ½ NSS is presumably due to the action of a complex of factors, including increased secretion of aldosterone, a direct intrarenal effect of angiotensin II (34,39), and hemodynamic adjustments. There is evidence that decreased glomerular filtration rate (33), and increased oncotic pressure (Table 3) occur during acclimation. The effects of aldosterone cannot be separated from those of other factors in this experimental design, however, the dynamic changes in secretion of aldosterone during the nonsteady state condition suggest a functional relationship between the two.

The initial natriuresis is over-compensated by increased conservation of sodium from day 3 of ½ NSS to the succeeding steady state, as indicated by its significantly greater half-life (Table 1), and plasma concentration (Table 3). The percentage increase in concentration is greater than the corresponding decrease in distribution volume. The elevated sodium concentration may produce movement of water from cells to the extracellular space, as indicated, at ¼ SS, by a disproportionately greater reduction in intracellular volume (i.e. total body water - ECV) in comparison to ECV losses (ECV - 6.4% loss versus intracellular volume loss of 14.3%, $P<0.05$). Additionally, an increase in plasma sodium may be secondary to the regulation of plasma potassium concentration. The influence of the aldosterone-potassium feedback control system in controlling plasma potassium concentration has been demonstrated in the dog (59). A similar relationship may pertain in house mice, since plasma potassium concentrations are not significantly different from control values throughout the acclimation sequence (Table 3). During hydropenia,

the ECV expansion which would normally result from sodium retention does not occur (Table 1), and this could blunt hemodynamic mechanisms (54) which aid in removing a sodium load, producing a persistent, elevated plasma sodium concentration.

A net loss of sodium and potassium, followed by a conservation or return to balance of both ions is a pattern common to both nonsteady states (Fig. 5), but the means by which the pattern is produced at ¼ NSS is different from that at ½ NSS and presumably reflects a pre-acclimation effect. The natriuresis, kaliuresis, spike of aldosterone excretion and slow initiation of an antidiuresis (Fig. 6) are absent at ¼ NSS, and the net loss of the ions is caused by an immediate and large decrease in food consumption at ¼ NSS (Fig. 1). Suggestions forwarded to explain the starvation rats and mice undergo during dehydration include maintenance of a constant proportion of body fluids to solids (2), maintenance of a constant ratio of water to food in the gastric contents (43), and an inability to moisten food before swallowing (12). Yin et al. (58) described, in rats with access to ad libitum food and water, changes in food consumption in response to a daily intragastric administration of hyperosmotic NaCl solution which parallel the responses of our water restricted mice. The solution, which transiently increased serum osmolality in the rats, and is equated to the effects of NSS water restriction on the mice in this comparison, produced a decrease in feeding followed by a return to control levels of food consumption over three days. A similar pattern pertains in mice adjusting to a reduced water ration from day 2 of ½ NSS and immediately at ¼ NSS. We postulate that increases in plasma osmolality depress feeding in a manner which

depends on the level of plasma osmolality prior to its increase, and that if the increasing osmolality is not too great (i.e. 100% water restriction) 'escape' from the inhibitory effect of hyperosmolality occurs over a period of days. We also suggest that the reduction of feeding during an initial period of ECV constriction may reduce the ion load, and as homeostatic mechanisms become more effective, increased food can be accommodated during the latter portion of the nonsteady state.

Urine volumes decrease rapidly at ¼ NSS (Fig. 6), suggesting that a decreased ion load allows a rapid, efficient antidiuresis, or that the release of ADH is faster and of a greater magnitude than at ½ NSS. The possibilities are not mutually exclusive.

Aldosterone excretion at ¼ NSS shows no increase from that at ½ SS (Fig. 7). A possible explanation for the lack of an additional response may be that the smaller relative water restriction (0.6 g/day deficit vs. 1.2 g/day at ½ NSS) does not produce as large of an increase in plasma potassium concentration and/or decrease in blood volume. Additionally, infusion of ADH to elevated, but physiological blood concentrations, inhibits the release of renin in dogs (38,55). Male rats also have decreased plasma renin activity when given daily injections of ADH (3). If a similar response to elevated ADH occurs in house mice, then the secretion of aldosterone at the initiation of a more severe water restriction at ¼ NSS may be partially suppressed.

Changes in sodium and potassium absorption by the intestine, in response to increasing water deprivation, involve a progressive augmentation of absorption of both ions (Fig. 4). Principles of small intestinal ion absorption have been reviewed (51) and the similarity

between the effects of oncotic and hydrostatic pressure on ion absorption across the intestinal and proximal renal tubule epithelia drawn. The ratio of sodium to potassium reflection coefficients in human jejunum and rabbit ileum is approximately 1.2 (51), which will, in the absence of elevated active transport of either ion, produce a net uptake of potassium greater than that of sodium. The data for house mice are suggestive that a reflection coefficient ratio pertains which similarly favors potassium uptake over sodium during control conditions, since the potassium:sodium ratio of 2 in the food is reduced to 1 in the feces, while it remains unchanged in the urine (Fig. 5). During nonsteady state conditions, an increase in plasma oncotic pressure (Table 3) and a possible decrease in hydrostatic pressure (secondary to hypovolemia), would favor the absorption of both ions, but in the absence of an increased active transport of sodium, potassium should be preferentially absorbed in comparison to sodium. In the rat, sodium absorption by the colon is increased by mineralocorticoids (11,20) while angiotensin II, by a direct effect, increases sodium uptake in jejunal preparations (15), and the ascending colon (37). Vasopressin also increases sodium transfer from the ascending colon (37). The relative contribution of each to the significantly increased fractional absorption of sodium relative to potassium observed at ½ NSS (Fig. 5) cannot be determined from our data, but the operation of these factors is demonstrated by the fact that absorption proceeds counter to that predicted by the presumed reflection coefficients of the two ions. The significantly greater absorption of both ions at ½ and ¼ steady states, with a maintained 1:1 ratio, suggests that the effects of dehydration on oncotic or hydrostatic pressures continues and that a small, but

continuous, active augmentation of sodium absorption is maintained. Alternatively, a greater degree of active sodium uptake could also increase potassium uptake by solvent drag (51).

The pattern of increased intestinal absorption of sodium and potassium during dehydration appears to be maladaptive to the purpose of reducing an ion load, and may be a non-regulatory response to the hemodynamic and hormonal changes detailed above. The additional ion load imposed by this response is minor in comparison to the renal modulation of the two ions (Fig. 4) and may have little effect on the daily balance of either. Rats produce significantly drier feces during acute dehydration than in a water replete state (18). A similar response was not demonstrated in house mice during an acclimation sequence (31), but the increased intestinal absorption of electrolytes (and associated water) may produce drier feces and aid in water balance during a more severe, acute water restriction.

A speculative relationship between ADH and aldosterone release after acclimation to water restriction is that if the release of ADH does not accommodate during chronic water restriction (9,29), then a 'tension' between ADH and aldosterone exists in the acclimated, steady state animals (½ and ¼ SS) in which ADH and aldosterone secretion are greater than control levels. The elevation, at least in the case of aldosterone, need not be great. If an additional water restriction is imposed, then ADH secretion is induced more rapidly than in a naive animal, and the pre-existing increment of aldosterone secretion, in concert with a reduction in food intake, may prevent the natriuresis, kaliuresis, and large urine volume characteristic of the initial ADH secretion in a non-acclimated animal. In support of this is the

observation that injection of deoxycorticosterone acetate (DOCA) abolishes the natriuresis of initially water-restricted rats (45). This effect of mineralocorticoids may require a hydropenic condition, since injection of DOCA prior to and infusion of deoxycorticosterone glucoside during the infusion of ADH into water diuresing dogs did not reduce the natriuretic effect of ADH (26).

In conclusion, the process of acclimation to water restriction by house mice involves dynamic changes in sodium and potassium balance. At initiation of each water restriction, both ions are excreted in excess of intake, although the means by which this occurs after acclimation to ½ ad libitum is different from that in naive mice. The excretory pattern is generally reversed to conservation of both ions during the latter part of the nonsteady states. In contrast to changes in renal handling of the ions, the gut displays a persistent, elevated absorption of sodium and potassium throughout the period of water restriction. Aldosterone secretion is significantly increased during the initial water restriction, and remains slightly elevated throughout the acclimation sequence. Definitive statements regarding the interplay of aldosterone, other hormonal mediators, and hemodynamic factors cannot be made at this time.

REFERENCES

1. Abraham, G. E., J. E. Buster, L. A. Lucas, P. C. Corrales, and R. C. Teller. Chromatographic separation of steroid hormones for use in radioimmunoassay. Anal. Lett. 5:509-517, 1972.

2. Adolph, E. F. Urges to eat and drink in rats. Am. J. Physiol. 151:110-125, 1947.

3. Balment, R. J., I. W. Henderson, and J. A. Oliver. The effects of vasopressin on pituitary oxytocin content and plasma renin activity in rats with hypothalamic diabetes insipidus (Brattleboro strain). Gen. Comp. Endocrinol. 26:468-477, 1975.

4. Beal, A. M. The effect of high rates of vasopressin administration on renal potassium and sodium excretion during potassium loading in the sheep. Brit. J. Pharmacol. 58:81-88, 1976.

5. Blair-West, J. R., A. H. Brook, and P. A. Simpson. Renin responses to water restriction and rehydration. J. Physiol. London 226:1-13, 1972.

6. Boyd, J. E., and P. J. Mulrow. Further studies of the influence of potassium upon aldosterone production in the rat. Endocrinology 90:299-301, 1972.

7. Buckalew, V. M., Jr., and K. A. Dimond. Effect of vasopressin on sodium excretion and plasma antinatriferic activity in the dog. Am. J. Physiol. 231:28-33, 1976.

8. Burch, G. E., S. A. Threefoot and J. A. Cronvich. Theoretic considerations of biologic decay rates of isotopes. J. Lab. Clin. Med. 34:14-30, 1949.

9. Castel, M. and M. Abraham. Effects of dry diet on the antidiuretic hormone content of the neurohypophysis in spiny mice as compared to the albino rat and mouse. Gen. Comp. Endocrinol. 19:48-55, 1972.

10. Chakmakjian, Z. H., W. W. Pryor, and G. E. Abraham. A radioimmunoassay for serum and urine aldosterone by celite column chromatography. Anal. Lett. 7:97-108, 1974.

11. Charney, A. N., M. D. Kinsey, L. Myers, R. A. Giannella, and R. E. Gots. Na^+-K^+- activated adenosine triphosphatase and intestinal electrolyte transport: Effect of adrenal steroids. J. Clin. Invest. 56:653-660, 1975.

12. Chew, R. M. and R. T. Hinegardner. Effects of chronic insufficiency of drinking water in white mice. J. Mammal. 38:361-374, 1957.

13. Conover, W. J. Practical Nonparametric Statistics. New York: Wiley, 1971, p. 206-215.

14. Corvol, P., E. Oblin, P. Degoulet, Ph. Fressinaud, and J. Menard. Effect of acute potassium loading on plasma renin and on urinary aldosterone in rats. Endocrinology 100:1008-1013, 1977.

15. Crocker, A. D. and K. A. Munday. The effect of the renin-angiotensin system on mucosal water and sodium transfer in everted sacs of rat jejunum. J. Physiol. London 206:323-333, 1970.

16. Davis, J. O. and R. H. Freeman. Mechanisms regulating renin release. Physiol. Rev. 56:1-56, 1976.

17. Debreceni, L. and B. Csete. Effect of change of body fluid volume on aldosterone and corticosterone production in the rat. Acta Physiol. Acad. Sci. Hung. 41:243-247, 1972.

18. Dicker, S. E. and J. Nunn. The role of the antidiuretic hormone during water deprivation in rats. J. Physiol. London 136:235-248, 1957.

19. Dluhy, R. G., M. Greenfield, and G. H. Williams. Effect of simultaneous potassium and saline loading on plasma aldosterone levels. J. Clin. Endocrinol. Metab. 45:141-146, 1977.

20. Dolman, D. and C. J. Edmonds. The effect of aldosterone and the renin-angiotensin system on sodium, potassium and chloride transport by proximal and distal rat colon in vivo. J. Physiol. London 250:597-611, 1975.

21. Drewes, P. A., J. A. Demetriou, and V. J. Pileggi. Measurement of urinary aldosterone by a simplified radioimmunoassay procedure. Clin. Biochem. 6:88-97, 1973.

22. Dunn, F. L., T. J. Brennan, A. E. Nelson, and G. L. Robertson. The role of blood osmolality and volume in regulating vasopressin secretion in the rat. J. Clin. Invest. 52:3212-3219, 1973.

23. Edwards, C. R. W., A. A. Taylor, C. K. Baum, and A. B. Kurtz. Evaluation of two new separation techniques for steroid radioimmunoassays. In: Steroid Immunoassay, edited by E. H. Cameron, et al. Cardiff: Alpha Omega, 1975, p. 229-234.

24. Ekins, R. P., G. B. Newman, and J. L. H. O'Riordan. Saturation analysis. In: Statistics in Endocrinology, edited by J. W. McArthur and T. Colton. Cambridge, MA: Colonial Press, 1970, p. 345-378.

25. Elkinton, J. R. and T. S. Danowski. The Body Fluids. Baltimore, MD: Williams and Wilkins, 1955.

26. Fejes-Toth, G., A. Magyar, and J. Walter. Renal response to vasopressin after inhibition of prostaglandin synthesis. Am. J. Physiol. 232:F416-F423, 1977 or Am. J. Physiol.: Renal Fluid Electrolyte Physiol. 1:F416-F423, 1977.

27. Finberg, J. P. M., R. Yagil, and G. M. Berlyne. Response of the renin-aldosterone system in the camel to acute dehydration. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 44:926-930, 1978.

28. Ganong, W. F., E. G. Biglieri, and P. J. Mulrow. Mechanisms regulating adrenocortical secretion of aldosterone and glucocorticoids. Rec. Prog. Horm. Res. 22:381-414, 1966.

29. George, J. M. Vasopressin and oxytocin are depleted from rat hypothalamic nuclei after oral hypertonic saline. Science 193:146-148, 1976.

30. Gordon, R. D. and C. G. K. Pawsey. Relative effects of serum sodium concentration and the state of body fluid balance on renin secretion. J. Clin. Endocrinol. Metab. 32: 117-119, 1971.

31. Haines, H., C. Ciskowski, and V. Harms. Acclimation to chronic water restriction in the wild house mouse Mus musculus. Physiol. Zool. 46:110-128, 1973.

32. Haines, H., T. M. McKenna, and J. E. Melton. Body fluid distribution in wild Mus musculus acclimated to water restriction. Am. J. Physiol. 235:R237-R242, 1978 or Am. J. Physiol.: Regulatory Integrative Comp. Physiol. 4:R237-R242, 1978.

33. Haines, H. and B. Schmidt-Nielsen. Kidney function in spiny mice (Acomys cahirinus) acclimated to water restriction. Bull. M. Desert Isl. Biol. Lab. 17:94-95, 1977.

34. Hall, J. E., A. C. Guyton, N. C. Trippodo, T. E. Lohmier, R. E. McCaa, and A. W. Cowley, Jr. Intrarenal control of electrolyte excretion by angiotensin II. Am. J. Physiol. 232:F538-F544, 1977 or Am. J. Physiol.: Renal Fluid Electrolyte Physiol. 1:F538-F544, 1977.

35. Haning, R., J. McCracken, M. St. Cyr, R. Underwood, G. Williams, and G. Abraham. The evolution of titer and specificity of aldosterone binding antibodies in hyperimmunized sheep. Steroids 20:73-88, 1972.

36. Hilfenhaus, M. Urinary aldosterone excretion rate and plasma aldosterone concentration in the rat: effect of ACTH, DOC, furosemide and of changes in sodium balance. Acta Endocrinol. 85:134-142, 1977.

37. Hornych, A., P. Meyer and P. Milliez. Angiotensin, vasopressin, and cyclic AMP: effects on sodium and water fluxes in rat colon. Am. J. Physiol. 224:1223-1229, 1973.

38. Johnson, M. D., L. B. Kinter, and R. Beeuwkes III. Effects of AVP and DDAVP on plasma renin activity and electrolyte excretion in conscious dogs. Am. J. Physiol. 236:F66-F70, 1979 or Am. J. Physiol.: Renal Fluid Electrolyte Physiol. 5:F66-F70, 1979.

39. Johnson, M. D. and R. L. Malvin. Stimulation of renal sodium reabsorption by angiotensin II. Am. J. Physiol. 232:F298-F306, 1977 or Am. J. Physiol.: Renal Fluid Electrolyte Physiol. 1:F298-F306, 1977.

40. Kley, H. K., W. Elsasser, H. Dehnen and H. L. Kruskemper. Evaluation of adrenal function in rats by the measurement of urinary free corticosterone, free aldosterone and free 11-deoxycorticosterone. Steroids 32:223-232, 1978.

41. Kurtz, A. B. and F. C. Bartter. Radioimmunoassays for aldosterone and deoxycorticosterone in plasma and urine. Steroids 28: 133-142, 1976.

42. Kurtzman, N. A., P. W. Rogers, S. Boonjarern, and J. A. L. Arruda. Effect of infusion of pharmacologic amounts of vasopressin on renal electrolyte excretion. Am. J. Physiol. 228:890-894, 1975.

43. Lepkovsky, S., R. Lyman, D. Fleming, M. Nagumo, and M. M. Dimick. Gastrointestinal regulation of water and its effect on food intake and rate of digestion. Am. J. Physiol. 188:327-331, 1957.

44. Lewis, V. J., W. L. Thacker, S. H. Mitchell, and G. M. Baer. A new technic for obtaining blood from mice. Lab. Anim. Sci. 26:211-213, 1976.

45. Luke, R. G. Natriuresis and chloruresis during hydropenia in the rat. Am. J. Physiol. 224:13-20, 1973.

46. Malvano, R., S. Orlandini, P. Cozzani, P. Duranti, N. Simonini, and A. Salvetti. Methodological simplifications in radioimmunoassay of urinary aldosterone. Clin. Chim. Acta 66:331-341, 1976.

47. Manlimos, F. S. and G. E. Abraham. Chromatographic purification of tritiated steroids prior to use in radioimmunoassay. Anal. Lett. 8:403-410, 1975.

48. Martinez-Maldonado, M., G. Eknoyan, and W. N. Suki. Natriuretic effects of vasopressin and cyclic AMP: possible site of action in the nephron. Am. J. Physiol. 220:2013-2020, 1971.

49. Newsome, H. H., and F. C. Bartter. Plasma renin activity in relation to serum sodium concentration and body fluid balance. J. Clin. Endocrinol. Metab. 28:1704-1711, 1968.

50. Rodbard, D., P. L. Rayford, J. A. Cooper, and G. T. Ross. Statistical quality control of radioimmunoassays. J. Clin. Endocrinol. Metab. 28:1412-1418, 1968.

51. Schultz, S. G., R. A. Frizzell, and H. N. Nellans. Ion transport by mammalian small intestine. Ann. Rev. Physiol. 36:51-91, 1974.

52. Scott, D., and J. J. Morton. Changes in urinary water and electrolyte excretion in sodium-loaded sheep in response to intravenous infusion of arginine vasopressin. Q. J. Exp. Physiol. 61:57-70, 1976.

53. Scott, D., and J. J. Morton. The effects of vasopressin on renal sodium excretion in pigs given intravascular hypertonic salt loads. Q. J. Exp. Physiol. 62:73-82, 1977.

54. Sealey, J. E., and J. H. Laragh. A proposed cybernetic system for sodium and potassium homeostasis: Coordination of aldosterone and intrarenal physical factors. Kidney Int. 6:281-290, 1974.

55. Shade, R. E., J. O. Davis, J. A. Johnson, R. W. Gotshall, and W. S. Spielman. Mechanisms of action of angiotensin II and anti-diuretic hormone on renin secretion. Am. J. Physiol. 224:926-929, 1973.

56. Shade, R. E., and L. Share. Volume control of plasma antidiuretic hormone concentration following acute blood volume expansion in the anesthetized dog. Endocrinology 97:1048-1057, 1975.

57. Tulchinsky, D., and G. E. Abraham. Radioimmunoassay of plasma estriol. J. Clin. Endocrinol. Metab. 33:775-782, 1971.

58. Yin, T. H., C. L. Hamilton, and J. R. Brobeck. Effect of body fluid disturbance on feeding in the rat. Am. J. Physiol. 218:1054-1059, 1970.

59. Young, D. B., R. E. McCaa, Yi-Jen Pan, and A. C. Guyton. Effectiveness of the aldosterone-sodium and-potassium feedback control system. Am. J. Physiol. 231:945-953, 1976.

Table 1. Distribution volumes and biological half-lives of ^{22}Na and $^{3}H_2O$

Condition	Weight (g)	Distribution Volume (ml)		Distribution Volume (% b.w.)		Biological Half-life (days)	
		^{22}Na	$^{3}H_2O$	^{22}Na	$^{3}H_2O$	^{22}Na	$^{3}H_2O$
Control	20.0	5.33	12.14	26.7	60.9	7.20	2.25
	1.0	.26	.63	.7	1.8	.46	.11
½ NSS*	18.1‡					9.43+	3.50‡
	.9					.50	.11
½ SS	18.5+	4.76+	10.63‡	25.7	57.5+	8.77	3.36‡
	1.0	.33	.59	.9	1.5	.66	.16
¼ NSS	17.1‡					9.33‡	4.38‡
	.9					.59	.17
¼ SS	17.7+	5.00	10.78+	28.5	61.2	8.78+	4.36‡
	1.0	.32	.75	1.4	2.4	.51	.18

Upper numbers are means, lower numbers are standard errors, N = 9. +P<0.05, ‡P<0.01

*½ NSS denotes that mice which are receiving ½ ad libitum water rations are in a nonsteady state condition, ½ SS indicates that mice are acclimated to ½ ad libitum water rations. ¼ NSS and ¼ SS denote nonsteady state and steady state conditions, respectively, in response to ¼ ad libitum water rations.

Table 2. Summary of trends during acclimation to water restriction*

	½ NSS		½ SS	¼ NSS		¼ SS
	day 1	remainder		day 1	remainder	
Na-K Balance	-	+	0	-	+	0
Aldosterone Excretion	+	+	0	0	0	0
Urine Volume	+	0	0	-	-	-
Food Consumption	0	-	-	-	-	0
^{22}Na Distribution (ml)			-			0
^{22}Na Half-life (days)		+	0		+	+
$^{3}H_2O$ Distribution (ml)			-			-
$^{3}H_2O$ Half-life (days)		+	+		+	+

* (+) indicates a generally greater or more positive response in comparison to control values, (0) denotes little change from control, (-) indicates a generally lesser or more negative response.

Table 3. Plasma characteristics of acclimating and acclimated house mice*

	Control	½ NSS	½ SS	¼ NSS	¼ SS
N	6	5	12	7	6
Osmolality	332.2	350.4	359.0^{+}	341.4	359.5
	13.5	4.4	5.6	3.7	8.3
Protein	67.2	80.4^{+}	77.5	83.6‡	67.8
	4.3	2.1	3.6	2.1	1.6
Na^{+}	148.8	178.4§	171.4‡	161.0‡	160.5‡
	1.9	5.2	5.2	4.7	2.4
K^{+}	5.8	6.4	5.8	6.9	6.4
	.6	.9	.4	1.1	.4

*Unpublished data, collected under the same conditions as those in this study. NSS samples were taken at day 4. Upper numbers are means, lower numbers are standard errors.

$^{+}P<0.05$, $^{\ddagger}P<0.01$, $^{\S}P<0.001$, t-tests for independent samples.

FIGURE LEGENDS

Fig. 1. Water ration, food consumption and body weights of mice throughout the acclimation sequence. Circles are means of 10 animals, vertical bars indicate one standard error of the mean. Closed circles indicate a significant difference from the mean of four days of control values at $P<0.05$. Heavy vertical lines denote a transition from a steady state to a successive nonsteady state condition. Responses of mice in nonsteady state and steady state conditions, while receiving ½ ad libitum water rations, are indicated under ½ NSS and ½ SS respectively. Nonsteady state and steady state responses while receiving ¼ ad libitum water rations are indicated under ¼ NSS and ¼ SS respectively.

Fig. 2. Sodium balance during acclimation to water restriction. Symbols as in Fig. 1. Solid line (O —— O) indicates daily input, dotted line (O ··· O) indicates daily urinary output, and dashed line (O — — O) denotes daily fecal output.

Fig. 3. Potassium balance during acclimation to water restriction. Symbols are as in Figs. 1 and 2.

Fig. 4. Concentration of sodium and potassium in feces (A, top panel), and contribution of fecal output to the total ion output (B, bottom panel). Measures of sodium (0 ··· 0) are slightly to the left of the potassium values (0 —— 0). The 95% confidence interval is indicated by vertical lines in bottom panel, otherwise symbols are as in Fig. 1.

Fig. 5. Output (O) and input (I) relations of sodium and potassium (A, upper panel) and fractional output of sodium in comparison to potassium (B, lower panel). The output-input relationship is expressed as a percentage of total output and input for each ion (i.e. if output = input, the percentage plotted = 50%). Symbols are as in Fig 4B. The fractional outputs of sodium in the feces (Δ) and urine (O) are graphed in the lower panel. Vertical lines indicate 95% confidence intervals.

Fig. 6. Daily urine volume and concentration of sodium (0 ··· 0) and potassium (0 —— 0). Symbols are as in Fig. 1.

Fig. 7. Daily urinary aldosterone excretion (free plus 'acid labile'). Symbols are as in Fig. 1. The sample size = 9 on days 2,3 of ½ NSS, due to inadequate urine sample size, otherwise n = 10.

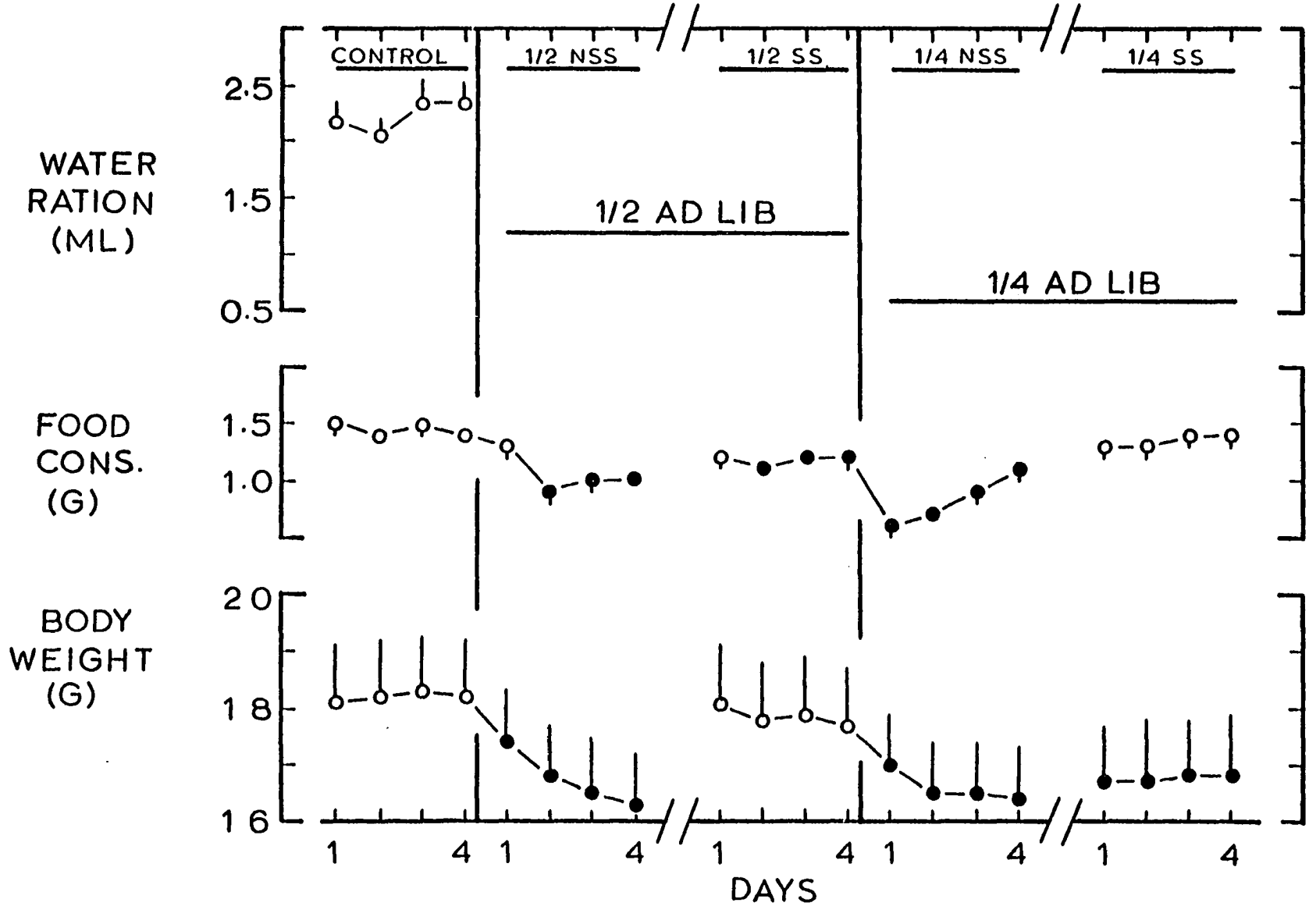
CONTROL
1/2 NSS
1/2 SS
1/4 NSS
1/4 SS
WATER RATION (ML)
2.5
1.5
0.5
1/2 AD LIB
1/4 AD LIB
FOOD CONS. (G)
1.5
1.0
BODY WEIGHT (G)
20
18
16
1
4
1
4
1
4
1
4
1
4
DAYS

Figure 1

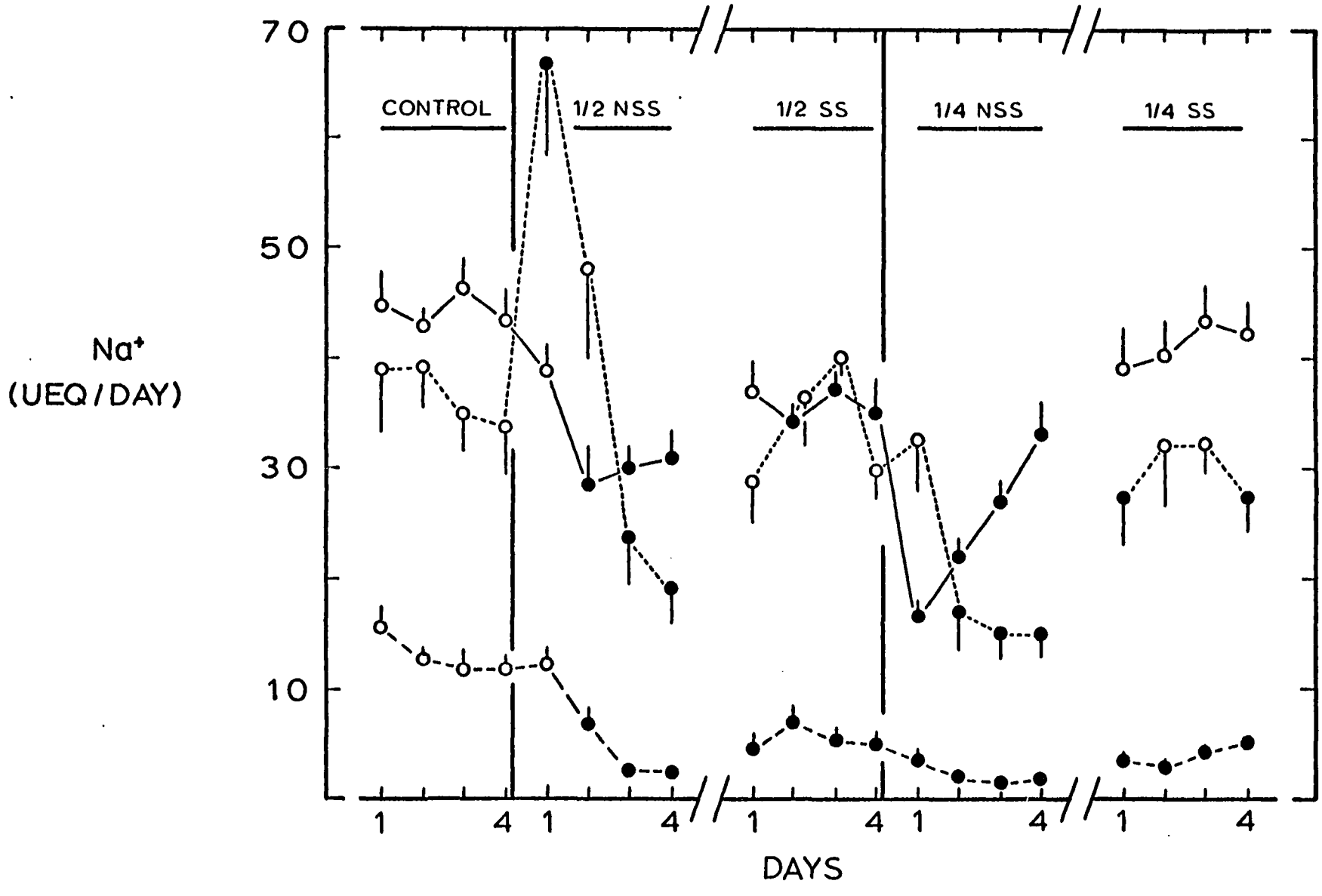
70
50
30
10
Na+
(UEQ/DAY)
CONTROL
1/2 NSS
1/2 SS
1/4 NSS
1/4 SS
1
4
1
4
1
4
1
4
1
4
DAYS

Figure 2

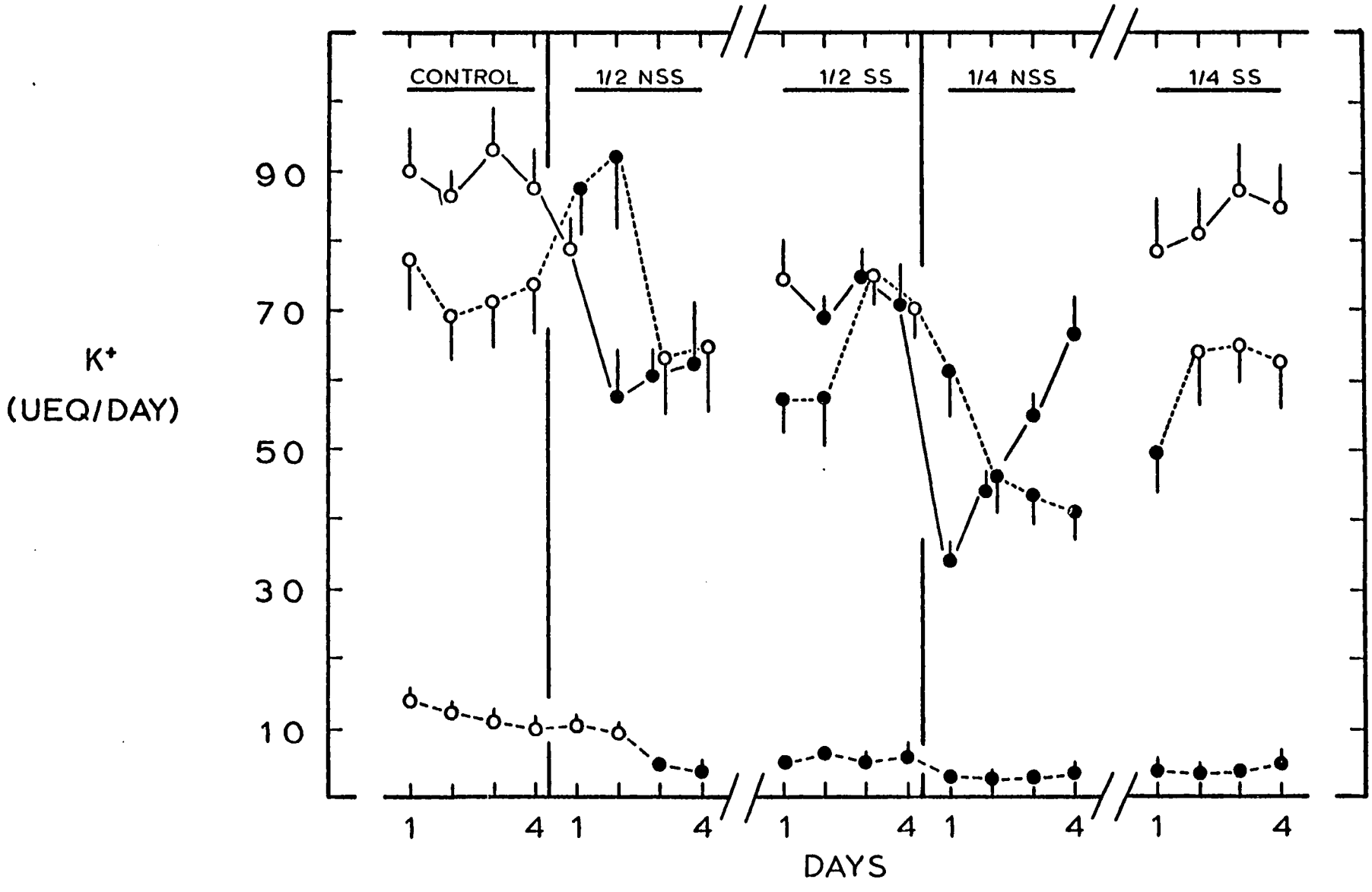
CONTROL
1/2 NSS
1/2 SS
1/4 NSS
1/4 SS
90
70
50
30
10
K+
(UEQ/DAY)
1
4
1
4
1
4
1
4
1
4
DAYS

Figure 3

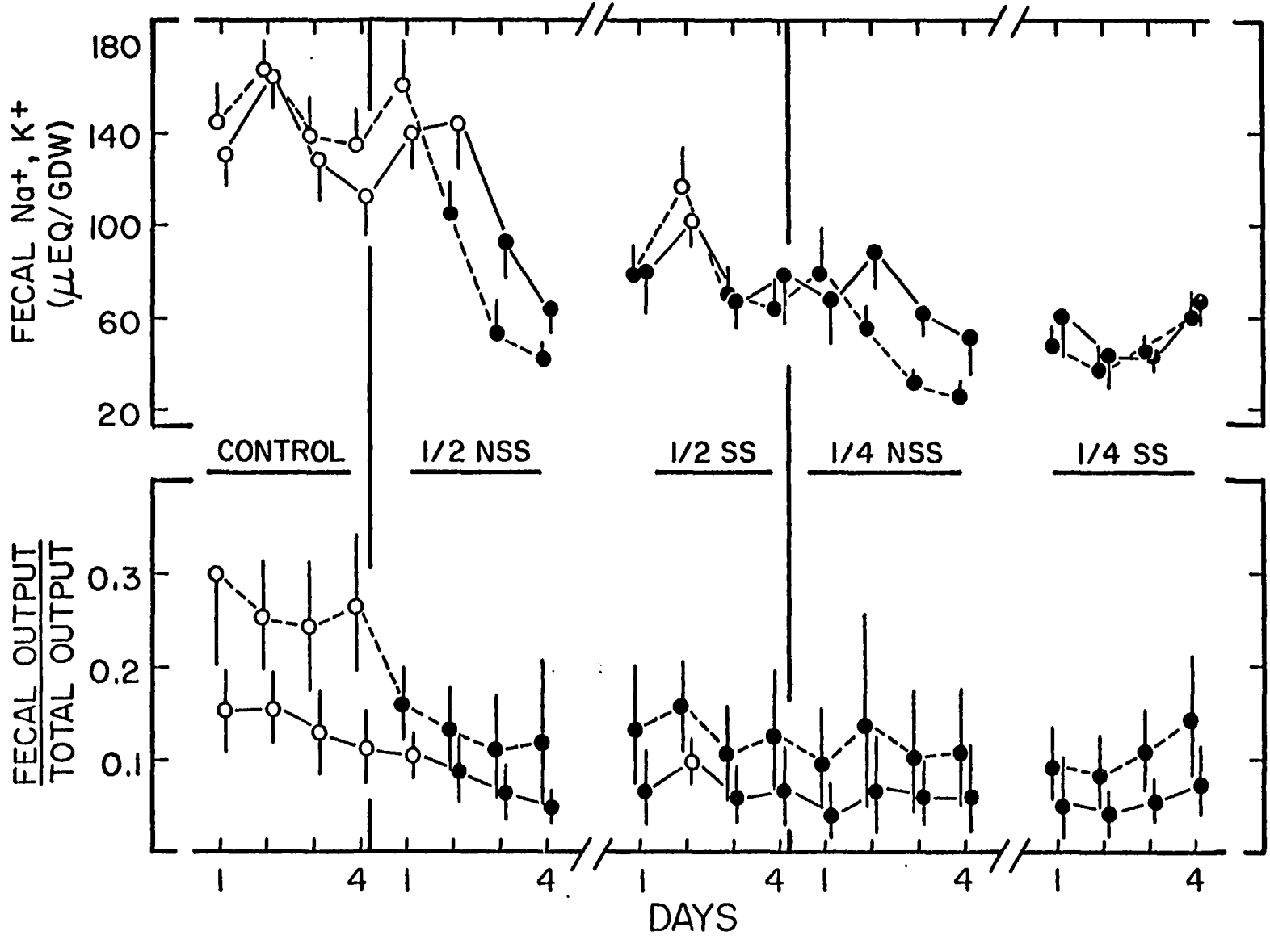
FECAL Na+, K+ (μEQ/GDW)
180
140
100
60
20
CONTROL
1/2 NSS
1/2 SS
1/4 NSS
1/4 SS
FECAL OUTPUT / TOTAL OUTPUT
0.3
0.2
0.1
1
4
1
4
1
4
1
4
1
4
DAYS

Figure 4

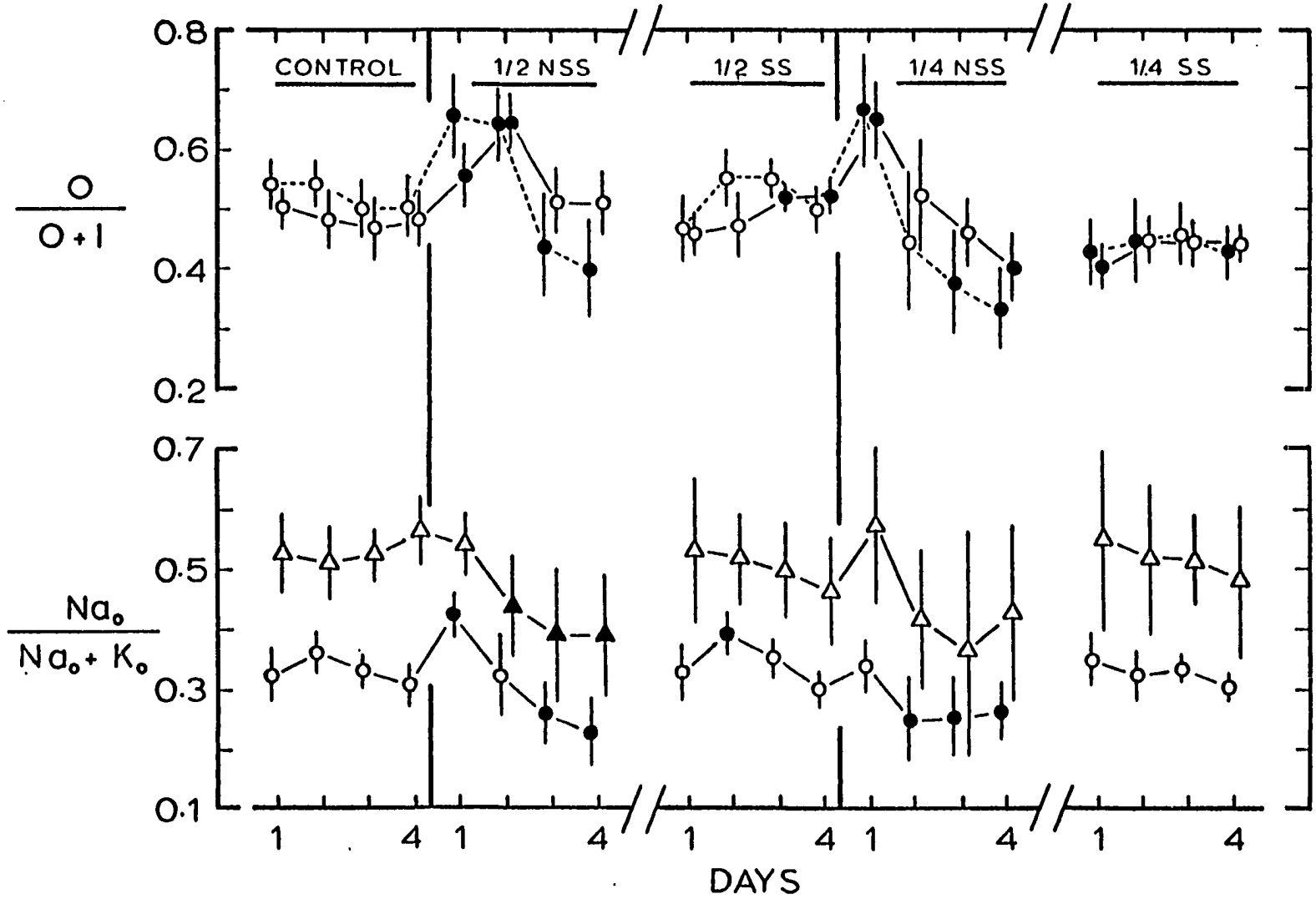
CONTROL
1/2 NSS
1/2 SS
1/4 NSS
1/4 SS
O / O+I
Na$_o$ / Na$_o$ + K$_o$
0.8
0.6
0.4
0.2
0.7
0.5
0.3
0.1
1
4
1
4
1
4
1
4
1
4
DAYS

Figure 5

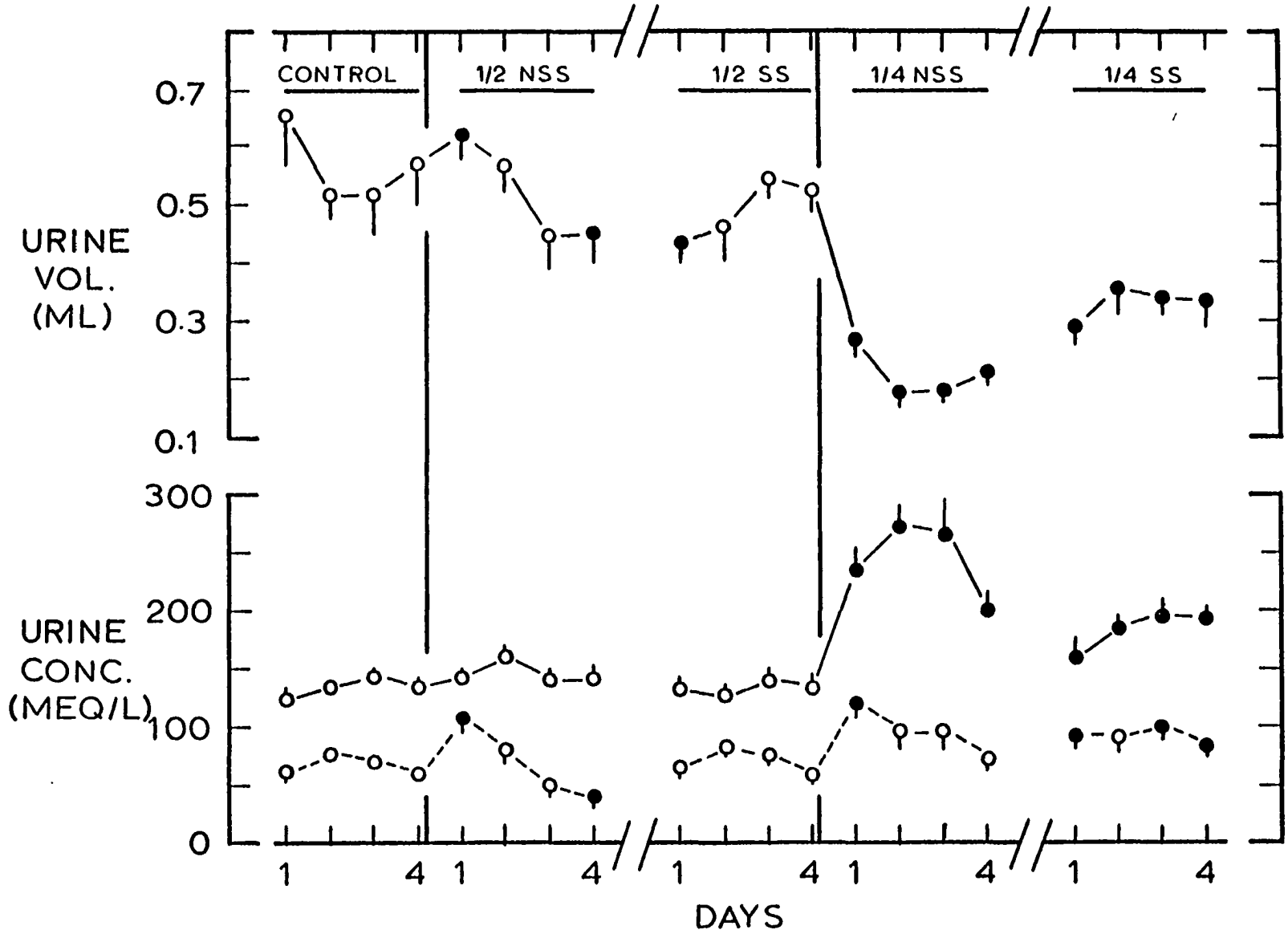
CONTROL
1/2 NSS
1/2 SS
1/4 NSS
1/4 SS
0.7
0.5
0.3
0.1
URINE VOL. (ML)
300
200
100
0
URINE CONC. (MEQ/L)
1
4
1
4
1
4
1
4
1
4
DAYS

Figure 6

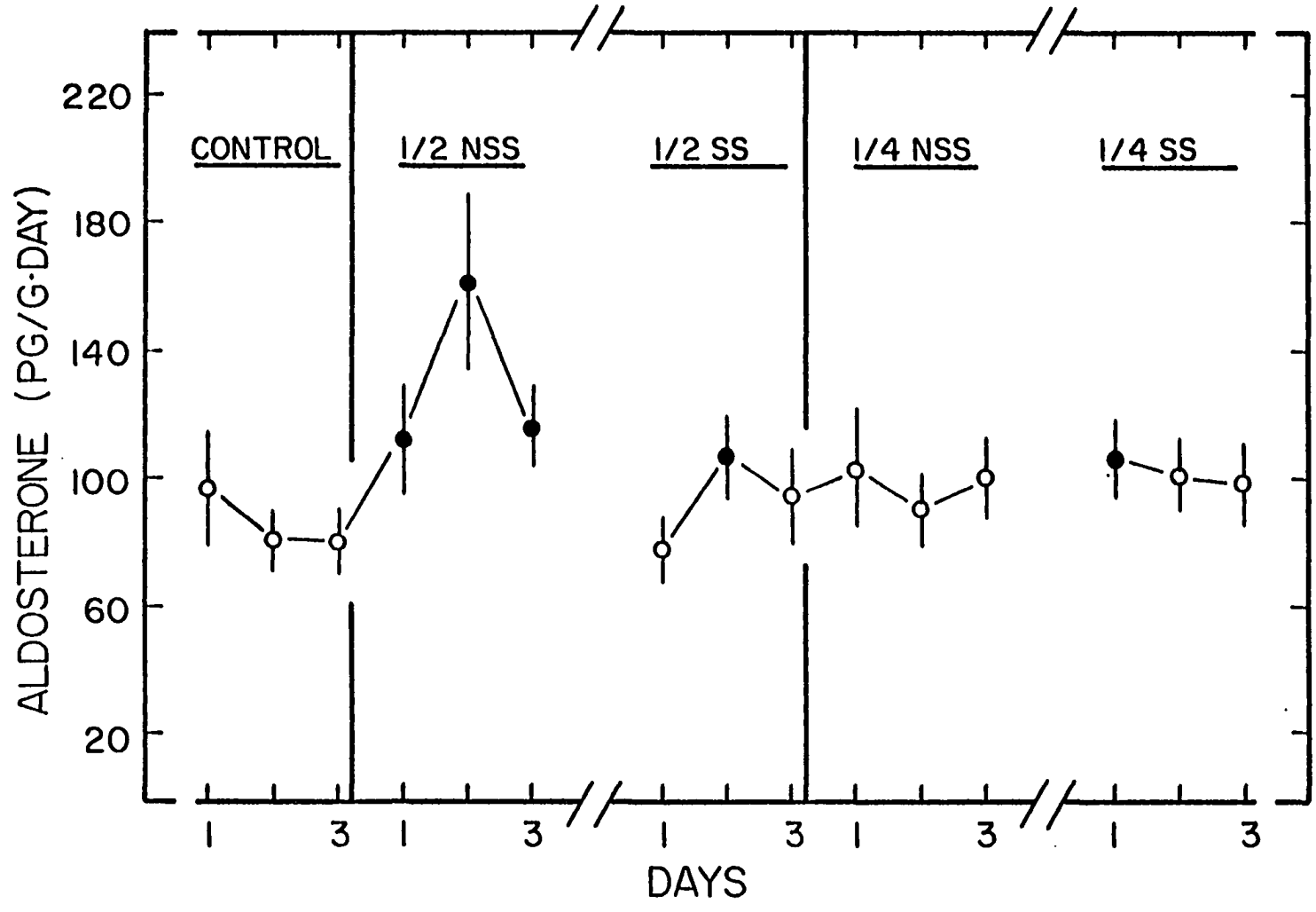
220
180
140
100
60
20
ALDOSTERONE (PG/G·DAY)
CONTROL
1/2 NSS
1/2 SS
1/4 NSS
1/4 SS
1
3
1
3
1
3
1
3
1
3
DAYS

Figure 7