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Reversal of Ethanol Induced Body Dehydration with Prolonged Consumption in Rats[1,2]

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WRIGHT, J. W. AND K. DONLON. *Reversal of ethanol induced body dehydration with prolonged consumption in rats.* PHARMAC. BIOCHEM. BEHAV. 10(1) 31–36, 1979.—Ethanol induced negative water balance has been previously inferred from fluid turnover rates upon initial exposure followed by restored normal fluid balance with prolonged exposure. The first two experiments of the present investigation noted hypovolemia and carcass dehydration during the initial few days of forced choice maintenance on 10% ethanol with recovery to near normal levels of intravascular volume and carcass water after 2 months of forced consumption. A third experiment attempted to test whether the alcohol induced dehydration could be corrected with the administration of exogenous ADH.

Ethanol consumption Hypovolemia Plasma volume determination Hematocrit
Serum Na^+ and osmolality Urine Na^+ and osmolarity Carcass water

ALCOHOL diuresis appears to have its effect by inhibiting antidiuretic hormone (ADH) release from the posterior pituitary gland [3, 4, 7–9, 12, 13, 15]. There is evidence of neurohypophysial hypertrophy which has been interpreted as the resulting effort of pituicytes to facilitate ADH release [19,20]. An initial negative water balance has been observed in rats with exposure to ethanol solutions [1], however, with prolonged consumption of 6 or 12%, but not 24% ethanol, adequate fluid balance appears to be restored [2]. Although this latter observation was based upon fluid consumption and urine excretion volumes and not upon direct body water analyses, it is clinically significant since chronically alcoholic human patients of several years duration reveal no signs of body dehydration [5,6]. Zeballos *et al.* [20] raised rats from weaning on 12% ethanol. As adults these animals were not different from normal rats with respect to urinary volume, osmolarity or sodium concentrations. This again agrees with human alcoholic patients who show no urinary differences compared with normals [9].

The present investigation was conducted in an effort to provide presently unavailable information concerning blood and carcass water deviations accompanying exposure to forced choice ethanol consumption. The research hypotheses centered upon the following questions: (1) Does body tissue dehydration occur with initial exposure to alcohol as predicted by the previously reported changes in fluid intake-urinary excretion ratios? (2) If body tissue dehydration is evidenced during alcohol exposure is normal body water balance restored with prolonged maintenance on ethanol? (3) Can alcohol induced body dehydration be corrected with the administration of exogenous ADH?

EXPERIMENT 1

The present experiment examined the influence of forced ethanol consumption on body water distribution. It was necessary to initially determine whether body dehydration occurs with ethanol intake, and secondly the persistance of the resulting dehydration over extended durations of consumption. It was considered appropriate in the first experiment to directly measure plasma volume loss by dye dilution technique and determine the best estimator of plasma volume (PV) from more easily obtained indices including plasma osmolality, protein and hematocrit for use in subsequent experiments.

METHOD

Animals

Twenty-four male Long-Evans hooded rats (120–140 days of age) were maintained at 23–24°C under constant illumination in single cages of steel and wire (24.5×18.0×18.0 cm) for a minimum adaptation period of 10 days. This procedure has been shown to abolish nocturnal rhythm of eating and drinking [21]. During the adaptation period the animals were provided demineralized water and rodent lab blox.

Procedure

The animals were randomly assigned to 1 of 2 groups, 12 each. Members of Group 1 were placed on forced intake of 10% ethanol (v/v with demineralized water) for a period of 6 days. The second group served as controls maintained on demineralized water. Both groups had rat lab blox available

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ad lib. At the completion of 6 days of treatment each animal was anesthetized with Equi-Thesin (Jensen-Salsbery Laboratories, 0.3 mg/100 g body weight) and plasma and blood volumes were determined by the Evans Blue dye technique [16]. The modified procedure entailed the exposure of both jugular veins from a point just rostral to the auxillary vein junction to the sternohyoideus muscle. An initial blood sample of 1.0 cm^3 was taken without the use of an indwelling cannula via a heparinized glass syringe prepared with a 25 ga 5/8 in. needle, immediately-followed by the injection of a weighed syringe volume (0.14–0.18 cm^3) of Evans blue dye (T-1824, 0.5% in normal saline, Warner-Lambert Company). Three successive 1.0 cm^3 blood samples were similarly collected at 10, 30 and 60 min following dye injection. The initial blood sample was used to prepare two microhematocrit samples centrifuged at 11,500 rpm for 10 min and immediately read. The remainder of the sample was centrifuged at 3300 rpm for 10 min and plasma osmolality was immediately determined by freezing point osmometer (Advanced Instruments, Model 3W). The sample was also used as a blank for the spectrophotometer (Bausch & Lomb, Spectronic 20) and to prepare a plasma dye standard for plasma volume determination. The percent optical transmittance of each post-dye injection plasma sample was read at 605 mμ against the plasma dye standard and were used to extrapolate percent transmittance at time zero. The value of dye concentration at time zero was then used to compute the plasma volume. Blood volumes were estimated using Wang's formula for true volume

$$BV = \frac{PV \times 100}{100 - HCT \times 0.95 \times 0.74}$$

At the conclusion of plasma volume determination each animal was killed by cervical dislocation, the gastrointestinal tract removed and the carcass was weighed then dried to constant weight at 85°C. The difference between wet and dry weights was then calculated on the basis of total weight at the time of surgery.

RESULTS AND DISCUSSION

The major purpose of this experiment was to determine the degree of plasma and blood volume loss induced by the forced consumption of 10% ethanol for 6 days and establish whether more easily obtained estimators of plasma volume could be utilized in subsequent experiments. Mean (± SD) plasma volumes of 3.55 ± 0.23 ml/100 g body weight for the controls and 2.49 ± 0.33 ml/100 g (based on 100 g of body weight at the time of surgery) for the alcohol treated animals were different, $t(22)=3.79$, $p<0.01$. Using the control group as the best estimation of normal plasma volume for the alcohol treated group this represents a 29.9% plasma volume loss. Pearson product moment correlations between determined plasma volume and each of the three blood measures taken, i.e., plasma protein, osmolality and hematocrit were r=–0.35, –0.77 and –0.85, respectively. Hematocrits were thus used to estimate PV in the following experiments. Figure 1 represents a scattergram of the relationship between observed hematocrit and plasma volume for each of the 24 rats utilized. The least squares line of best fit yielded a regression equation of PV=9.18–0.129 (Hct) where Hct is hematocrit. Mean hematocrits were 44.7 and 51.4 for the control and treated groups, respectively. Utilizing Wang's

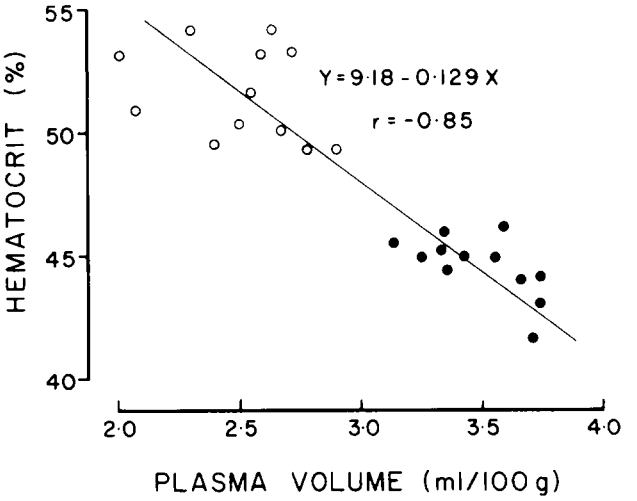

FIG. 1. Scattergram showing the relationship between plasma volume (ml/100 g body weight) and hematocrit (% RBC). The line is a least squares line of best fit. The filled circles represent ad lib animals, the open circles the six day forced choice ethanol animals of Experiment 1.

[16] formula, mean blood volumes were determined to be 5.18 ml/100 g body weight in the control group and 3.89 ml/100 g in the alcohol treated group, representing a 24.9% vascular volume loss. The alcohol treated animals also revealed a loss of carcass water with a mean (± SD) of 57.2 ± 1.7 ml/100 g body weight as compared with the control group's level of 59.8 ± 2.2, $t(22)=4.62$, $p<0.001$.

This experiment indicated intravascular volume loss and carcass water dehydration accompanying short periods of forced choice alcohol consumption. Hematocrit was shown to correlate reasonably closely with plasma and blood volume losses and may subsequently be used as an estimator of intravascular volume.

EXPERIMENT 2

The temporal persistence of ethanol induced hypovolemia was addressed in a second experiment. Crow [2] has indicated that rats increased their consumption of 6 and 12% ethanol over a 10 day period until they overcame an initial negative water balance. There are no data available concerning concomitant adaptation of intravascular volume and carcass water maintenance. The present experiment was designed to examine forced choice ethanol animals over the course of two months with particular attention given to blood and tissue water changes.

METHOD

Animals

Sixty male Long-Evans hooded rats (120–140 days old) were maintained as described in Experiment 1 for a minimum adaptation period of 10 days.

Procedure

The animals were randomly assigned to 1 of 6 groups with resulting group sizes reported in Table 1. Groups 1–3 were placed on forced intake of 10% ethanol for 3, 9 and 64 days, respectively. Groups 4–6 served as non-ethanol treated con-

TABLE 1

FLUID CONSUMPTION FOR EACH GROUP OF EXPERIMENT 2 DURING THE LAST THREE DAYS OF TREATMENT

Treatment	3 Day Group			9 Day Group			64 Day Group		
	day1	day 2	day 3	day 7	day 8	day 9	day 62	day 63	day 64
Demineralized water									
N	10			10			8		
ml/100 g	7.7 ± 0.8*	8.3 ± 0.7	6.1 ± 0.4	7.9 ± 0.6	8.1 ± 0.5	8.6 ± 0.8	8.2 ± 0.6	9.0 ± 0.5	9.0 ± 0.5
Ethanol									
N	12			12			8		
mg/100 g	6.4 ± 1.4	7.8 ± 0.8	6.2 ± 1.0	7.2 ± 0.6	6.1 ± 0.4	6.3 ± 0.2	8.1 ± 0.5	9.2 ± 0.7	9.3 ± 0.8

* Mean ± SE

TABLE 2

ETHANOL INFLUENCES UPON BLOOD AND CARCASS WATER MEASURES DURING THE EXPOSURE PERIODS OF EXPERIMENT 2

Fluid	Measure	Treatment Groups (Days) 3	6	9	64
Demineralized Water	N size	10	10	10	8
	Hematocrit (% RBC)	45.9 ± 0.9†	44.7 ± 1.3	46.6 ± 2.1	46.0 ± 1.5
	Plasma Vol. (ml/100 g)	3.36 ± 0.19*	3.55 ± 0.23	3.37 ± 0.34	3.34 ± 0.26
	Protein (g/100 ml)	7.0 ± 0.2	6.2 ± 0.4	6.9 ± 0.3	7.2 ± 0.3
	Serum Na^+ (mEq/L)	143.3 ± 3.7	—	142.8 ± 3.3	144.1 ± 1.9
	Serum Osmolality (mOsm/Kg)	—	304.2 ± 2.7	—	—
	Carcass water (ml/100 g)	57.3 ± 1.8	58.7 ± 1.3	59.3 ± 1.9	58.8 ± 3.9
10 Percent Ethanol	N Size	12	12	12	8
	Hematocrit (% RBC)	49.3 ± 2.5	51.4 ± 2.1	51.2 ± 2.3	46.4 ± 1.2
	Plasma Vol. (ml/100 g)	2.82 ± 0.36	2.49 ± 0.33	2.58 ± 0.36	3.28 ± 0.23
	Protein (g/100 ml)	7.2 ± 0.2	6.5 ± 0.5	6.9 ± 0.6	7.1 ± 0.3
	Serum Na^+ (mEq/L)	146.4 ± 2.4	—	148.6 ± 3.2	148.4 ± 3.4
	Serum Osmolality (mOsm/Kg)	—	330.9 ± 8.6	—	—
	Carcass water (ml/100 g)	56.2 ± 2.6	57.5 ± 2.1	58.5 ± 1.9	57.1 ± 3.8

*Derived from regression equation of Experiment 1, PV=9.18−0.129(Hct).
†Mean ± SD

trols that remained on demineralized water. Fluid consumption was measured once daily between 0900 and 1100 hr. All groups were provided lab blox ad lib. At the completion of each specified duration of ethanol exposure members of the appropriate treatment group and its control group, were heart puncture blood sampled under light ether anesthesia. This change in location of blood withdrawal and anesthetic as compared with Experiment 1 does not appear to change the value of hematocrit as a predictor of intravascular volume [18]. A 1 to 1.5 ml sample was taken via a 2 ml glass syringe prepared with a No. 25 needle. Latency from time of removal from the cage until the completion of blood sampling was less than 120 sec. A portion of the sample was used to prepare two microhematocrit capillary tubes per animal. The remainder of the sample was centrifuged at 3300 rev/min for 5 min, serum drawn off and protein level determined by refractometer (American Optical Corp., Model 10400). Serum sodium concentration was measured in duplicate by flame photometer (Instrumentation Laboratories, Model 143). Plasma volume (PV) was estimated for each animal using the regression equation PV=9.18–0.129 (Hct) as determined in Experiment 1.

At the conclusion of blood sampling each animal was sacrificed by cervical dislocation and carcass water measured according to the procedure of Experiment 1. The difference between wet and dry weights was calculated on the basis of total body weight at the time of heart puncture.

RESULTS AND DISCUSSION

Table 1 includes mean (± SE) daily fluid intakes (ml/100 g body weight) for the last three days of treatment for each group of this experiment, i.e., 3, 9 and 64 days of treatment. These data were submitted to a Groups×Treatment ANOVA. Although there were no differences comparing the demineralized water groups with the ethanol groups, $F(1,45)=2.37$, $p>0.10$; there were differences with duration of treatment, $F(2,45)=9.55$, $p<0.001$. Those groups placed on ethanol for 3 and 9 days were different from the other groups (Interaction $F(2,45)=3.89$, $p<0.05$; Newman-Keuls, $ps<0.05$).

Table 2 presents mean (± SD) group blood measures. Hematocrits were used to estimate the plasma volume of each animal. These data plus the blood measures taken from

the animals treated with 6 days of alcohol exposure in Experiment 1 were submitted to a Groups×Treatments ANOVA. The animals on forced intake of ethanol revealed lower plasma volumes, $F(1,72)=87.93$, $p<0.001$, when compared with their control groups. Further, there were differences between groups with respect to duration on alcohol, $F(3,72)=7.59$, $p<0.001$, with the 64 day exposure animals different from each of the other alcohol groups (Newman-Keuls, $p<0.05$) but not different from the normal control group (Newman-Keuls, $p>0.05$).

There were no differences in plasma protein concentrations between control and alcohol treated groups, $F(1,88)=2.13$, $p>0.10$. The groups differed over days of exposure, $F(3,88)=22.66$, $p<0.001$, with both 6 day groups differing from the other groups (Newman-Keuls, $p<0.05$). The interaction was not significant, $F(3,88)=p>0.10$.

Serum sodium concentrations were measured for the 3, 9, and 64 day groups and revealed consistent mean elevations for the alcohol treated groups, $F(1,66)=10.33$, $p<0.005$, as compared with the controls.

There were decreases in carcass water for the alcohol groups as compared with the control groups, $F(1,88)=5.83$, $p<0.05$, and there were differences between groups over days of alcohol exposure, $F(3,88)=3.42$, $p<0.05$; however, the interaction of these factors was not significant, $F(3,88)=0.22$, $p>0.10$.

The two immediate goals of this experiment were to: (1) replicate the intravascular volume losses noted in the first experiment with short durations of forced ethanol consumption; and (2) determine whether extended exposure to forced ethanol consumption results in recovery of normal plasma and blood volumes. The results indicate the presence of hypovolemia and tissue dehydration upon initial exposure to the forced intake of a 10% ethanol solution. Following two months of adaptation to ethanol, plasma and blood volumes appeared normal, however, there was the suggestion of continued minor carcass water deficiencies. It should be noted that the 3 day demineralized water group also revealed an unexplained depressed carcass water level. We have found mean (± SD) ad lib carcass water values to be approximately 59.6 ± 1.4 ml/100 g body weight [17].

EXPERIMENT 3

The prior experiments of this report indicated an initial hypovolemia and diminished fluid intake accompanying the forced consumption of a 10% ethanol solution with subsequent recovery. As referenced earlier there have been numerous suggestions that the ingestion of ethanol results in the inhibition of ADH release. If this is the major consequence of alcohol intake upon body water balance then a readily testable hypothesis may be formulated: Exogenous administration of ADH should relieve or prevent the ethanol induced hypovolemia. The present experiment addressed this question.

METHOD

Animals

Twenty-four male Long-Evans hooded rats (120–140 days of age) were maintained in unit rodent metabolism cages (Acme Metal Products) under the previously described conditions. Powdered rodent lab chow was provided ad lib.

Procedure

The animals were randomly assigned to 1 of 4 groups, 6 each. The rats of Group 1 were given a 10% ethanol solution as the only fluid available for a 6 day period. Members of Group 2 served as nontreated controls and remained on demineralized water. Group 3 animals were provided 10% ethanol as their only fluid for 6 days and also received intramuscular injections of Pitressin twice daily at 12 hr intervals (dosage 25 mU/100 g). This dosage was set according to Rolls' [10,11] suggestion that the lowest dose of ADH be used that reliably suppresses urine flow since high doses may reduce fluid intake in rats. Members of Group 4 were provided 10% ethanol and were given injections of peanut oil in equivalent volume and at the same times as the Group 3 animals.

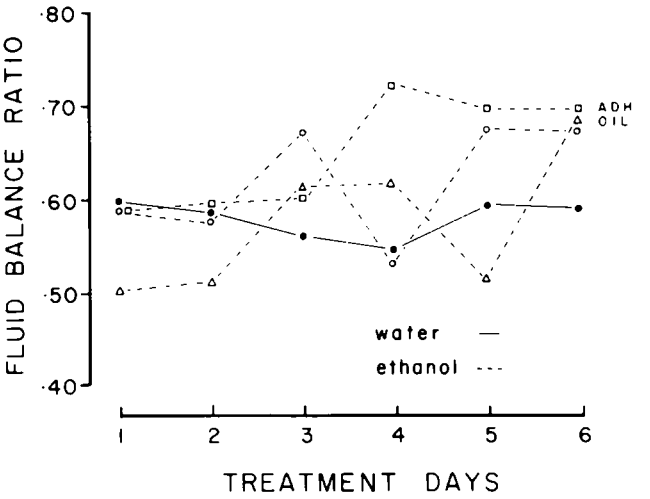

FIG. 2. Mean daily fluid balance ratios (Fluid intake - Fluid output)/Fluid intake for each group of Experiment 3. Filled circles represent demineralized water group; open circles: 10% ethanol group; squares: 10% ethanol, ADH treated group; triangles: 10% ethanol, peanut oil treated group.

At the conclusion of 6 days of treatment all animals were heart puncture blood sampled as previously described. Plasma and blood volumes were estimated from hematocrits using the regression equation of Experiment 1. Plasma osmolality was measured by freezing point osmometer (Advanced Instruments, Model 3W). The animals were then sacrificed by cervical discloation and carcass water determined by desiccation. During each day of treatment, body weight, food, fluid and urine volumes were measured for each animal. A urine sample was collected under mineral oil and retained daily from each animal and sodium concentration was determined by flame photometry, urine osmolarity was measured by freezing point osmometer.

RESULTS

The four groups revealed no differences in body weight during the six treatment days, $F(3,120)=0.027$, $p>0.10$. Therefore, it was considered appropriate to analyze the food and fluid measurements without transformation to g (ml)/100 g body weight. There were no differences between groups' daily food consumption, $F(3,120)=1.45$, $p>0.10$; however, the groups did differ in fluid volume consumed over treatment days, $F(3,120)=12.32$, $p<0.001$, with all ethanol groups indicating depressed intakes (Newman-Keuls, $p<0.05$).

TABLE 3

THE INFLUENCE OF ADH OR OIL INJECTIONS UPON BLOOD AND CARCASS WATER MEASURES IN THE ETHANOL TREATED GROUPS OF EXPERIMENT 3

Treatment Group	N	Hematocrit (% RBC)	Plasma Volume (ml/100 g)	Protein (g/100 ml)	Plasma Osmolality (mOsm/Kg)	Carcass water (ml/100 g)
Ethanol	6	49.6 ± 0.7*	2.78 ± 0.10	6.87 ± 0.19	307.2 ± 6.1	57.1 ± 1.3
Demineralized water	6	44.7 ± 0.9	3.42 ± 0.13	6.33 ± 0.29	303.3 ± 1.7	59.6 ± 2.2
Ethanol Exogenous ADH	6	47.1 ± 1.1	3.11 ± 0.14	7.38 ± 0.18	305.7 ± 6.2	57.6 ± 1.7
Ethanol Exogenous Oil	6	49.1 ± 0.8	2.85 ± 0.10	7.13 ± 0.28	303.7 ± 5.1	59.8 ± 1.8

* Mean ± SD

Urine volume also differed between groups, $F(3,120)=22.14$, $p<0.001$, with the ethanol groups excreting depressed volumes (Newman-Keuls, $p<0.05$). Figure 2 summarizes the fluid balance data for each group over the six days of testing. These daily group means were obtained by the application of the following formula to each animal's daily fluid consumption and excretion measures: Fluid intake-Fluid output/Fluid intake. Thus, greater mean ratios indicate higher fluid retention. It was not surprising to see elevated retention ratios demonstrated in the ethanol group administered ADH particularly during the last 3 days of treatment, however, the control group placed on forced ethanol consumption unexpectedly indicated reasonably high retention levels for 3 of the 6 days. And, unfortunately, the ethanol group administered oil injections demonstrated a high retention level on Day 6 of treatment resulting in perhaps an unusually elevated carcass water level (Table 3).

There were group differences in urinary sodium concentration during treatment, $F(3,120)=3.27$, $p<0.05$; the demineralized water control group revealed a mean of 150.0 m Eq/L, compared with significant dilutions in concentrations of 126.6 and 133.4 mEq/L evidenced by the 10% ethanol and pitressin treated 10% ethanol groups, respectively (Newman-Keuls, $p<0.05$). The peanut oil treated group exposed to 10% ethanol revealed a mean concentration of 146.8 mEq/L and did not differ from the demineralized water controls and Pitressin treated ethanol group but was different from the group given only 10% ethanol (Newman-Keuls, $p<0.05$). Group urine osmolarity concentrations were different, $F(3,120)=55.37$, $p<0.001$, with the ADH and oil treated ethanol groups revealing mean values of 3429 and 3095 mOsm/kg, while the control ethanol and demineralized water groups indicated 2317 and 1742 mOsm/kg, respectively.

The plasma and body water determinations were most relevant to the hypotheses tested in this experiment and replicated the findings of Experiment 1 with declines in estimated plasma volume indicated in all three groups provided 10% ethanol for six days, $F(3,20)=36.47$, $p<0.001$; Newman-Keuls, $p<0.05$; Table 3) as compared with the demineralized water control group. Although members of the Pitressin treated group indicated some reparation of plasma volume their levels remained below those of the demineralized water control group (Newman-Keuls $p<0.05$). Although there were no differences in plasma osmolality, $F(3,20)=0.74$, $p>0.10$, there were group differences in carcass water, $F(3,20)=3.93$, $p<0.05$. Surprisingly the 10% ethanol, and pitressin ethanol groups revealed carcass water dehydration compared with both the demineralized water control animals and the peanut oil treated animals provided 10% ethanol.

GENERAL DISCUSSION

The results of these experiments extend the earlier findings of Beard and Knott [1] in that the previously observed negative water balance with initial exposure to alcohol is accompanied by hypovolemia and tissue dehydration. The present findings also supplement Crow's [2] indication of restored normal fluid balance with prolonged consumption of moderate concentrations of ethanol.

Returning to the primary questions asked in this investigation the present data indicate that (1) tissue dehydration does occur with initial exposure to alcohol; and (2) prolonged forced ethanol exposure results in the return of normal body water balance as measured by plasma and blood volumes and carcass water indices; but the question as to whether alcohol induced dehydration can be corrected with the administration of exogenous ADH remains unanswered. It is clear from Experiment 3 that ethanol induced hypovolemia is reduced with ADH treatment, however there remains a significantly diminished carcass water level. These data are difficult to interpret given the unexplained normal carcass water level revealed by the ethanol group treated with peanut oil injections. It is known that certain stressors such as the pain accompanying daily injections can result in exaggerated ADH release [14]. This does not explain the depressed carcass water level of the ADH treated alcohol group that presumably experienced equivalent pain stress. At least two additional possibilities come to mind. The dosage of ADH may have been insufficient to insure reparation of both blood volume and carcass water. And perhaps the observed elevations in serum sodium in the alcohol treated groups indicate altered aldosterone levels that interact with changed ADH release resulting in carcass dehydration. These hypotheses will require further evaluation.

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