vantage of chemical structures that can be completely defined. In fact $GgOs_3Cer$ has been synthesized chemically (*7*). Many glycolipids are present in various organs of different species, thus providing a source of glycolipid antigen for immunization; for example, the $GgOs_3Cer$ used to prepare the monoclonal antibodies described in this report was obtained from guinea pig erythrocytes (*4*). Recently, the neutral glycolipid ganglio-*N*-tetraosylceramide ($GgOs_4Cer$) was defined as a marker for human acute lymphocytic leukemia cells (*8*). The present study in mice may serve as a model for future therapeutic attempts to use this human tumor glycolipid marker.

Most attempts to use specific antibodies for tumor immunotherapy have met with limited success [for a review, see (*9*)]. However, using the hybridoma technique (*10*) to generate large amounts of specific antibodies, several investigators have demonstrated suppression of tumor growth by serotherapy (*11, 12*). In another study (*13*) serotherapy was only effective with IgG antibodies to Thy-1; monoclonal IgM antibodies were not effective. Similarly, in the present study, IgM antibody to $GgOs_3Cer$ plus complement caused only a marginal increase in the survival time of mice (Fig. 1, A and B), even though this antibody mediated complement-dependent lysis of L51781A1 in vitro. Thus, the effect of the IgG3 antibody to $GgOs_3Cer$ in our system may be due to antibody-dependent cellular cytolytic (ADCC) mechanisms, because IgG in vitro mediates ADCC but IgM does not (*13*). In fact, Tracey and Silberman (*14*) recently found host ADCC effector cells present among the ascites cells of mice bearing the L5178Y lymphoma. Alternatively, IgM may not readily diffuse to tumor cells in vivo.

The successful suppression of L51781A1 cells with IgG_3 antibodies may be partially due to the greater susceptibility of lymphoma cells to destruction by antibody compared to solid tumors, and use of a similar approach to the suppression of solid tumors (fibrosarcoma) bearing the same glycolipid marker (*15*) would be interesting. We and others have described the presence of a trace of $GgOs_3Cer$ in mouse spleen (*15, 16*). Thus, injection of antibody to $GgOs_3Cer$ may modify host lymphoid components which then could retard tumor growth.

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Water Intake in Hypovolemic Sheep: Effects of Crushing the Left Atrial Appendage

Abstract. *Sheep increased their water intake in proportion to the amount of protein-free, isosmotic fluid that was removed from their blood by ultrafiltration. This behavioral response to hypovolemia was eliminated by crushing the left atrial appendage of the heart. The surgical maneuver had no effect on basal water intake or on the drinking response to a salt load. These findings suggest that left atrial stretch receptors, which influence secretion of antidiuretic hormone when stimulated, may also play an important role in mediating thirst during hypovolemia.*

Loss of plasma volume (hypovolemia) provokes compensatory increases in the secretion of antidiuretic hormone (*1*) and the consumption of water (*2, 3*). The nature of the thirst stimulus remains uncertain; increased concentrations of angiotensin II (*4*) and altered neural activity in vascular baroreceptors (*3, 4*) have been proposed as important factors. The secretion of antidiuretic hormone in response to plasma volume deficits is believed to be stimulated in large part by the activation of stretch receptors in the area of the left atrium of the heart (*5*). This report presents evidence that the receptors may also mediate thirst during hypovolemia.

We performed the experiments on female mixed-breed sheep (*6*). Acute intravascular dehydration was produced in conscious animals by ultrafiltration of the blood with a parallel flow dialyzer (*7*). During a 5-hour period in which the sheep were deprived of food and water, their blood was continuously pumped from a saphenous vein catheter through the filter and back into the animal via the contralateral catheter. Protein-free, isosmotic fluid was separated under negative pressure and collected. Simultaneous measures of arterial blood pressure (ABP), glomerular filtration rate (GFR), plasma sodium (PNa) concentration, plasma protein (PP) concentration, he-

Table 1. Effects of hypovolemia on ABP, GFR, PNa, and PP concentrations, Hct and PRA in unoperated sheep, sheep with damaged left atrial appendages (operated), and unoperated sheep treated with furosemide. Values are means ± standard errors.

Treatment group	ABP (mm-Hg)	GFR (ml/min)	PNA (mEq/liter)	PP (g %)	Hct (%)	PRA (ng AI/ml-hour)
Unoperated (N = 8)						
Before filtration	88 ± 1	67 ± 4	147 ± 1	6.3 ± 0.2	29 ± 1	1.0 ± 0.2
After filtration	71 ± 7*	22 ± 7†	147 ± 1	7.7 ± 0.2†	39 ± 2†	19.9 ± 5.8†
Operated (N = 11)						
Before filtration	84 ± 1	70 ± 4	145 ± 1	6.7 ± 0.2	25 ± 1	1.7 ± 0.4
After filtration	58 ± 3†	6 ± 4†	144 ± 2	9.1 ± 0.7†	35 ± 3†	28.4 ± 5.7†
Furosemide (N = 4)						
Before filtration	84 ± 2	72 ± 4	145 ± 1	6.2 ± 0.1	30 ± 1	0.7 ± 0.1
After filtration	61 ± 6*	13 ± 8†	145 ± 2	8.3 ± 0.3†	38 ± 1†	25.8 ± 7.2†

*Significantly different from the control value at $P < .05$. †Significantly different at $P < .01$.

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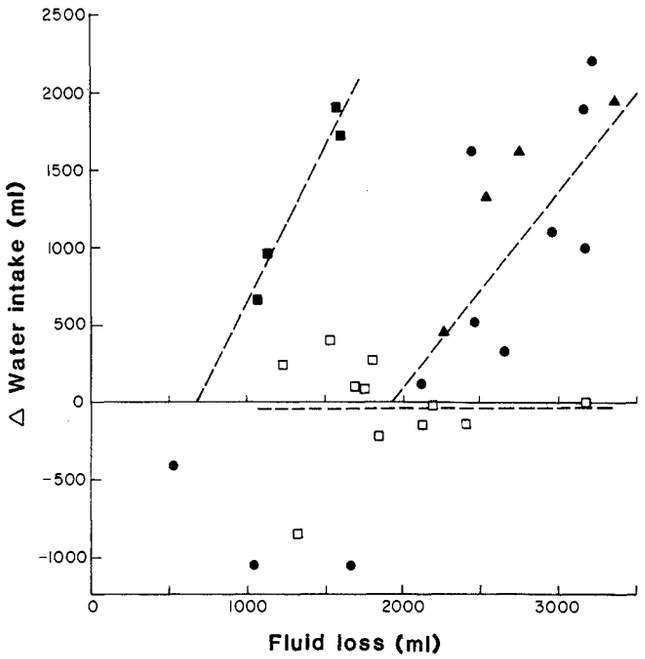

Fig. 1. Water intake of sheep after ultrafiltration, shown in relation to their net fluid fluid losses. Regression equations are as follows: for intact controls (●), $y = 1.29x - 2484$; for sheep with crushed atrial appendages (□), $y = 0.01x - 44$; for furosemide-treated controls (■), $y = 2.01x - 1369$. The data for the four sham-operated controls are also presented (▲).

matocrit (Hct), and plasma renin activity (PRA) were made throughout the filtering period (*8*). Afterward, all blood in the extracorporeal system was returned to the animals, the apparatus was disconnected and removed, and the catheters were filled with heparinized saline. Water and food were then made available, and water intake was measured for 2 hours and cumulatively over the next 16 hours (*9*).

Between 515 and 3208 ml of extracellular fluid was removed through ultrafiltration in 11 experiments on nine sheep. Hypovolemia was indicated by significant increases in PP, Hct, and PRA and by decreases in ABP and GFR; as expected, there were no changes in PNa concentration (Table 1). Estimated deficits in plasma volume (*10*) were exponentially related to net fluid losses ($r = .91$), with sharp increases in the plasma deficit when fluid loss exceeded 2000 ml. Losses of less than 2000 ml, which produced plasma volume deficits of only 5 to 10 percent, occurred in three experiments, and each time the animal failed to increase its drinking. In the other eight experiments fluid loss exceeded 2000 ml, plasma volume deficits were 15 to 32 percent, and the sheep increased their water intake in proportion to the net quantity of filtrate removed—both during 2 hours ($r = .69$) (Fig. 1) and 18 hours ($r = .88$) of water availability.

Fluid removal through ultrafiltration is clearly an effective means for producing thirst in sheep. To determine whether the drinking is mediated by low-pressure cardiac stretch receptors, we attempted to denervate the appropriate area of the heart by crushing the left atrial appendage (*11*) in a second group of nine sheep. A left thoracotomy was performed between the fourth and fifth ribs, and the fifth rib was removed. Six thoracic forceps were fixed around the appendage for 10 to 15 minutes to produce maximal tissue damage. Four additional female sheep were prepared as controls: a left thoracotomy was performed on each, but in two animals a pair of atraumatic clamps was placed around the atrial appendage and in the other two the appendage was not clamped.

The sheep were given 2 weeks to recover (*12*) and then were subjected to ultrafiltration like the first group. The removal of fluid from these animals reduced plasma volume more than in the unoperated animals (mean deficit in unoperated sheep, 22 ± 3 percent; in operated sheep, 28 ± 5 percent) and had more pronounced effects on ABP, GFR, PP, and PRA (Table 1). Nevertheless, the sheep with crushed atrial appendages did not increase their drinking, even during 18 hours of water availability (Fig. 1). It should be emphasized that these animals did not look distressed during the experiments, ate normally, and consumed water in quantities similar to their basal intake. The four operated control sheep, in contrast, increased their water intake after plasma volume reduction and drank in proportion to their net loss of fluid, as did the unoperated animals (Fig. 1).

Although mean plasma volume deficits and arterial hypotension were greater in the sheep with crushed atrial appendages than in the unoperated animals (Table 1), the net loss of extracellular fluid was less than 2000 ml in seven of the experiments (Fig. 1). We assumed that the operated sheep began the test with diminished fluid reserves to buffer the losses that occurred during filtration (*13*), and attempted to reproduce this effect in four unoperated sheep by treating them with the diuretic furosemide 5 to 6 days before ultrafiltration (*14*). In these animals, removal of 1070 to 1600 ml of extracellular fluid had effects on ABP, GFR, PP, and PRA that were similar in magnitude to those observed in the sheep with crushed atrial appendages (Table 1). However, the furosemide-treated sheep increased their water intake and drank in proportion to their net loss of fluid during 2 hours ($r = .97$) and 18 hours ($r = .95$) of water availability. The amounts were similar to those consumed by the control animals (Fig. 1).

These results indicate that crushing the left atrial appendage eliminates the enhanced drinking behavior of hypovolemic sheep. The fact that it does so despite greater arterial hypotension and more elevated PRA than heretofore observed is noteworthy because hypotension and angiotensin have been reported to stimulate thirst in rats (*4, 15*). However, neither of these putative stimuli seems to be effective in provoking water consumption by sheep (*16*). Thus, the stimulus for thirst in our experiments evidently resulted predominantly from the induced plasma volume deficits, with stretch receptors in the area of the left atrium presumably providing a critical afferent signal of hypovolemia.

An alternative interpretation might hold that cardiac surgery nonspecifically disrupts the drinking response of sheep to acute dehydration. If so, thirst arising from hypernatremia and cellular dehydration should also be affected. To test this hypothesis, we infused 4*M* NaCl (1.1 ml/min for 30 minutes) into the right carotid artery of five sheep before and after crushing the left atrial appendage, and monitored drinking for 2.5 hours. The animals drank 341 ± 165 ml before the surgery and 721 ± 140 ml afterward ($P < .05$) (*17*). Since the salt load increased body fluid osmolality by only 3 percent, it appears that sheep remain capable of responding even to slight dehydration after damage to the left atrial appendage.

After testing was concluded (10 to 20 days after the atrial appendage was crushed), the sheep were killed and their hearts were removed for examination. The ventricles and the right atrial appendage were indistinguishable from those of unoperated animals. However,

the traumatized appendage had become severely atrophied and 80 to 90 percent of it was necrotic, so it is likely that the innervation of the area was drastically affected (*11*).

In conclusion, hypovolemia produced by ultrafiltration of the blood is a sufficient stimulus for thirst in sheep, and crushing the left atrial appendage eliminates this behavioral response. These findings add to the advances made during the past decade in elucidating the physiological bases of thirst—especially in the identification of relevant stimuli and the location of receptors on which they act (*4, 15, 18*). To date, however, destruction of such receptor sites in the central nervous system has invariably disrupted the drinking response to more than one stimulus (*19*). Our results may constitute the first example of neural damage that specifically eliminates the drinking response to one stimulus for thirst.

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10. Changes in plasma volume were calculated as $(PP_2 - PP_1/PP_2) \times 100$ [E. M. Stricker, *Physiol. Behav.* **3**, 379 (1968)], where PP_1 is the basal protein concentration and PP_2 is the protein concentration at the end of the filtration period.
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13. Operated sheep lost 1.5 kg in body weight despite normal daily intakes of food and water. Some portion of this undoubtedly represents extracellular fluid lost during surgery and not subsequently replaced (the sheep were maintained on a diet of alfalfa pellets and tap water, which provided only 8 mEq of sodium per day).
14. Furosemide was administered intravenously in isotonic saline (0.21 ml/min for 4 hours). The diuretic was given on two consecutive days at doses of 250 and 125 mg per sheep. Urine collected through a bladder catheter contained 125 ± 8 mEq of sodium.
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17. Due to their maintenance on a low-sodium diet (*13*), neither group excreted much of the salt load. The significant difference in their intakes reflects the fact that the unoperated sheep did not drink enough water to dilute the remaining salt load to isotonicity, whereas the sheep with crushed atrial appendages did. It is possible that the surgery abolished tonic inhibitory impulses from low-pressure volume receptors and thereby lowered the threshold for osmoregulatory drinking [S. Kozlowski and E. Szczepanska-Sadowska, in *Control Mechanisms of Drinking*, G. Peters, J. T. Fitzsimons, L. Peters-Haefeli, Eds. (Springer-Verlag, New York, 1975), pp. 25-35].
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Nonenzymatic Browning in vivo: Possible Process for Aging of Long-Lived Proteins

Abstract. *The incubation of lens proteins with reducing sugars leads to the formation of fluorescent yellow pigments and cross-links similar to those reported in aging and cataractous human lenses. Called nonenzymatic browning or the Maillard reaction, this aging process also occurs in stored foods. Reducing sugars condense with the free amino group of proteins, then rearrange and dehydrate to form unsaturated pigments and cross-linked products. Although most proteins in living systems turn over with sufficient rapidity to avoid nonenzymatic browning, some, such as lens crystallins and skin collagen, are exceptionally long-lived and may be vulnerable.*

The initial stage of the Maillard reaction, nonenzymatic glycosylation, has been shown to occur in vivo (*1*). Glucose or glucose-6-phosphate reacts with the terminal amino group or the ϵ-amino group of lysine residues in proteins. The condensation product, an aldosylamine, undergoes an Amadori rearrangement to form a 1-deoxyfructosyl adduct. Amadori products have been detected in hemoglobin (*2*), erythrocyte membrane protein (*3*), lens crystallins (*4*), collagen (*5*), albumin, and serum proteins (*6*). In the later stages of the Maillard reaction, during nonenzymatic browning, the Amadori product undergoes multiple dehydrations to form yellow-brown fluorescent products and protein cross-links that decrease the protein solubility (*1*).

Similar changes have been observed in the aging and cataractous human lens. These include protein aggregation (*7*), decreased protein solubility (*8*), oxidation of sulfhydryl groups (*9*), production of nondisulfide covalent cross-links be-

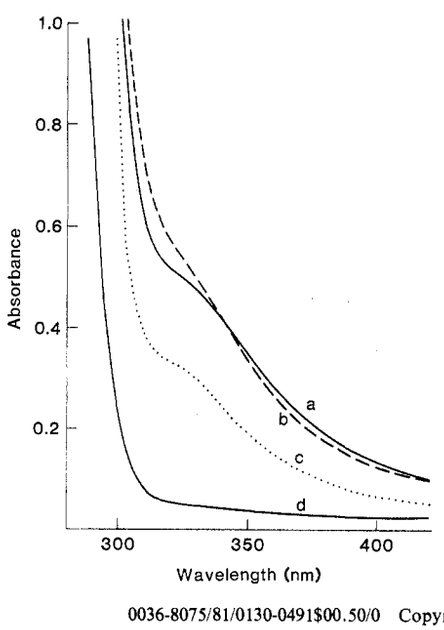

Fig. 1. Absorption spectra of human and bovine lens protein digests, as recorded with a Beckman Acta CIII spectrophotometer. The concentration in all samples was 8 mg/ml in 50 m*M* ammonium bicarbonate buffer (*p*H 8.75). Spectra: *a*, cataractous human lens proteins; *b*, bovine lens proteins incubated with 5 m*M* glucose-6-phosphate; *c*, bovine lens proteins incubated with 5 m*M* glucose; and *d*, control lens (incubated without hexose). The spectrum of the digests from young noncataractous human lenses was identical with spectrum *d*.

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