Reduced Renal Concentrating Capacity During Isotonic Saline Loading.* (30777)

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It has been demonstrated by several recent studies that saline loading in the dog may result in decreased net tubular reabsorption of sodium independent of mineralocorticoids (1-5). On the basis of recent studies from this laboratory it was suggested that decreased tubular reabsorption of sodium during saline loading may result *in part* from an increased renal medullary blood flow(4,5). Increased medullary blood flow should decrease medullary interstitial hypertonicity, and, in turn, the passive loss of water from the descending limb of Henle's Loop should diminish. The resulting increased rate of flow of fluid with a lower concentration of sodium could limit the absolute reabsorption of sodium by the ascending limb(4,5). If extracellular volume expansion does increase renal medullary blood flow, and if the reabsorption of sodium by the ascending limb of Henle's Loop is influenced by the concentration of sodium in the tubular fluid, then the concentrating mechanism (as measured by $T^c_{H_2O}$) should be influenced by both extracellular volume expansion and by the concentration of sodium in the glomerular filtrate. The present studies were designed to compare the concentrating capacity during different types of solute loading and during different degrees of extracellular volume expansion. It was observed that at high rates of solute excretion the concentrating mechanism was less efficient during *isotoinc saline* loading than during hypertonic mannitol loading, and that *hypertonic saline* loading was associated with even more efficient urinary concentration than observed with hypertonic mannitol.

Methods. Experiments were performed in 6 unanesthetized female mongrel dogs (weighing 19 to 21 kg) trained to stand with the support of loose slings. Animals were deprived of water for 2 days and of food the day before experiments. Vasopressin (Pitressin® tannate) in oil 5 units and desoxycorticosterone acetate (DOCA) 10 mg were given intramuscularly 12-18 hours before the experiments. A maintenance infusion of 0.9% saline (acidified to pH 5-5.5) was begun at a rate of 0.3 ml per minute, 60 to 90 minutes prior to collections. This infusion delivered inulin at 10 mg per minute, p-aminohippurate (PAH) at 3 mg per minute, vasopressin at 50 milliunits per kg per hour, and desoxycorticosterone at 20 μg per minute. Venous blood samples were collected at the midpoint of urine collections. Animals were studied at intervals of no less than 7 days, and the sequence of protocols described below was alternated within the group. Urine was collected during 2 to 4 control periods of 30 minutes each prior to the infusion of different loading solutions. The maintenance of a urine flow less than 0.25 ml per minute and a urine osmolality greater than 1500 mOsm were considered necessary control conditions before the loading infusion was begun. After control periods, the experiments were continued according to one of the protocols outlined below. Each dog was studied by the first 3 methods, and 2 dogs were studied also by the fourth method.

1) *Isotonic saline:* A modified, slightly hypertonic, Ringer solution containing 160 or 170 mEq/l Na, 148.5 mEq/l Cl, 3.5 mEq/l K, and 15 or 25 mEq/l HCO_3 was infused at a constant rate of 30 to 45 ml per minute in 6 experiments in 6 dogs. Collection periods of 6 to 20 minutes each (depending upon urine flow) were made as urine flow increased to 20 to 30 ml per minute. Total volumes infused ranged from 3.5 to 5 liters.

2) *Hypertonic mannitol:* 15% Mannitol containing 50 mM/l NaCl was infused at stepwise rates to 10 or 15 ml per minute,

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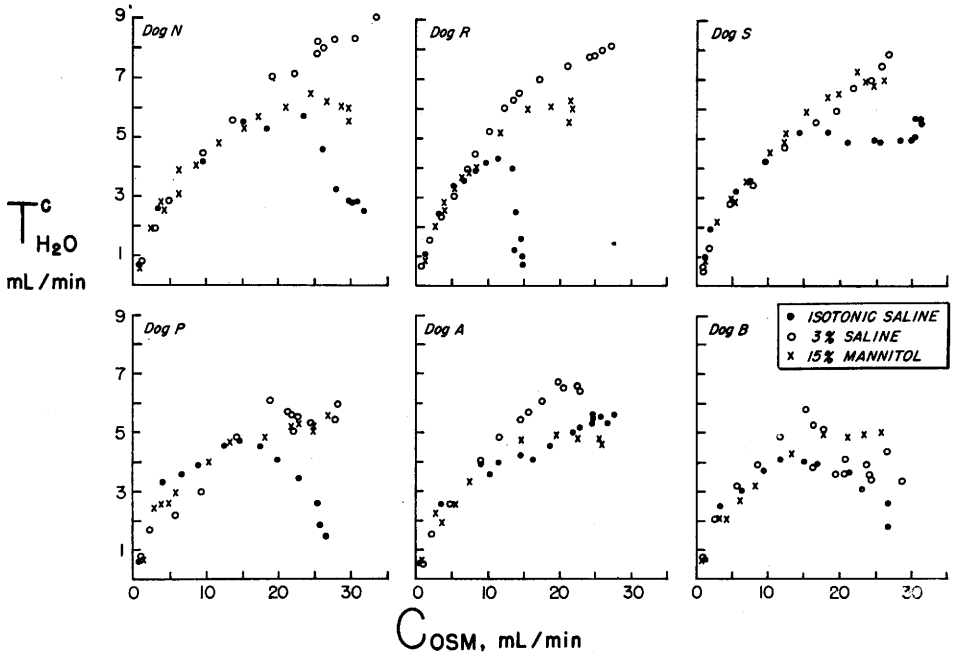

FIG. 1. $T^c_{H_2O}$ *during different types of solute loading.* Three separate studies in each of 6 dogs are shown. At low rates of solute clearance $T^c_{H_2O}$ was independent of the loading solutions. At higher rates of solute clearance, the general pattern was that $T^c_{H_2O}$ was lowest during isotonic saline infusion and highest during hypertonic saline infusion.

with collection of 2 to 3 periods at each rate of infusion. This resulted in maximal urine flows of 20 to 30 ml per minute. Total volume infused was approximately one liter. This experiment was performed once in each of the 6 dogs.

3) *Hypertonic ("3 per cent") saline:* A solution containing 538 mEq/l Na, 516.5 mEq/l Cl, 3.5 mEq/l K, and 25 mEq/l HCO_3 was infused at 10 to 15 ml per minute with frequent collection periods as the flow of urine increased to levels of 20 to 30 ml per minute. This experiment was performed once in each of the 6 dogs.

4) *Isotonic saline followed by hypertonic ("5 per cent") saline:* In 2 experiments after urine flows of 25-30 ml per minute were reached during "isotonic saline" loading (as described above) a solution containing 890 mEq/l Na, 25 mEq/l HCO_3, 868.5 mEq/l Cl, 3.5 mEq/l K was begun at 30 ml per minute, and at the same time the infusion of isotonic saline was discontinued. This hypertonic infusion was continued until the flow of urine increased to approximately 50 ml per minute.

Analytical procedures were performed by methods previously described for this laboratory(4,5).

Results. On different days $T^c_{H_2O}$ was compared in each of the 6 animals through a wide range of solute excretion during loading with isotonic saline, hypertonic mannitol, and hypertonic saline. These results are shown in Fig. 1. As osmolal clearance increased to approximately 12 ml per minute, $T^c_{H_2O}$ increased and was independent of the loading solution in each animal. However, as osmolal clearance continued to increase, further changes in $T^c_{H_2O}$ appeared to relate to the specific loading solution. In 4 of 6 experiments during *isotonic* saline loading, $T^c_{H_2O}$ decreased as osmolal clearance continued to increase. In one experiment $T^c_{H_2O}$ remained relatively stable as osmolal clearance increased, and in only one experiment did $T^c_{H_2O}$ continue to increase with increasing solute excretion. During hypertonic mannitol loading $T^c_{H_2O}$ usually reached a plateau at solute clearances above 15 ml per minute, and in all but one animal $T^c_{H_2O}$ at high rates of osmolal clearance during hypertonic man-

TABLE I. Effect of Different Loading Solutions on Renal Concentrating Mechanism.*

			Isotonic saline		Hypertonic mannitol		Hypertonic saline	
Dog N	GFR	(ml/min)	119	122	78	78	98	105
	C_{Osm}	(ml/min)	30.8	31.9	30.0	30.0	30.8	33.5
	T^c_{H2O}	(ml/min)	2.8	2.5	5.6	6	8.3	8.8
	U_{urea}	(mM/l)	13	12	12	12	10	
	P_{Na}	(mEq/l)	152	153	135	132	174	174
	†ΔECV	(ml)	+1752	+1790	+434	+118	+779	+747
Dog S	GFR		108	114	82	82	102	100
	C_{Osm}		25.4	28.3	24.6	26.0	25.9	26.5
	T^c_{H2O}		4.9	4.9	6.8	7.0	7.5	7.6
	U_{urea}		23	20	20	18	27	25
	P_{Na}		147	150	126	126	194	195
	ΔECV		+3287	+3657	+1664	+1800	+2840	+2987
Dog R	GFR		99	93	74	71	105	113
	C_{Osm}		14.8	14.9	12	15.6	14.2	17.2
	T^c_{H2O}		1.0	.7	5.2	6.0	6.6	7.1
	U_{urea}		20	18	46	30	34	25
	P_{Na}		151	151	136	134	149	146
	ΔECV		+2092	+2277	+187	+333	+2780	+3122
Dog P	GFR		95	116	65	69	80	81
	C_{Osm}		25.6	26.4	24.8	26.9	24.5	28.0
	T^c_{H2O}		1.8	1.4	5.1	5.6	5.5	5.5
	U_{urea}		16	15	12	14	25	23
	P_{Na}		154	154	126		186	193
	ΔECV		+1576	+1681	−160	−160	+599	+681
Dog A	GFR		116	116	69	70	117	106
	C_{Osm}		22.0	22.7	22.0	25.3	22.8	22.4
	T^c_{H2O}		5.1	5.2	4.8	4.9	6.6	6.4
	U_{urea}		15	15	14	12	18	17
	P_{Na}		152	149	129	126	182	186
	ΔECV		+2240	+2429	+407	+548	+1357	+1520
Dog B	GFR		103	100	69	71	79	95
	C_{Osm}		26.9	26.8	23.3	25.7	21.0	26.3
	T^c_{H2O}		2.7	1.8	4.9	5.0	4.2	4.3
	U_{urea}		17	16	18	16	15	13
	P_{Na}		151	150	124	124	204	
	ΔECV		+2457	+2497	+260	+284	+1503	+1623

* Experiments are those shown in Fig. 1, and data are from 2 consecutive collection periods at maximal comparable solute clearances achieved with each loading solution.

† Calculated change in extracellular volume (assuming that the solute infused and excreted is limited to the extracellular space):

$$\Delta ECV = \frac{(ECV_1 \times P_{Osm1}) + Osm_{net}}{P_{Osm2}} - ECV_1$$

$ECV_1 = .19 \times$ body wt; P_{Osm1} = initial plasma osmolality; Osm_{net} = solute infused − solute excreted; P_{Osm2} = final plasma osmolality.

nitol loading clearly exceeded $T^c_{H_2O}$ during isotonic saline loading. During *hypertonic* saline loading, $T^c_{H_2O}$ continued to increase with increasing osmolal clearance in all but one experiment. In the remaining 5 animals the highest observed values for $T^c_{H_2O}$ clearly occurred during loading with *hypertonic* saline. Thus, the pattern observed in these 6 animals was that at high rates of osmolal clearance, the highest values for $T^c_{H_2O}$ were during loading with *hypertonic* saline, and the lowest values were during loading with *isotonic* saline. $T^c_{H_2O}$ during loading with hypertonic mannitol was usually intermediate between these highest and lowest values. Data from consecutive collection periods demonstrating differences in $T^c_{H_2O}$ at similar rates of solute clearance for the 3 loading solutions are given in Table I.

During the control periods prior to loading, glomerular filtration rate (GFR) in the same animal was similar on each of the days

of study. GFR prior to loading ranged from 57 to 88 ml per minute in all animals, and the maximal differences observed in each animal averaged 10 ml per minute. During loading with isotonic saline GFR increased by an average of 52%, during loading with hypertonic saline GFR increased by an average of 40%, and during loading with hypertonic mannitol GFR changed little, (average change— 1%). Thus, there was no relationship between GFR and the observed differences in $T^c_{H_2O}$, (Table I). In 3 dogs clearances of PAH increased 100% during isotonic saline loading, 51% during hypertonic mannitol loading and 51% during 3% saline loading. Plasma sodium decreased to an average of 129 mEq per liter during hypertonic mannitol loading, was essentially unchanged during isotonic saline loading, and was strikingly increased during hypertonic saline loading. The urinary concentration of urea in the periods shown in Table I averaged 18.7 mM/l, and no relationship existed between differences in $T^c_{H_2O}$ and urinary concentrations of urea.

The estimated change in extracellular volume was calculated for each of the loading solutions. This value was greatest during isotonic saline loading in all but one animal, and was least during hypertonic mannitol loading.

In 2 experiments at the peak diuresis during isotonic saline loading, the infusion was changed to "5 per cent" saline (880 mEq/l Na). This resulted in a further large increase in urine flow and solute excretion, and a rise in $T^c_{H_2O}$ of 1.2 and 2.3 ml/min in the 2 experiments. Plasma sodium increased from 154 and 148 mEq/l to 218 and 200 mEq/l, respectively. Despite previous expansion of the extracellular volume (isotonic saline loading) and urine flow rates in excess of 50 ml per minute, $T^c_{H_2O}$ increased during the infusion of hypertonic saline. One of these experiments is shown in Fig. 2.

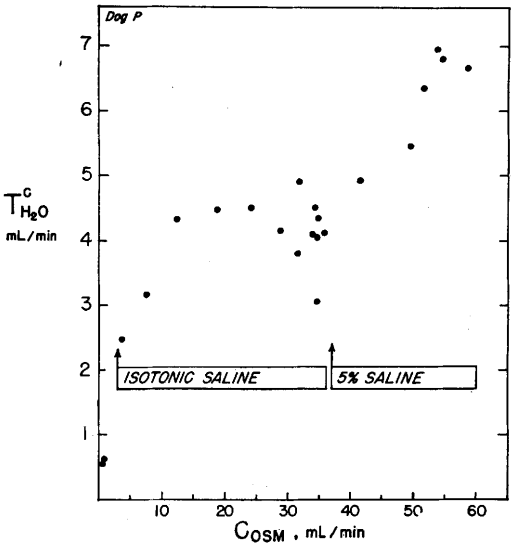

FIG. 2. *$T^c_{H_2O}$ during isotonic saline infusion followed by hypertonic saline infusion.* At solute clearances of about 20 ml per min $T^c_{H_2O}$ failed to increase further and began to decrease at solute clearances above 30 ml per min. When the infusion was changed to hypertonic saline $T^c_{H_2O}$ promptly increased and remained maximal at solute clearances above 50 ml per min (see text).

Discussion. The variability of $T^c_{H_2O}$ in dogs undergoing different types of solute diuresis is well recognized(6-9). In the present study attempts were made to maintain completely uniform control conditions, and studies were not continued when spontaneous fluctuations in urinary concentration were observed. It is assumed, therefore, that the differences in concentrating capacity observed in the present studies related to the infused solutions.

It is known that $T^c_{H_2O}$ may decrease at high rates of solute excretion, and several explanations have been offered. 1) A lower GFR may in some way be involved in the limited values of $T^c_{H_2O}$ at high rates of solute clearance(7). However, such a mechanism cannot account for the lower values of $T^c_{H_2O}$ observed in the present studies since GFR was invariably higher during isotonic saline loading than during hypertonic mannitol loading, and also the lowest values for $T^c_{H_2O}$ in each animal were usually associated with the higher values of GFR. 2) $T^c_{H_2O}$ may decrease at high rates of solute clearance due to a failure of water to equilibrate passively in the distal convolution and collecting duct (6,7). Although this must be the case when hypotonic urine is excreted during high rates of urine flow (despite hydropenia and vasopressin), it would not appear that this mechanism could explain the differences in $T^c_{H_2O}$

observed in the present studies, unless the permeability of the distal nephron was different in the presence of the different loading solutions. If such failure of equilibration is dependent entirely on the high rates of flow through the distal nephron, then $T^c_{H_2O}$ should decrease independently of the loading solutes employed in the present study. In the experiments with hypertonic saline, $T^c_{H_2O}$ continued to increase in 5 of the animals when solute clearance was greater than 25 ml per minute. In the 2 experiments in which infusion of hypertonic saline was superimposed on the isotonic saline load, urine flow reached 54 and 52 ml per minute, and a further increase in $T^c_{H_2O}$ occurred.

If it is assumed that the permeability to water of the distal nephron is not different under the different experimental conditions, then the degree of medullary interstitial hypertonicity may differ. Factors known to affect medullary interstitial hypertonicity include: 1) The rate at which water is added from the collecting duct and descending limb of Henle's Loop. If relatively little solute is reabsorbed in the collecting duct and if delivery from the distal convolution is isotonic then water added to the medullary interstitium from the collecting duct would relate directly to the rate of solute excretion. 2) The rate at which solute (sodium) is added to the medullary interstitium. This factor could be affected by an absolute change in rate of transport by the ascending limb of Henle's Loop or by a change in delivery of sodium to the ascending limb. In the present studies such delivery of sodium to the ascending limb should have been greater during isotonic saline loading than during hypertonic mannitol loading. However, $T^c_{H_2O}$ was usually lower under the former condition. The possibility cannot be excluded that during saline loading transport by the ascending limb is directly affected (decreased), and thereby the efficiency of the concentrating mechanism diminished. If such a decrease in active transport does occur it is probably not mediated by a decrease in mineralocorticoid effect, since the present observations were made during the infusion of large amounts of a mineralocorticoid. On the other hand, if the rate of transport by the ascending limb is influenced by the substrate (sodium) concentration, then absolute reabsorption at this site could be greater as the concentration of sodium in the tubular fluid increases, as should occur with the rising concentration of sodium in plasma during hypertonic saline loading. Higher values for $T^c_{H_2O}$ during hypertonic saline loading as compared to hypertonic mannitol loading have been observed in man also(9,10), and have been attributed generally to the greater delivery of sodium to the transport sites involved in maintenance of medullary hypertonicity. However, Goldberg and associates recently have suggested also that the concentration of sodium in the fluid reaching the ascending limb may be a factor influencing reabsorption at this site(10).

In the present studies, delivery of sodium to the distal nephron must not be the major factor resulting in the lower values for $T^c_{H_2O}$ during isotonic saline loading as compared to loading with hypertonic mannitol. Therefore, additional factors must be invoked to account for the diminished concentrating ability at high rates of solute excretion during isotonic saline loading. Since medullary interstitial hypertonicity is inversely related to medullary blood flow(11), the lower concentrating capacity during isotonic saline loading could be due to a higher rate of medullary blood flow. It has been suggested on the basis of measurements of total renal blood flow and extraction ratios for PAH, that isotonic saline loading may be associated with large increases in medullary blood flow(5). It is suggested that the present data are consistent with the view that at high rates of solute excretion the concentration of sodium delivered to the distal nephron (ascending limb) may become one factor limiting the extent to which urine may be concentrated.

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1. deWardener, H. E., Mills, I. H., Clapham, W. F. C., Hayter, C. J., Clin. Sci., 1961, v21, 249.

2. Levinsky, N. G., Lalone, R. C., J. Clin. Invest., 1963, v42, 1261.

3. Rector, F. C., Jr., Van Giesen, G., Kiil, F., Seldin, D. W., ibid., 1964, v43, 341.
4. Earley, L. E., Friedler, R. M., ibid., 1964, v43, 1928.
5. ———, ibid., 1965, v44, 929.
6. Earley, L. E., Kahn, M., Orloff, J., ibid., 1961, v40, 857.
7. Goldsmith, C., Beasley, H. K., Whalley, P. J., Rector, F. C., Seldin, D. W., ibid., 1961, v40, 2043.
8. Goodman, B., Cohen, J. A., Levitt, M. F., Kahn, M., Am. J. Physiol., 1964, v206, 1123.
9. Stein, R. M., Levitt, H. B., Goldstein, M. H., Porush, J. J., Eisner, G. M., Levitt, M. F., J. Clin. Invest., 1962, v41, 2101.
10. Goldberg, M., McCurdy, D. K., Ramirez, M. A., ibid., 1965, v44, 182.
11. Hargitay, B., Kuhn, W., Z. Electrochem., 1951, v55, 539.

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19S and 7S Anti-Toxoplasma Antibodies in Diagnosis of Acute Congenital and Acquired Toxoplasmosis.* (30778)

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Because newborn infants with congenital toxoplasmosis may exhibit clinical signs and symptoms often found in other disease states, serological methods and/or isolation procedures have usually been employed in an attempt to establish the diagnosis. If specific therapy is to be used in such cases, in hope that it will prevent further tissue destruction by the parasite, early diagnosis (usually during the first days of life) is imperative. Unfortunately, early serologic diagnosis of toxoplasmosis in the newborn is complicated by the fact that maternal antibody is passively transferred to the fetus *via* the placenta. Because none of the serologic methods presently available differentiate between maternal and fetal antibody, a high anti-*Toxoplasma* antibody titer in the serum of an infant may merely reflect infection in the mother and does not therefore establish the diagnosis of congenital toxoplasmosis. This problem of interpretation of serologic tests is magnified in infants who do not exhibit the classical clinical picture of hydrocephalus, jaundice, hepatosplenomegaly and chorioretinitis often associated with fulminant congenital toxoplasmosis. In such infants, in whom irreparable damage may not yet have occurred, therapy may prevent crippling sequelae such as mental retardation and blindness.

An additional method used in diagnosis is the demonstration of *Toxoplasma* by isolation techniques. However, if the infecting strain is relatively avirulent for laboratory animals, the isolation procedure may not reveal the diagnosis in time to allow early initiation of specific therapy.

A no less complicated situation confronts physicians attempting to establish a diagnosis of acute acquired toxoplasmosis in individuals with signs and symptoms of this disease who, on initial examination, exhibit high serologic test titers to *Toxoplasma*. Since clinical manifestations in cases of acquired toxoplasmosis may mimic those of a variety of disease states and since dye test, hemagglutinating and complement fixing anti-*Toxoplasma* antibody titers may remain elevated for years following the acute infection(1,2), a high serologic test titer in such individuals does not necessarily indicate that the antibodies are related to the present illness. In addition, because the cyst form of *Toxoplasma* persists in extraneural tissues of chronically infected humans for years and perhaps for life following acute infection(3,4), successful isolation of the parasite from a patient with a high serologic test titer is difficult to evaluate and

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