Lymphology 10 (1977) 204–208
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Effect of Hypotonic Volume Expansion with Water Diuresis on Renal Lymph

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Summary

Water diuresis was induced in dogs, under chloralose anesthesia, by hypotonic volume expansion of 5 to 10% body weight. The average diuretic response was 12.5 ml/hr/kg body weight with a urine osmolality of 175 mOsm/L. Renal hilar lymph flow and thoracic duct lymph flow increased significantly by an average of 125% and 150% respectively. The $Na^{\oplus}$ concentration in plasma and lymph fell by approximately 7.0 mEq/L whereas that for $Cl^{\ominus}$ was relatively unaffected. Control lymph to plasma ratios of approximately 1.050 for $Na^{\oplus}$ and 1.130 for $Cl^{\ominus}$ were not significantly altered even when plasma concentrations fell significantly. Urea, glucose and $K^{\oplus}$ concentrations were little affected. The results are consistent with the hypothesis that renal hilar lymph reflects in part the composition of the outer medullary interstitium which is directly influenced by the electrolyte pump in the ascending thick limb of *Henle.*

The relationship between diuresis and renal lymph flow is not consistent because of the different factors which independently influence the formation of lymph and urine. Mannitol, by a variety of mechanisms, increases both urine and lymph formation (1). In contrast, a comparable diuresis induced by furosemide or ethacrynic acid is not accompanied by greater lymph production unless renal blood flow is significantly increased (1, 2). The effect of water diuresis is less clear. According to one report, it increases renal capsular lymph in the unanesthetized dog (3), whereas a more recent study showed it to have no significant effect upon capsular lymph in the anesthetized animal (4). This finding is surprising in view of the general increase in lymph flow which accompanies the volume expansion and associated dilution of plasma proteins in the induction of water diuresis.

The effect of mannitol and furosemide diuresis upon the composition of renal lymph has been described (1) but comparable data are not available for water diuresis. Thus it is not known whether water diuresis alters the $Na^{\oplus}$ and $Cl^{\ominus}$ concentration difference, which exists under control conditions, between renal lymph and plasma (5). This question is of importance because water diuresis has been shown to dissipate the intramedullary $Na^{\oplus}$ gradient, although a tissue $Na^{\oplus}$ concentration difference between cortex and outer medulla is maintained (6, 7, 8). If, as is believed (1, 5), hilar lymph derives a component from the outer medulla, then water diuresis, like mannitol diuresis (1), might be expected to reduce but not abolish the comparatively higher concentrations of $Na^{\oplus}$ and $Cl^{\ominus}$ in renal lymph compared to plasma.

The study reported here concerned the effect of water diuresis, induced by hypotonic volume expansion, upon the flow and composition of renal lymph. It was found that flow was significantly increased and that $Na^{\oplus}$ and $Cl^{\ominus}$ concentrations, even when reduced in both lymph and plasma, maintained a lymph to plasma ratio greater than one. No major change in the other components of lymph that were studied, was detected.

Materials and methods

Experiments were performed on 10 dogs of either sex with an average weight of 17 kg. The animals were anesthetized with 1% chloralose (*Kuhlman, Paris, France*) using an initial dose of 170 mgm/kg and a sustaining dose of 35 mgm/kg/hr approximately. The left common carotid artery was cannulated for sample col-

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lection, and the thoracic duct was cannulated through the same incision. The left renal pedicle was exposed through a loin incision and a catheter placed in the left ureter. Renal venous blood samples were obtained from a cannula passed through the gonadal vein into the renal vein towards the kidney. One or more hilar lymphatics were cannulated with nylon (Portex) tubing (I.D. = 0.02 in.).

Each animal served as its own control for a period of about two hours, before volume expansion was induced. During the control periods, isotonic saline was infused at about 1 ml per min, carotid and renal venous plasma samples were collected at 30 minute intervals, and urine, thoracic duct lymph and hilar lymph were collected throughout. During control and experimental periods a small sample of thoracic duct lymph was removed at 30 minute intervals and the remainder reinjected intravenously.

Volume expansion was achieved by intravenous infusion of hypotonic saline (0.4%, or 0.23% saline with 2.5% glucose) amounting to 5–10% of body weight. This was brought about either by continous rapid infusion over a 90 minute period or by a series of injections of up to 500 ml in volume. Urine loss was compensated for by additional saline infusion. Urine and lymph samples were collected more frequently during volume expansion than during control periods because of increased flow.

Significance was estimated by the Student t test for paired groups. For the statistical analysis the means of the concentrations and ratios were calculated for each experiment: the tests were then applied to the overall sets of means.

Sample analysis

$Na^{\oplus}$ and $K^{\oplus}$ were measured with an IL 343 automatic readout flame photometer with a built-in dilutor. $Cl^{\ominus}$ was measured with a Corning Model 920 M chloride meter. Osmolality was obtained with an Advanced Instrument osmometer (Model 3L). Protein was estimated using an AO refractometer. Glucose estimations were made by the routine O-toluidine in glacial acetic acid technique. Blood urea nitrogen was estimated by the condensation of urea with diacetyl monoxmine using the Harleco blood urea nitrogen sets. All optical densities were read on a Coleman 55 spectrophotometer.

Results

The typical time course of events which followed the administration of hypotonic fluid can be seen in Figure 1 which illustrates a representative experiment.

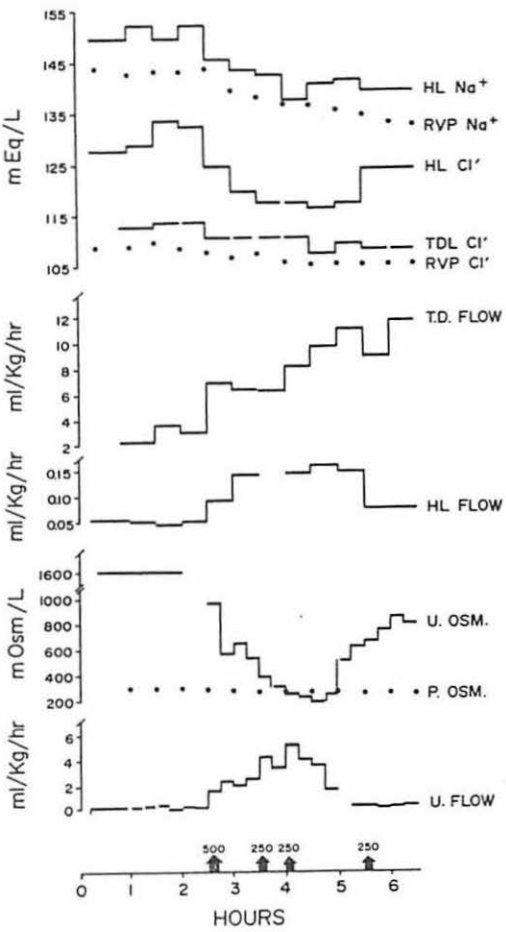

Fig. 1 Representative experiment illustrating the effects of hypotonic volume expansion. The arrows on the abscissa indicate the time and volume (ml) of infusion.

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Table 1 Effect of volume expansion on hilar lymph (HL), thoracic duct lymph (TDL) and urine (U) flow in ml/hr/kg body weight.
"Diuresis" values are the average values obtained during the several consecutive periods of maximum diuretic response.
N = number of animals; % = percentage increase.

	HL		TDL		U	
	Control	Diuresis	Control	Diuresis	Control	Diuresis
$\bar{x}$	0.078	0.176*	3.2	8.2*	1.1	12.4*
SE	0.022	0.053	0.32	1.5	0.19	2.6
N	10	10	9	9	10	9
%		125		153		990

* $p < 0.01$

Table 1 summarizes the effects upon lymph and urine flow in all the experiments. Hilar and thoracic duct flow increased by about 125% and 150% respectively at a time when urine flow increased by an average of 1000 fold. As urine flow increased, Uosm fell to reach a minimum value of 100–150 mOsm/L in most experiments. In both tables 1 and 2 the term "diuresis" alludes to the several consecutive periods when urine flow attained a maximum.

both lymph and plasma were not significantly affected even though the $Cl^{\ominus}$ content of the infusate was only 70 mEq/L: however, in some experiments, a temporary reduction did occur after the onset of the infusion. In any event, the $Cl^{\ominus}$ lymph to plasma ratio was not significantly affected.

Urea, glucose and $K^{\oplus}$ were also measured. No difference was detected between the hilar lymph and plasma concentrations of urea or $K^{\oplus}$ dur-

Table 2 A comparison of renal venous plasma (RVP), thoracic duct lymph (TDL) and hilar lymph (HL) during control and diuretic periods. The values represent the means ± S.E.
HL was significantly different from RVP and TDL in both control and diuresis ($p < 0.01$).

		$Na^{\oplus}$	$Cl^{\ominus}$	Protein	Urine Osmolality
Control	RVP	142.4 ± 1.40	110.9 ± 1.67	5.89 ± 0.296	1084.6 ± 113
	TDL	141.4 ± 1.71	111.7 ± 2.01	3.72 ± 0.53	
	HL	148.9 ± 0.955	125.3 ± 1.68	2.08 ± 0.18	
	HL/P	1.049 ± 0.008	1.130 ± 0.010	0.354 ± 0.024	
Diuresis	RVP	135.9 ± 2.40	110.1 ± 2.22	4.50 ± 0.324	173.7 ± 21.46
	TDL	135.2 ± 2.72	110.3 ± 2.60	2.37 ± 0.44	
	HL	141.9 ± 2.33	122.5 ± 2.21	1.05 ± 0.296	
	HL/P	1.041 ± 0.007	1.119 ± 0.009	0.220 ± 0.049	

Table 2 illustrates changes which occurred in lymph, plasma and urine composition. The control values conformed to those obtained in other studies in our laboratory (1, 5). The concentrations of $Na^{\oplus}$ and $Cl^{\ominus}$ in hilar lymph exceeded those in thoracic duct lymph and plasma both before and during diuresis ($p < 0.01$). Thus despite a reduction in plasma sodium of about 7.0 mEq/L, there was no change in the hilar lymph to plasma ratio. Surprisingly, the mean $Cl^{\ominus}$ concentrations for

ing either control or diuretic states. On the other hand, the glucose concentrations of hilar lymph were consistently about 10% less than those in plasma ($p < 0.01$). These findings conformed with previously published data from our laboratory (5).

The protein concentration of plasma was reduced by an average of 23%, and that for hilar lymph by 50% during volume expansion.

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Discussion

Renal hilar lymph flow, in contrast to composition, varies widely from one lymphatic to another as well as between successive animals. Individual variations in the number and size of lymphatics cannot fully account for this, so that other, as yet unexplained, factors must participate. As a consequence comparison of flow between different animals is less informative than the changes which occur between control and experimental periods in any given animal. The latter comparison was used in this study and showed that water diuresis when induced by volume expansion of 5–10% body weight increased the flow of lymph by an average of 125% (Table 1, Figure 1). The increase can be readily understood from hemodynamic principles, including the associated dilution of plasma proteins (Table 3), without invoking any specific effect of the diuresis. In this context, the failure of *Le Brie* and *Gotshall* (4) to obtain an elevated renal lymph flow, when "water" diuresis was induced by glucose infusion, is hard to understand. One difference between their work and ours concerned the source of renal lymph – they sampled capsular whereas we collected hilar. However, this does not provide a convincing explanation since volume expansion has a generalized effect, and also because *Henry* et al. (3), obtained an increase, comparable to that obtained by us, when they sampled capsular lymph in the unanesthetized dog. Scrutiny of *Le Brie* and *Gotshall*'s (4) results shows considerable variation between individual experiments. For example in three of four experiments depicted, there was an increase in lymph flow during diuresis, and the overall mean change in 21 animals studied was an increase of about 100%, although this was not found to be significant. Thus an increase in renal lymph flow during water diuresis does not appear to be entirely inconsistent with their results.

Of particular interest in the present study was the finding that lymph to plasma differences for $Na^{\oplus}$ and $Cl^{\ominus}$ were maintained in the face of changes in their plasma levels. This demonstrated that, whatever is the process responsible for the lymph to plasma difference, it was relatively unaltered by electrolyte concentration changes, volume expansion, and water diuresis. Although no direct information was obtained on the nature of the process (which cannot be explained solely on the basis of the Gibbs-Donnan phenomenon) previous work (1) has implicated the $Cl^{\ominus}$ pump in the thick ascending limb of *Henle*. According to this theory the outer medullary interstitium, into which $Cl^{\ominus}$ is pumped in excess of water, contains a fluid relatively rich in $Na^{\oplus}$ and $Cl^{\ominus}$, and it is this fluid which reaches lymphatics that enter the hilar system and thereby elevate its electrolyte content. Evidence (6, 7, 8) derived from tissue slice analysis, indicates that the $Cl^{\ominus}$ pump maintains its activity in water diuresis. Thus *Perlmutt* (7) showed that in water diuresis in dogs the cortico-medullary gradient was maintained although the intramedullary stratification was abolished. *Eknoyan* et al. (8) obtained somewhat different results in that they found the osmotic gradient to increase, because of increased interstitial $Na^{\oplus}$, in moderate water diuresis. In both studies it is evident that the pump in the thick ascending limb of *Henle* was not abolished. Functional studies on the activity of the electrolyte pump during water diuresis are as yet equivocal (9), although there is some indication that it may be reduced slightly in the diluting segments of the nephron. The results of the present study are, therefore, consistent with the hypothesis that hilar lymph to plasma ratios, greater than one for $Na^{\oplus}$ and $Cl^{\ominus}$, stem at least in part from the outer medullary interstitium. This interstitium is in turn influenced by the electrolyte pump in the ascending thick limb of *Henle* which is not abolished in water diuresis.

Supported by a grant from the National Institutes of Health # AM 18473.

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EDITORIAL

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