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Influence of Protein Content upon the Electrolyte Composition of Lymph and Plasma[1]

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Summary

The concentration of Na^+ and Cl' is higher in renal hilar lymph (HL) than in arterial (P) or renal venous plasma or thoracic duct lymph (TDL). The purpose of the present study was to test the theory that this is the consequence of differences in protein content of lymph and plasma rather than a reflection of renal function. Samples of P, HL and TDL were obtained from dogs and analysed for Na^+, Cl', K^+ and Ca^{++}. The samples were then centrifuged through an ultrafiltration membrane to remove the protein and reanalysed for electrolyte content. Ca^{++} concentrations were reduced by 30-40% in the (protein-free) filtrate. This was attributed to protein binding. Na^+ and Cl' concentrations were raised minimally (2.0 mEq/L and 5.0 mEq/L respectively) in protein-free filtrate of plasma, but not in protein-free filtrate of lymph. It was concluded that the relative protein concentration in lymph and plasma are not an important influence on Na^+ and Cl' concentrations.

Introduction

The electrolyte content of renal lymph has attracted considerable attention in recent years. The main reason for this interest is the unresolved question of whether or not the renal medulla has a lymphatic system. It has been argued that if the medulla is a source of lymph then that lymph should reflect the high concentrations of Na^+, Cl' and urea which are characteristic of the medulla.

Although some early studies failed to detect any difference between the electrolyte concentration of hilar lymph and plasma, it is now well established that in the dog at least, hilar lymph has a higher concentration of Na^+ and Cl' than does simultaneously collected renal venous plasma, capsular lymph or thoracic duct lymph (1, 2, 3). However because these hilar lymph to plasma concentration differences are relatively small (average 5-8 mEq/L for Na^+, and 15-23 mEq/L for Cl') it is possible that they are more a consequence of relative protein concentration differences than a result of medullary function. More specifically, since lymph contains less than half the protein concentration of plasma, it might be expected to have a higher electrolyte content if measured in mEq/l of sample. Valid comparisons between lymph and plasma, so this argument goes, can only be made if samples are corrected for total solute content so that electrolyte concentrations may be expressed in mEq/L of water. Application of this type of correction does indeed reduce the apparent renal lymph to plasma concentration difference significantly (3). However it does not take into account electrolyte binding of proteins which if present could require correction in the opposite direction: nor does it consider the effects of the Gibbs-Donnan phenomenon. Resolution of the controversy is of fundamental importance in the interpretation of functional studies involving renal lymph. The present study was designed to elucidate the problem by determining experimentally the effect of removal of protein from lymph and plasma samples on Na^+, Cl', K^+ and Ca^{++} concentrations.

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Material and Methods

Sample Collection and Analysis

Samples were obtained from nine dogs of either sex anaesthetized with sodium pentobarbital (25-30 mgms/kg body wt). Fourteen blood samples were collected by cannulation of the carotid artery in eight of the dogs and centrifuged immediately at 2000 rpm for 10 minutes. Thoracic duct lymph (25 samples) was obtained from the neck in nine dogs, and renal lymph (7 samples) by cannulation of hilar lymphatics through left loin incisions in four dogs. Throughout the periods of sample collection 0.9% saline was infused intravenously at 1 to 2 ml/min and 500 units of heparin were administered every hour. In most instances samples were refrigerated overnight for analysis the next day, care being taken to avoid evaporation. All samples were analysed for Na^+, K^+, Cl' and protein concentration, and many were analysed for Ca^{++}. They were then filtered through Centriflo Ultrafiltration Membrane Cones (Aminco 2100 CF50). Each cone was soaked in either distilled water or Ringer's lactate solution for approximately 2 hours prior to use, and then centrifuged for 6 minutes at 1000 rpm to remove excess fluid (approximately 0.1 ml). Samples (5.0 ± 2.0 ml) were centrifuged at 1000 rpm until approximately 50% of the sample had been filtered (30 ± 10 minutes). During centrifugation the tubes containing the filter cones and samples were covered with parafilm to prevent evaporation. The filtrate (protein free) and the supernatant (protein rich) were then analysed for Na^+, K^+, Cl', Ca^{++} and protein. In 6 cases after this second analysis the supernatant, still in its filter cone, was supported in a small beaker containing the filtrate for about two hours. Both filtrate and supernatant were then reanalysed for Na^+, K^+, Cl', Ca^{++} and protein. The purpose of this was to permit any possible Gibbs-Donnan effect to occur accross the filter membrane.

Analytical Methods

Na^+ and K^+ concentrations were measured on an IL flame photometer (Model 343) with a built-in automatic dilutor. Cl' was measured using a Corning chloride meter (920 M), and Ca^{++} concentrations were obtained with an IL 253 atomic absorption spectrophotometer. Protein concentrations in most samples were measured with an AO 10-400 refractometer, and electrophoretically using cellulose acetate strips and a Beckman R111 densitometer. Protein analysis of filtrate samples, because of the low concentrations were estimated by the trichloracetic acid technique (4) after concentration in an Amicon A25 minicon concentration chamber.

Statistical analysis was performed using the Student t test for paired groups.

Results

The normal protein content of plasma, thoracic duct lymph and hilar lymph as measured with the refractometer is shown in Table 1 and as measured by electrophoresis in Table 2. It can be seen from Table 1 that the Centriflo filter cones were effective in removing almost all the protein. The manufacturers specifications claim retention of 97% of the protein, a figure which conforms with our results.

Figure 1 shows the normal lymph and plasma concentrations of Na^+, K^+, Cl' and Ca^{++} and the effect of protein separation. Control Na^+ and Cl' concentrations in HL were significantly higher than in plasma or thoracic duct lymph. No significant differences however were found for K^+ concentrations. Ca^{++} concentrations were lower in lymph than in plasma.

Table 1 Protein concentrations of plasma (P), thoracic duct lymph (TDL) and hilar lymph (HL) before and after protein separation (gms/100 ml).

		P	*TDL*	*HL*
Control	$\bar{x}$	6.29	3.92	2.47
	SE	0.21	0.28	0.20
	N	11	18	7
Filtrate	$\bar{x}$	0.009	0.009	0.001
	SE	0.004	0.003	0.006
	N	11	21	5
Supernatant	$\bar{x}$	11.18	9.0	6.7
	SE	0.69	0.9	1.08
	N	9	9	7

Table 2 Electrophoretic analysis of the protein content of plasma (P), thoracic duct lymph (TDL) and hilar lymph (HL) (gms/100 ml).

		Total	*Alb*	α_1	α_2	β	γ
P	$\bar{x}$	6.63	1.92	0.22	0.72	2.50	1.31
	SE	0.10	0.11	0.01	0.09	0.12	0.16
	N	12	12	12	12	12	12
TDL	$\bar{x}$	4.69	1.75	0.16	0.48	1.56	0.91
	SE	0.23	0.10	0.01	0.09	0.13	0.13
	N	11	12	12	12	12	12
HL	$\bar{x}$	2.5	1.06	0.10	0.22	0.84	0.32
	SE	0.29	0.15	0	0.04	0.13	0.02
	N	5	5	5	5	5	5

Samples when centrifuged in cones which had been soaked in water showed a slight (e.g., 2-3 mEq/L) fall in some of the electrolyte concentrations. Since this was attributed to dilution by water absorbed onto the cone (approximately 0.25 ml), the results shown in Figure 1 were obtained from experiments in which cones were presoaked in Ringer's lactate solution. However, when the results obtained using water soaked cones were corrected for the dilution, they conformed with those shown in Figure 1. A small (2-3 mEq/L) but significant ($P < 0.01$) increase in plasma Na^+ concentration was effected by protein removal. No statistically significant change was detected for thoracic duct or hilar lymph, although the average hilar lymph concentration increased by about 1.0 mEq/L. The average HL/P Na^+ ratio changed from a control value of 1.059 to a value of 1.046 after protein removal. Both plasma and thoracic duct filtrate had a higher Cl^- concentration than their respective control values but this was not true for hilar lymph. As expected

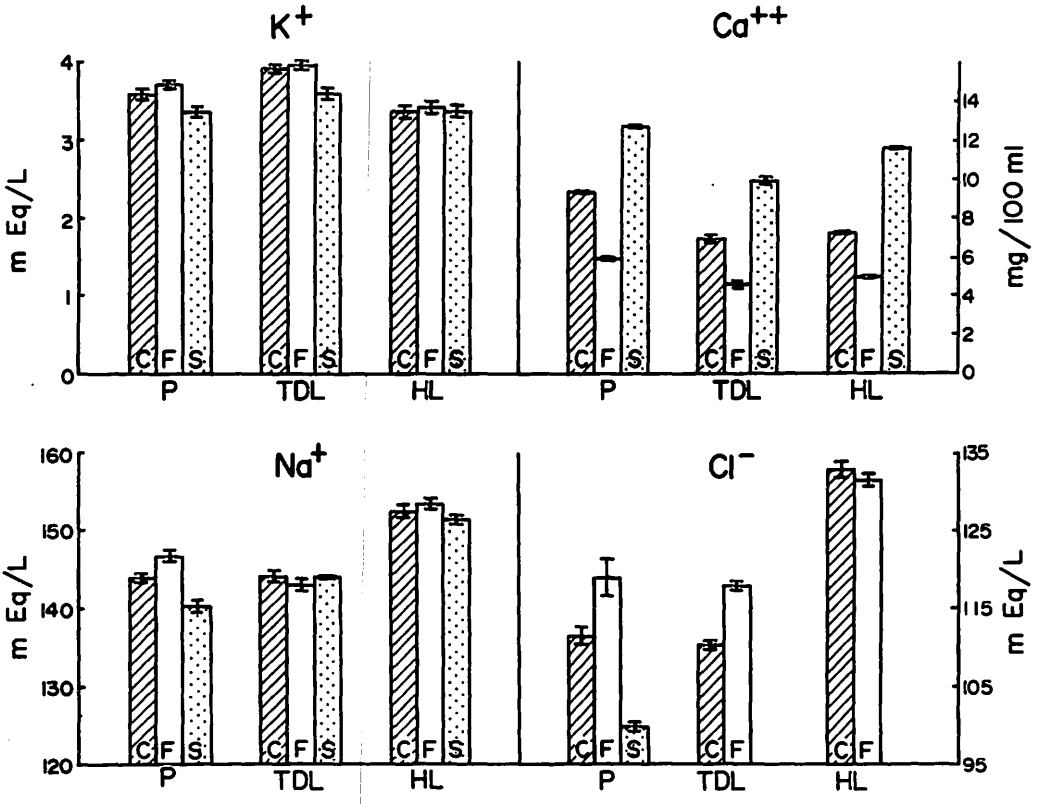

Fig. 1 Electrolyte concentrations of plasma (P), thoracic duct lymph (TDL) and hilar lymph (HL) in the original samples (C), the protein-free filtrate (F) and the protein-rich supernatant (S).

Ca^{++} concentrations were reduced by 30%-40% upon removal of the protein.

Supporting the cone, containing supernatant, in the filtrate for two hours after centrifugation had no additional effect upon electrolyte concentrations.

Discussion

In preliminary experiments of this study precipitation techniques were used to remove protein from plasma and lymph but with unsatisfactory results. Chemical precipitation by, for example, $ZnSO_4$-$Ba(OH)_2$ (5) was unsuitable because the accompanying dilution required a correction factor which in itself introduced errors as large as the theoretical effect of protein removal on electrolyte concentration. Comparable problems arose from evaporation when precipitation was induced by heat. At first protein removal by filtration through Aminco filters also produced bizarre results in that electrolyte concentrations of both filtrate and supernatant fell soon after the onset of centrifugation and then progressively increased to exceed their initial values. However it was discovered that the early fall was caused by water adherence to the cone since the filter cones had to be soaked before use, and the subsequent rise was due to evaporation from uncapped tubes during centrifugation. Once these preliminary problems had been solved protein separation by centrifugation produced consistent results. Protein removal was essentially complete and electrolyte concentrations in the protein free filtrate was reproducible. Measurement of Na^+ and K^+ in the protein rich supernatant however often proved to be difficult because the viscous fluid tended to clog the inlet tube of the flame photometer or the pipette used in Cl' analysis.

Calcium was measured in the present study as a standard of comparison since it is known that 30% to 50% of Ca^{++} in plasma is protein bound. As therefore expected protein removal from both lymph and plasma caused a reduction in Ca^{++} concentration of 30% to 40%. In contrast protein removal had minimal effects upon Na^+ and K^+ concentrations. This latter finding suggests that these electrolytes bind to protein in almost equivalent concentration per unit volume as their respective concentrations in the accompanying fluid. The Cl' findings were less clear cut in that protein removal had no effect on lymph concentrations but increased plasma concentrations by about 5 mEq/L. Even this difference would appear to be unimportant functionally since total removal of protein from plasma or lymph is physiologically unrealistic. Normally (Tables 1, 2) lymph contains between 30% and 50% as much protein as plasma. On this basis Cl' concentrations, because of protein differences, might be 2-3 mEq/L higher in plasma than lymph. In cat hilar lymph Cl' normally exceeds plasma Cl' by about 20 mEq/L (Figure 1). Comparison with lymph of nonrenal origin shows that only a small part of this can be explained by the Gibbs-Donnan phenomenon.

The findings of this study show that normal hilar lymph to plasma electrolyte concentration differences cannot be explained by differences in protein content (Figure 1), and thus provide confirmation that they are a real indication of intrarenal function regardless of protein concentration.

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