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Influence of Venous Congestion on Blood-lymph Transport of Fluid and Large Molecules in the Heat-injured Dog's paw[1]

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Summary

Hind paws of anesthetized dogs were exposed to moderate continuous heat (45–52 °C). Lymph flow (L) was increased to 5.61 (SD 3.26) times control levels, lymph:plasma concentration ratios for total plasma protein (R_T) were increased to 3.23 (SD 1.33), and for exogenous Dextran-110 (R_D) to 8.41 (SD 4.00) times control levels. Selectivity of the blood-lymph barrier for individual plasma proteins was also decreased. Elevation of venous pressure during heating resulted in an increase of L in 5 out of 8 experiments, with no consistent change in R_T or R_D. Increased protein flux was thus closely coupled to increased volume flow. These observations are consistent with the presence and persistence of intercellular openings or gaps in the microvascular endothelium.

Introduction

Heat is one of several agencies known to increase blood-lymph transport of plasma proteins and other large molecules (1, 2). The effects of heat are believed to be exerted mainly on microvascular endothelium (3). Morphological studies show persistent intercellular gaps in venules and capillaries (4, 5, 6). However, in view of the possibility of other endothelial pathways for large molecules (7), it seems desirable to test the characteristics of blood-lymph macromolecular transport in heat injured tissues, to see if they are consistent with the morphological observations. To do this, we investigated the coupling of protein and fluid transport when capillary hydrostatic pressure was raised by venous congestion (8).

Methods

Paw lymph was collected from pre-popliteal lymphatics of 12 anesthetized mongrel dogs. To promote flow, the legs were flexed passively at the ankle, 100 times per minute. Lymph flow (L) was measured as weight/time. In 6 dogs Dextran-110 (Pharmacia) was infused i.v. Plasma and lymph samples were analyzed for total protein (T) and for Dextran when present (D). Lymph concentrations expressed as ratios to plasma (R_T and R_D). Individual protein components were resolved by disc electrophoresis. Lower leg venous pressures (P_V) were measured by a plastic catheter in a branch of the lateral saphenous vein. P_V was elevated by tightening a soft rubber tourniquet around the thigh until the desired pressure was attained. The tourniquet was central to the level of lymphatic cannulation, and thus did not interfere with lymph collection. Details of these methods are given in earlier publications (9, 10, 11).

The paw to be heated was clipped, and thermistors were taped to dorsal and ventral surfaces. The ventral thermistor was used to control surface temperature (Yellow Springs Model 71A Controller), the dorsal to monitor

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Table 1 Influence of sustained heat on flow and composition of dog paw lymph. Effects of increased venous pressure[a]

	Control					Heat Ratio to Control					Heat + venous Congestion Ratio to Control				
EXP	T_S	P_V	L	R_T	R_D	T_S	P_V	L	R_T	R_D	T_S	P_V	L	R_T	R_D
1[b]	33	16.5	3.11	.250	.072	47.	17.5	*2.00*	*0.78*	*1.04*	47	32	*2.20*	*.65*	*1.07*
2[b]	31	0.5	1.13	.431	.085	51.5	2.	*1.69*	*0.90*	*1.67*					
3	32	15.	1.88	.133	.039	45.	15.	*7.04*	*5.04*	*10.51*	45	30	*7.11*	*5.94*	*14.08*
4	33.5	7.	1.14	.259	.050	50.5	8.	*5.61*	*3.26*	*13.36*	50.5	25	*11.84*	*2.80*	*9.44*
5	34	9.	1.09	.214	.086	50.	10.	*12.72*	*3.21*	*5.72*	(50	21.5	*3.18*	*3.72*	*6.23*)
6	34.5	7.	0.26	.520	.275	52.	11.	*4.15*	*1.61*	*3.19*	52	18	*11.87*	*1.04*	*2.99*
7	33	13.5	1.18	.254	.080	49.	18.5	*6.58*	*3.53*	*9.30*	50	31	*4.78*	*3.64*	*8.83*
8	32		1.30	.250		50.		*2.97*	*2.80*						
9	31	9.	4.70	.298		52.	15.	*3.28*	*1.78*		52	44	*5.51*	*1.82*	
10	31	14.	3.65	.237		48.	12.	*1.62*	*2.36*		48	32	*5.01*	*1.92*	
11	33	10.	4.62	.113		50.	17.5	*6.54*	*5.51*		50	37.5	*8.18*	*6.23*	
12[c]	34	18.	5.35	.149							51	40	*2.14*	*2.50*	

[a] T_S = surface temperature of paw, °C. P_V = venous pressure, mmHg, L = lymph flow ml/sec x 10^4, R_T = lymph/plasma concentration ratio for total protein, R_D for exogenous Dextran-110 (where infused). Values of L, R_T and R_D for the experimental priods (*italics*) are expressed as *ratios* to control.

[b] Experiments 1 and 2 did not show an "injury" response; see text. Their data are not included in group averages in text.

[c] In this experiment P_V was increased before heat was applied. The data for increased P_V alone are P_V = 40 mmHg, L/L_C = 1.60 and $R_T/R_{T,C}$ = 0.664. The data for this experiment are not included in group averages in text

surface temperature (T_S). The paw was then wrapped loosely in a single layer of surgical gauze, over which was loosely coiled a flexible heating strip (Briskeat BIH 2-1/2) extending from toes to ankle. The opposite paw served as an unheated control.

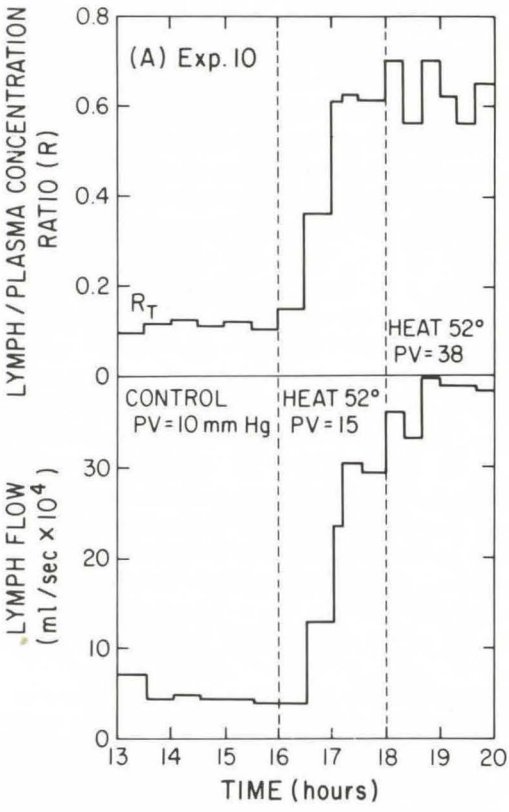
Results

Table 1 summarizes experimental data under three headings: control, heat, and heat plus venous congestion. Before heating, T_S ranged from 31°C to 34.5°C in an ambient of 22°C–26°C, and for experiments 3–11, in which the full protocol was followed, mean lymph flow was 2.20 x 10^{-4} ml/sec (SEM 0.56). Lymph:plasma concentration ratios for total plasma protein (R_T) fell between 0.11 and 0.52, roughly in inverse relation with L (12). $R_{D\text{-}110}$ was always less than R_T.

Our aim was to produce a mild degree of heat injury in which elevation of lymph flow and protein transport could be sustained for 2 to 4 hours. Accordingly, we approached raising of paw temperature gingerly, and in the first two of 12 trials, failed to produce unmistakable evidence of permeability change: L was doulbed at T_S 47 °C and 51.5 °C but R_T fell slightly. R_D was slightly elevated in the second experiment. In subsequent trials, more substantial increases in L, R_T and R_D were obtained.

Sensitivity to heat seemed to be variable, since marked responses were obtained in some experiments at temperatures lower than 51.5 °C or even 47 °C. Onset of the changes was usually sudden, and occurred between 30 and 90 min after application of the indicated level of heat. The full response was developed 1 to 3 hours after onset. In experiments 3–11, the mean increase in L was 5.61 fold (SEM 1.09), in R_T 3.23 fold (SEM 0.44), in experiments 3–7 the increase in R_D was 8.41 fold (SEM 1.79). Figure 1A and B shows two examples: in B, there were abortive changes in R_T and R_D at 45 °C and 47.5 °C without any change in L before definitive increases were produced at 50.5 °C. In the opposite (control) paws, L, R_T and R_D remained steady over the period of heating.

Figure 2 presents control and heat-lymph R's



individual protein components and D-110 as functions of molecular size. The logarithmic ordinate scale shows proportional changes as equal vertical displacements. Heat increased all R's, those for the larger plasma proteins and D-110 more than for the smaller components. Diminished slopes of the lines connecting the major components albumin, immunoglobulin G and fibrinogen-macroglobulin demonstrate reduced selectivity of the blood-lymph barrier, in agreement with previously published descriptions of the effects of somewhat more severe heat injury (1, 2, 9).

In experiment 12, *unheated,* elevation of venous pressure by 22 mmHg increased L 1.6 fold and reduced R_T to 0.66 its control value. These results are similar to those previously reported for normal dog paws (8). Venous congestion was applied to 9 preparations during heating. One of these (expt. 1) appeared unresponsive to the level of heat applied, another (expt. 5) did not achieve a stable level of response, and venous congestion was applied while L was falling. Response of the other 7 to elevation of P_V was varied, In one case (expt. 7) L decreased with little change in R_T or R_D. In another (expt. 3) there was no change in L, while R_T and R_D increased

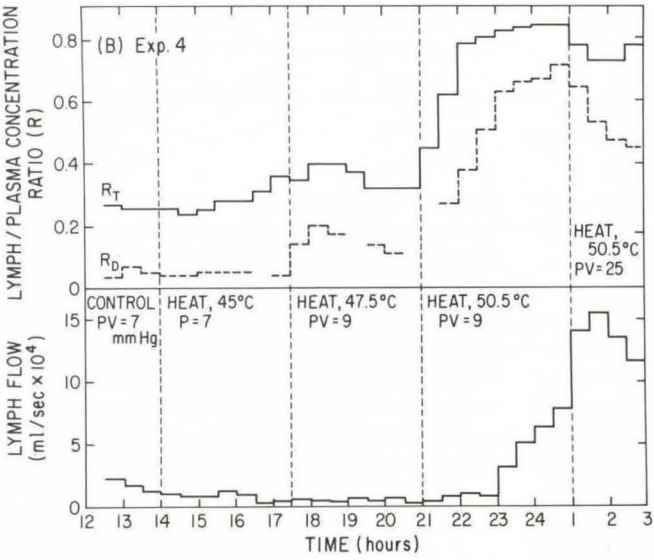


Fig. 1 Examples of dog paw lymph response to mild sustained heating. Experiment numbers refer to Table 1

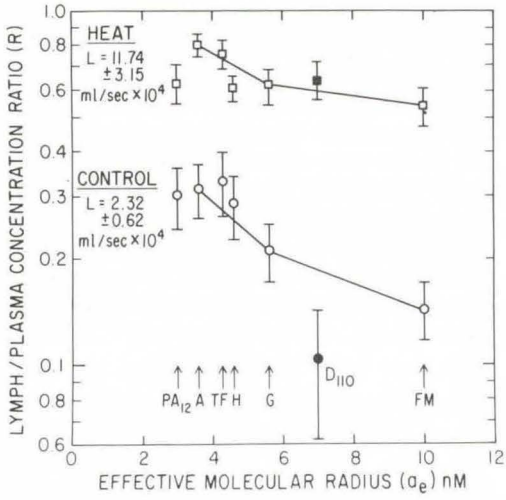


Fig. 2 *Lymph/plasma concentrations* ratio for six plasma protein components (open figures) and for Dextran-110 (filled figures). Means and SEM's of experiments 3–7 with Dextran, also 9–11 without. Protein components are as follows: PA_{12} alpha globulins, A albumin, TF transferrin, H haptaglobin, G immunoglobulin G, FM fibrinogen and macroglobulin. Effective radii were calculated from free diffusion coefficients by the Einstein-Stokes equation (see 11).

slightly. The remaining 5 experiments showed increases in L ranging from 1.25 to 3.09 fold (mean 2.2) with small and variably directed changes in R_T and R_D. In these experiments the increases in P_V ranged from 7 to 29 mmHg (mean 22). The relation of R to molecular size continued to show the same pattern as for heat injury without P_V elevation (Fig. 2).

A more consistent relation between large molecule transport and lymph flow in heat-injured paws could be shown by plotting the product of R and L as a function of L, when the latter was varied by raising microvascular pressure. The product designates a solute clearance, the apparent volume of plasma which transfers its content of the solute to lymph in a given time. Total protein clearances (LR_T) for the 5 heat-injured paws in which L was increased by venous congestion are shown in Fig. 3, before and during this procedure. Graphs for D-110 and for individual plasma proteins are qualitatively similar. Slopes of the lines connecting pairs of points are coupling coefficients, comparable to $1-\delta_f$, where δ_f is the solvent drag reflection coefficient of the blood-lymph barrier (8, 13). Observed slopes for heat-injured paws range from 0.4 to 1.0. For normal paws (expt. 12 and previously published experiments) coupling slopes lie between 0 and 0.2.

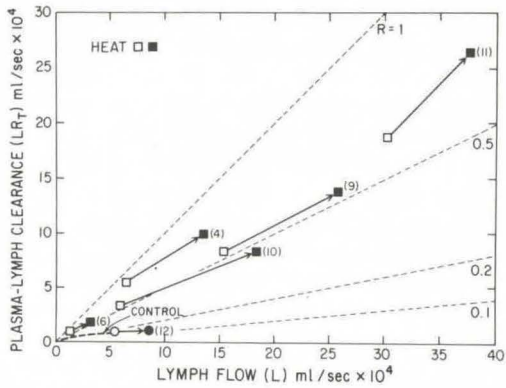


Fig. 3 Coupling of protein and volume flows into lymph during heat injury. The open squares show total protein clearances at spontaneous values of P_V, the closed squares at elevated P_V, for the five heated paws in which venous congestion increased lymph flow. Open and closed circles show the effect of P_V elevation on an unheated paw (Expt. numbers in parentheses refer to Table 1). The heavy dashed line near these two points represents the average relation for 8 normal paws from a previous publication (8). The lightly dashed lines indicate various proportionality coefficients

Discussion

Our observations are in agreement with the reports of others showing sustained increased in blood-lymph transport of macromolecular solutes in heat-injured tissues, and diminution of molecular-size selectivity of the blood-lymph barrier. Variability of temperature and time of onset, and occasional observation of abortive responses (Fig. 1B) are consistent with a triggered process, rather than a graded effect of temperature. The initial magnitudes of volume and solute fluxes after the response occurs must be many times larger than the increases in L and LR observed, since tissue volume is

expanding rapidly. However, the relatively steady levels attained after 1–1.5 hours may provide reasonable estimates of the composition of "injury filtrate" or interstitial fluid.

Reduced selectivity of macromolecular transport into lymph could be accounted for by one or more of the following processes: (a) an increase in the number and size of non-selective trans-endothelial leaks (b) increased turnover of endothelial cell vesicles (14) or (c) augmentation of vesicular transport by large vacuoles not normally present in endothelial cytoplasm (15). Since transport by turnover of individual vesicles or vacuoles is not a hydraulically conductive process (7), their activation by heat injury would not be expected to lead to an increase in the coupling of macromolecule transport to fluid flow. However, transport through trans-endothelial channels formed by fusion of vesicles or vacuoles, or by separation of intercellular tight junctions is consistent with our observation of substantially increased coupling coefficients (Fig. 3). Thus our observations are consistent with morphological observations of the latter kind of lesion in capillary and venular endothelium (3, 4, 5, 15). The fact that the coefficients were less than unity in four out of five cases indicates, however, that a substantial part of the fluid entering lymph during venous congestion moves through pathways maintaining some degree of molecular sieving.

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