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EFFECT OF ACUTE VENOUS HYPERTENSION ON ERYTHROCYTE, LEUKOCYTE, AND PLASMA PROTEIN EXTRAVASATION IN THE DOG HINDLIMB

P. Bryla, W. Piotrowicz, H. Galkowska, W.L. Olszewski

Department of Surgical Research and Transplantation, Medical Research Center, Polish Academy of Science, Warsaw, Poland

ABSTRACT

The effect of acute venous hypertension on the extravasation of plasma proteins, erythrocytes, and leukocytes into regional lymph was studied in the dog hindlimb. Although the lymph protein transport sharply rose with hindlimb phlebohypertension, capillary permeability was unchanged with retention in draining lymph of a normal proportion of large to small molecular weight proteins. Leukocyte transport also increased initially but then progressively decreased despite persistent venous hypertension. Lymph transport of erythrocytes was also high during venous hypertension but in contrast to white cells, it remained at that level as long as venous pressure was elevated. These findings suggest that different pathways and mechanisms are involved in the capillary extravasation of plasma proteins and cells capable of intrinsic motility (i.e., leukocytes) and those without independent motion (i.e., erythrocytes).

The flow and composition of peripheral lymph reflect changes in the net transcapillary transport of plasma proteins (1) and migratory cells (2,3) into the interstitial space. Increased microvascular permeability with inflammation is demonstrated by draining lymph with an augmented transport of proteins and blood-borne cells such as leukocytes and erythrocytes. "Stretching of pores" or capillary disruption facilitates greater extravasation of higher molecular weight proteins such as IgM and α-2-macroglobulins. Normally, these proteins are found in lymph at low concentrations but with inflammation the concentration of these proteins in draining lymph approaches that in plasma. Circulating immune cells such as granulocytes, monocytes, and lymphocytes attracted by chemotactic factors migrate to the site of inflammation at an increased rate. A portion of these cells can be found in lymph exiting the inflammed area. These migratory cells are also accompanied by cells without intrinsic motion such as erythrocytes. The mechanism of escape of passive cells from the blood compartment into the interstitium, however, is unclear. For example, if erythrocytes are forced through large gaps in the capillary membrane, plasma protein should also escape through these gaps in an unrestricted fashion and lymph protein concentration should rapidly reach the protein level of plasma. This occurrence, however, is never observed. Thus, in this study we examined the mechanism(s) responsible for different rates of capillary extravasation of plasma proteins, erythrocytes, and leukocytes in normal tissues during acute venous hypertension.

MATERIALS AND METHODS

Six mongrel dogs weighing 18-22kg were anesthesized with pentobarbital (30mg/kg). Combelen (0.5mg/kg) was also given for muscle relaxation. An afferent lymphatic paralleling the saphenous vein was cannulated for flow and pressure measurements and for determination of protein and cellular composition of regional lymph. The femoral vein was isolated for temporary occlusion. The paw and foot were placed in a plaster cast just above the ankle to prevent greater tissue compliance, as fluid and cells accumulated in the engorged interstitial space during the period of venous stasis.

Experimental setting

Dogs were kept upright. The observation time lasted 6 hours and was divided into 3 two-hour periods. During the first period, normal venous pressure was maintained; in the second, a clamp was placed on the femoral vein to elevate venous pressure in the foot; in the third, the clamp was removed and peripheral venous pressure was allowed to return to normal. During each experimental segment, after an hour equilibration, the leg was passively flexed and relaxed for 20 minutes with use of an external mechanical device. (Movement of the foot sharply increases distal venous pressure when the femoral vein is occluded as there is no alternative superficial venous pathway in the canine.)

Parameters measured

Venous pressure (Pv), lymph pressure (Pl), lymph flow (LF), total protein (TP), albumin (A) and globulin (G), IgG and IgM concentrations and the amount transported in lymph were measured. Erythrocyte (E) and leukocyte (L) concentrations and the amount transported in lymph were also determined.

Statistical evaluation

Results were expressed as means ± SD. For evaluation of differences between the mean, student t test for pairs was used. The following ratios were calculated; A/G, IgM/IgG output, E/L output. Correlations were determined between E output and LF, L transport and LF, TP and E transport, and L and E transport.

RESULTS

Venous pressure, lymph pressure, and flow

Venous pressure with the dog standing quietly was 29.0±6.0mmHg and oscillated during foot movements between 23.0±4.7 and 37.0±5.6mmHg. With femoral vein occlusion ("venous stasis"), this pressure rose to 67.0±21.8mmHg. With passive flexion of the foot, it increased further to 83.7±17.5mmHg and decreased during extension to 60.5±15.5mmHg. With declamping, venous pressure returned promptly to control values.

Pl during rest was 10.2±7.7mmHg and increased during passive movement to 110.0±74.0mmHg ($p<0.05$) *(Fig. 1)*. With venous "stasis" resting Pl increased to 31.7±13.8mmHg (Pl resting with venous stasis vs. resting with free venous flow ($p<0.05$). With passive hindlimb movement Pl reached 177.5±119.3mmHg (Pl movement with venous stasis vs. movement with free venous flow $p<0.05$).

LF was 6.3±5.2μl/min in the initial resting period, increased during hindlimb movement to 160.0±136.0μl/min ($p<0.01$) *(Fig. 1)*. In the resting phase of venous stasis LF was 18.4±11.2μl/min, significantly higher than in the control resting period ($p<0.05$). Foot movement raised LF during venous stasis to 207.0± 189.1μl/min, significantly above the level of LF observed during foot movement with uninterrupted venous flow ($p<0.05$). After restoring free venous flow, Pl and LF returned to normal values.

A/G ratio, TP, IgG and IgM lymph transport

The A/G ratio decreased from 0.53±0.04 to 0.49±0.01 during foot move-

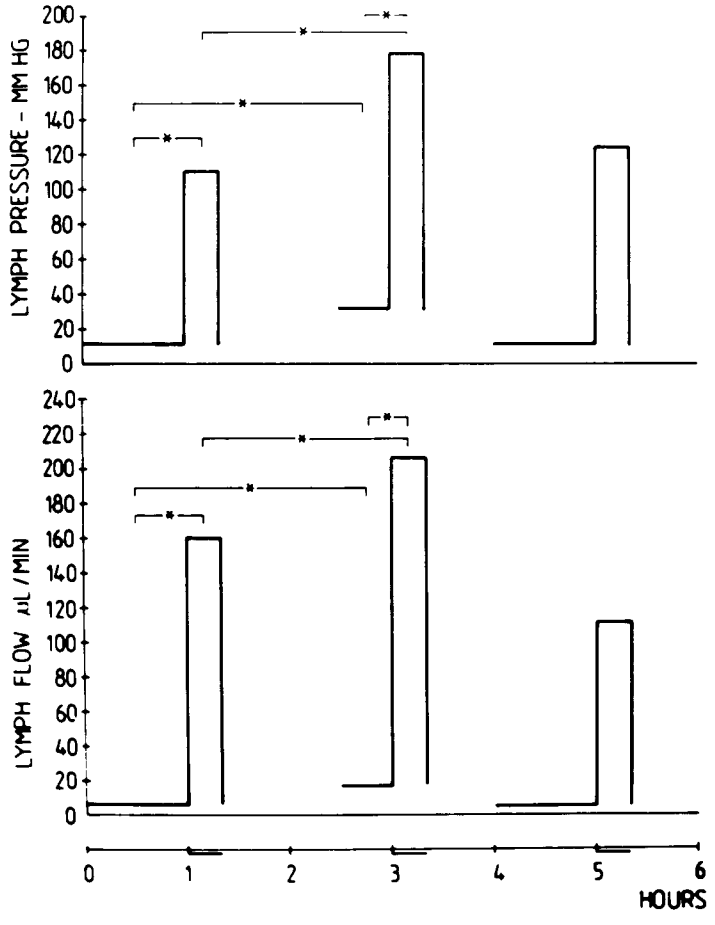

Fig. 1. Lymph pressure and flow in dog foot lymphatic with venous hypertension, 0-2h normal pressure, 2-4h femoral vein occluded, 4-6h venous clamp removed. At 1, 3 and 5h leg was moved passively for a period of 20min. Values are means of 6 experiments ($p<0.05$).

ment. During venous stasis it was 0.56±0.12 and 0.54±0.24, respectively. After restoration of uninterrupted venous flow the A/G ratio rose to 0.67±0.33 and 0.59±0.24, respectively; however, the differences were not statistically significant.

TP lymph transport rose from the initial 0.14±0.12mg/min to 2.24±2.14mg/min with foot movement. During venous stasis it rose from 0.24±0.2mg/min to 2.5±2.4mg/min. There was a statistically significant difference between TP transport in the resting period without and with venous stasis ($p<0.05$). TP transport increased during foot movement both with and without venous stasis by a mean factor of 16.

The lymph transport of IgG increased during foot movement over the resting value by a mean factor of 36.9, decreased during the resting period of venous stasis to 3.8 and rose again during hindlimb movement to 36.9. The output of IgM increased by 13.5, 1.5, and 20-times, respectively.

The calculated IgM/IgG ratio in the comparable experimental periods was 0.15, 0.083, 0.05, 0.09, 0.17, and 0.07. The decreased ratio during the periods of increased lymph flow was due to the higher

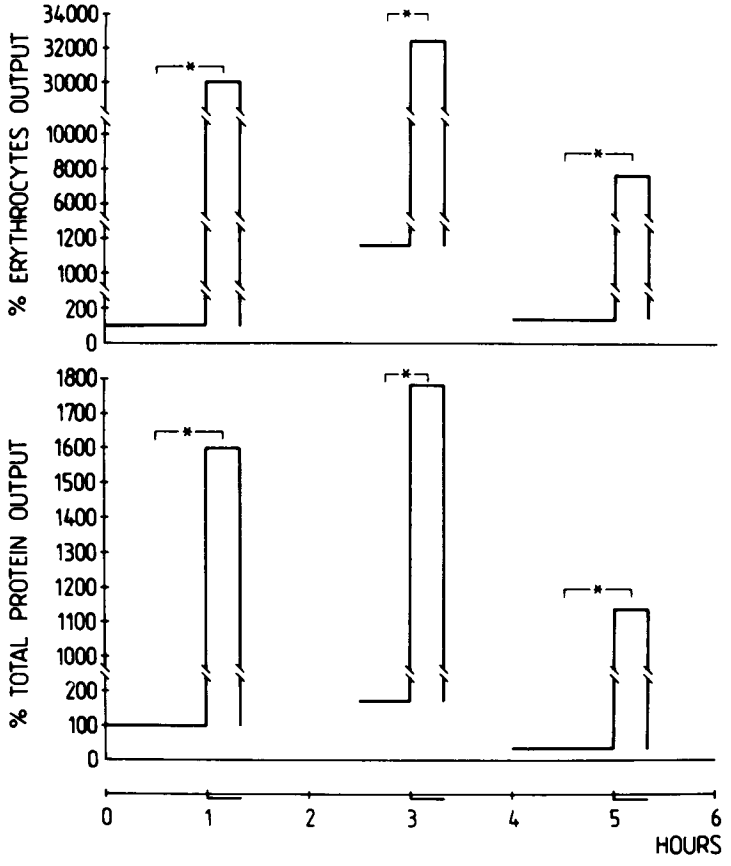

Fig. 2. Erythrocyte and protein transport from dog foot lymphatic with venous hypertension. For details see Fig. 1.

transport of IgG over IgM.

Erythrocyte (E) and leukocyte (L) output

The E transport in lymph rose from 1.9±2.2x10^3 cells/min to 570±701x10^3 cell/min (x300) during foot movements *(Fig. 2)* and decreased during the resting phase of venous stasis to 21.5±30.5x10^3 cells/min (x11.5), followed by a rise during subsequent movements to 613±988x10^3 cells/min (x323). After restoration of venous flow the E transport was 4.53±8.13x10^3 cells/min (x2.38) and during foot movement 338.2±702.4x10^3 cells/min (x178). Leukocyte output was respectively 0.2±0.17x10^3 cells/min, 46±23.7x10^3 (x230), 0.36±0.12x10^3 (x1.8), and 34.3±18.9x10^3 (x171) *(Fig. 3)*. Release of the venous clamp was followed by a further drop to 0.08±0.046x10^3 cells/min (x0.04) and during foot movement was 7.2±3.9x10^3 cell/min (x34.2). The calculated mean E/L transport ratio was 9.9, 12.4, 60, 17.9, 56.5, and 46.9, respectively. The correlation coefficient of the E and L transports was $r=0.799$ ($p<0.001$).

Cellular transport and lymph flow

Leukocyte transport increased during the consecutive periods of the experiment by factors of 230, 1.8, 171, 0.3, and 40, whereas lymph flow rose 25.3, 2.9, 32.8, 0.71, and 17.6 times, respectively. There was poor correlation between the changes in leukocyte transport and lymph flow ($r=0.444$; $p<0.05$). Erythrocyte output rose by a factor of 300, 11.5, 323, 2.6, and 204. The correlation coefficient with

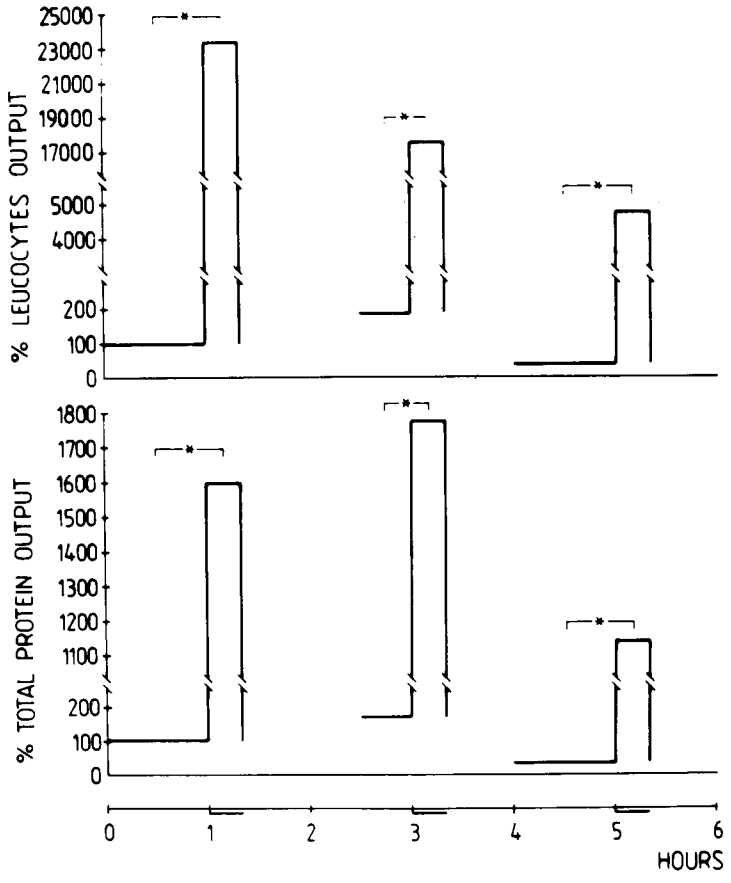

Fig. 3. Leukocyte and protein transport from dog foot lymphatic with venous hypertension. For details see Fig. 1. *Note a progressive decrease in white cell output ($p < 0.05$).*

lymph flow changes was $r = 0.590$ ($p < 0.001$)*(Fig. 4)*.

Total protein and erythrocyte transport

TP transport increased during consecutive periods of the experiment 16, 1.7, 17.8, 0.35, and 11.4 times and the transport of erythrocytes was 300, 11.5, 323, 2.6, and 204 times, respectively. The correlation coefficient of TP to erythrocyte transport was $r = 0.962$ ($p < 0.001$)*(Fig. 5)*.

DISCUSSION

The main question raised in this study was whether increased microvascular hydrostatic pressure during acute venous hypertension increased permeability of the normal capillary membrane in terms of "stretching pores", (i.e., widening of the interendothelial gaps) and/or accelerated vesicular transport. Had this occurred, there would have been almost unrestricted influx of plasma proteins and cells into the tissue space. This microcirculatory sequence of events occurs with inflammation but not at the sites of tumor growth where abundance of host leukocytes and immune proteins may be desirable. Designing a method to augment extravasation of these host constituents

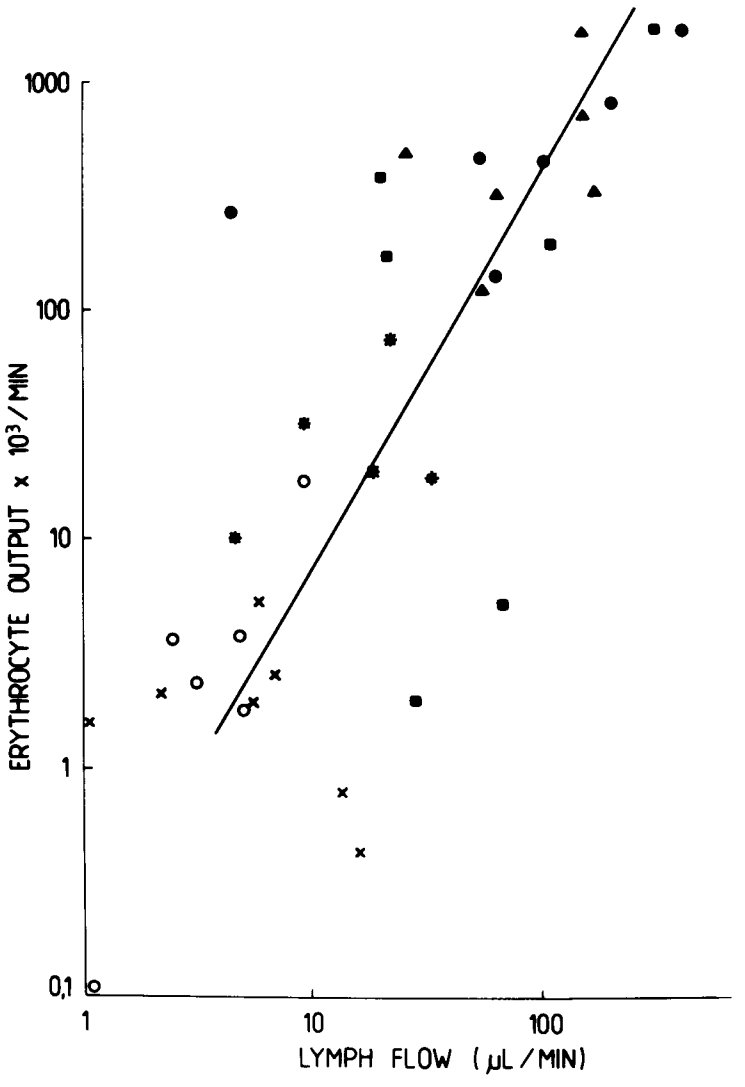

Fig. 4. Erythrocyte output plotted against lymph flow during rest (x) and movements (▲) in the period of normal venous pressure, rest (*) and movements (●) during venous hypertension and rest (o) and movements (■) after restoration of venous outflow, n = 6, r = 0.590, p < 0.001.

into noninflammed tissues therefore is of considerable practical usefulness. Raising local venous and hence capillary hydrostatic pressure is one possible method. However, as shown in this study, whereas acute venous hypertension promotes a modest increase in tissue extravasation of plasma proteins, leukocytes, and erythrocytes, capillary permeability is unaltered. Moreover, the pathways and kinetics of transcapillary migration of plasma proteins, leukocytes and erythrocytes are apparently different and complex during venous hypertension. Thus, we found an increase in total protein lymph transport but no change in the lymph A/G ratio; the lymph transport of IgG exceeded that of IgM; erythrocyte but not leukocyte transport correlated with lymph flow while changes in erythrocyte output corre-

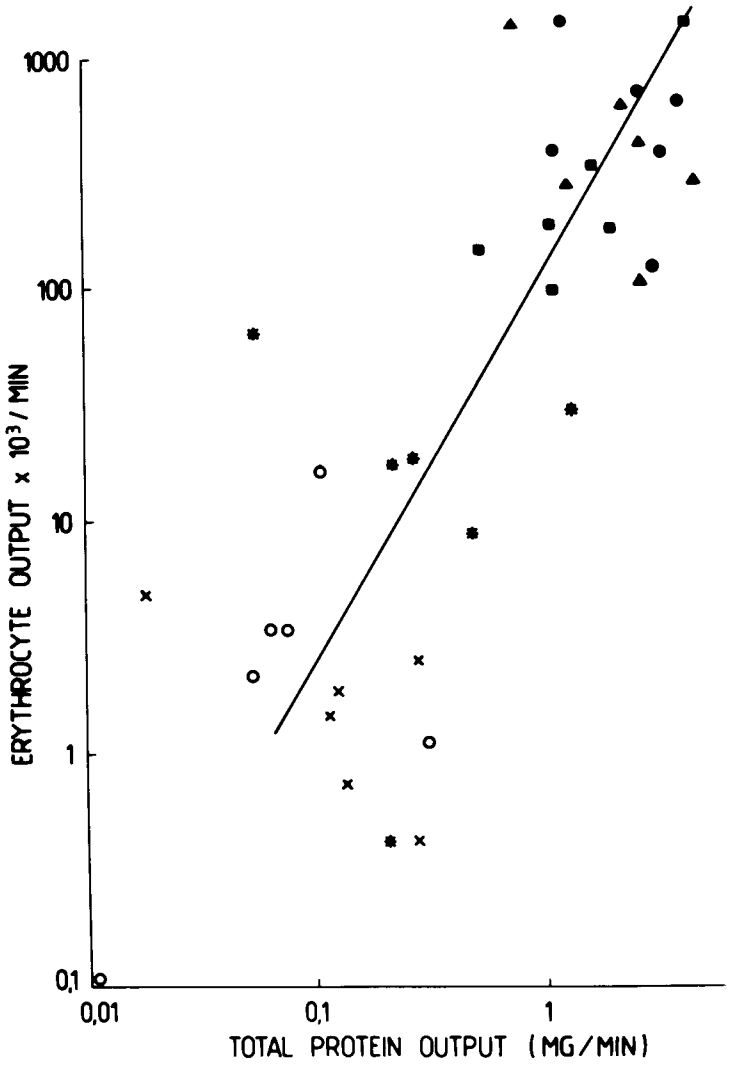

Fig. 5. Erythrocyte output plotted against protein output, n=6, r=0.962, p<0.001. For details see Fig. 4.

lated with those of total protein transport.

A 2-3 fold increase in venous pressure brought about a mean 16-fold rise in net total protein transport in lymph, a finding which is supported by the results of others (4). As expected, the concentration of lymph proteins decreased. The question remains, however, whether the total volume of proteins or their concentration in tissues is key for immune homeostasis. The augmented protein transport in lymph is not the result of enhanced microvascular permeability, but rather greater microvascular exchange surface area, that is, an increased capillary radius with opening of previously closed capillaries. With "stretched pore", high venous pressure would be expected to force across the capillary membrane proportionally greater amounts of larger molecular weight proteins such as IgM than smaller weight molecules such as IgG or albumin. But this did not occur with "molecular sieving" persisting in lymph despite heightened microvascular hydrostatic pressure with venous obstruction. Lack of increased permeability of capillaries to macromolecules in venous hypertension

has been also found by others (5-8). A combination of heightened venous pressure with intraarterial histamine infusion (8), however, results in a great influx of macromolecules into tissue fluid and lymph. This finding suggests that it is not mechanical "stretching of pores" but rather chemically regulated contractions and relaxations of endothelial cells that regulate the macromolecular transport across the capillary membrane.

A different pattern of transcapillary transport emerged with examination of migrating and non-migrating blood cells (i.e., leukocytes and erythrocytes). Leukocytes (approximately 80% of them being lymphocytes) displayed an increased extravasation with venous hypertension and muscular contraction at first and a gradual diminution in transcapillary passage with time. Probably both intracapillary trapped and extravasated pools of leukocytes became gradually diluted by an increased volume of capillary filtrate and tissue fluid. Thus, two factors, intrinsic migratory properties and solvent drag were involved in augmenting lymph leukocyte transport. Somewhat different findings had been observed in human subjects (2). In these individuals, the output of lymphocytes did not depend on the net capillary filtration. Only after leg muscular contractions promoted a transient increased in lymph flow with a high number of lymphocytes did a prolonged resting period result in accumulation of lymphocytes in the interstitium.

Another transcapillary pattern of transport occurred with erythrocytes, where transcapillary migration was directly related to the rate of lymph flow. Although it may be assumed that hydrostatic pressure was responsible for erythrocyte extravasation, the exact microvascular locus of this red cell leakage was not defined. Lymphocytes and granulocytes probably traversed capillaries through the interendothelial junctions and likely also depended on active motion of the white cells. This process is in distinct contrast with erythrocytes, cells not possessing intrinsic motility. Thus, erythrocytes leave the capillary either along the pathways "opened" by migrating lymphocytes or leaked via sporadically damaged capillaries. A third possibility is extravasation of red cells from the *vasa vasorum* of lymphatic collectors directly into the lumen of a lymph vessel. If the first two possibilities are valid, plasma would likely leak in bulk and flood the tissue space. Moreover, protein concentration in lymph would tend to high values and the proportion of high to low molecular weight proteins in lymph would approach that of plasma. This scenario, however, is not observed. Whereas there is a direct correlation between E and TP transport, the rate of changes of protein transport is much less than that of E transport. The appearance of erythrocytes in leg lymph is a physiologic phenomenon with small numbers invariably detectable in peripheral lymph (2). With moderate exercise (e.g., walking) there is a large increase in erythrocyte concentration in peripheral lymph in healthy humans (2). A high erythrocyte count in thoracic duct lymph was also observed by El-Gendi (10) in patients with portal hypertension in schistostomiasis. Extravasation of these red blood cells correlated with increased capillary filtration rate as reflected in augmented central lymph flow. Taken together, these data suggest that there are different pathways for transport of plasma proteins, erythrocytes, and leukocytes from blood capillaries into the tissue space under the conditions of acute venous hypertension. Capillary permeability for plasma proteins is not increased despite a greater transport of lymph protein. Leukocyte transport into lymph increases sharply at first but then gradually decreases. The concentration and lymph transport of erythrocytes, on the other hand, increases with acute venous hypertension but remains elevated as long as venous pressure is high.

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W.L. Olszewski, M.D.
Department of Surgical Research and Transplantation
Medical Research Center
Polish Academy of Sciences
02004 Warsaw
5 Chalubinski, POLAND