Lymphology 14 (1981) 161–166

Lymphatic Clearance during Compressive Loading*

G.E. Miller and J. Seale

Bioengineering Program Texas A&M University, College Station, Texas 77843

Summary

Lymphatic clearance of radioactive sulfur colloid is measured as a function of externally applied pressure on the hind limb of mongrel dogs. A dead weight device is placed over the site of subcutaneous injection. A solid state Si (Li) is placed into a a slot at the bottom of the device to continuously record activity of the tracer. An exponential decrease in activity is modeled as a dual decay resulting from both tracer half life decay and lymphatic clearance of the tagged sulfur colloid. External pressure is seen to enhance lymph clearance until a critical closing pressure is reached, whereupon the vessel collapses and lymph flow is drastically reduced. A closing pressure of 60 mmHg is observed for several experiments. Lymph flow per tissue volume is seen to rise from a mean of 0.324 ml/hr/ml for uncompressed tissue to 0.96 ml/hr/ml for fully enhanced flow in other experiments at 60 mmHg. Results at a pressure of 75 mmHg show almost no lymph clearance suggesting complete vessel closure.

Introduction

The role of the microcirculation in the progression of decubitus ulcers (bedsores) has been studied for many years. The effects of compressive forces on the immobilized tissue of bedridden or wheel-chair bound patients are to disrupt capillary blood flow and obstruct contractile lymph flow. The consequences of the former are a loss of nutrients to the compressed tissue. The consequences of the latter are an accumulation of metabolic waste products in the compressed region. Both factors can lead to tissue necrosis.

Many investigations have been conducted into the restriction of capillary flow with little or no effort into the study of lymphatic effects. Recently, *Miller* et al. (1) recorded the lymphatic clearance of a radioactive sulfur colloid in uncompressed tissue in a dogs hind limb. The present work extends these experiments to measure the effect of compressive forces on terminal lymphatic function.

Methods and Materials

Thirty-one experiments were conducted with mongrel dogs each weighing approximately 20 kg. Anesthesia was induced by injection of sodium pentabarbitol 25 mg/kg in the cephalic vein. A 1 ml bolus of sulfar colloid tagged with Technetium 99m (Tesuloid, ER Squibb & Sons) was injected subcutaneously in the upper thigh of a hind limb with the animal in lateral recumbancy. Bolus actiyity averaged 500 μCi. The detection system used to monitor the rate of clearance of the tagged sulfur colloid from the injection site consisted of a Si (Li) solid state detector shielded by 3.2 mm of lead except of a 6.4 mm diameter hole at the front face. The detector is placed directly over the injection site so that any minor movement of the animal did not change the radioactive source-detector solid angle. Similarly, to avoid motion artifacts, anesthesia of the animal during the experimental procedure must be maintained. The system electronics have been described in a previous study (1).

Lymph clearance data measured in this manner compared favorably with those of other studies (2–6). The use of radioactive tracers has the advantage of being noninvasive to the lymph vessels. Thus, lymph flow is not disturbed by the measurement technique. A dis-

*Supported by RSA Grant 23-P-5788-6

0024–7766/81 1600–0161 $ 02.00

cussion of the validity of the tracer system in the measurement of lymph clearance appears in *Miller* et al. (1).

Radioactivity at the injection site was assumed to decay from two sources: the natural decay of the Technetium (with a half life of six hours), and the clearance of the tagged sulfur colloid by the terminal lymphatics. A decay model was developed similar to that of *Daly* (12),

$$N(t) = N_0 e^{-[\frac{F}{V} + \frac{\ln 2}{T^1/_2}]t}$$

where N is the number of radioactive counts at time t (measured from a count rate meter), N_0 is the initial count rate, $\frac{F}{V}$ is the lymphatic clearance flow rate per unit tissue fluid volume, $T^1/_2$ is the radioactive half life, and t is time. N(t) is measured at frequent time intervals (10–15 minutes). Since $T^1/_2$ is known, the data can be fit to a curve approximating the above equation and $\frac{F}{V}$ can be computed. Thus, lymph flow may be calculated from the rate of tracer clearance. Previous studies on uncompressed tissue have shown a 99% correlation to the above model. Use of the model assumes tissue fluid volume to be constant with time.

In order to assess the effects of compressive loading, pressure is applied to the tagged tissue area by a simple dead weight device (Fig. 1). This device is cylindrical in shape and is set onto the skin with a known area of contact. The weight of the device is varied by adding lead shot to the volume provided within the device. Since the weight and area are known, the applied pressure can be calculated. The cylinder end which is in contact with the skin is covered with clear plastic. This is slotted to allow placement of the radioactive detector above the plastic disk.

Lymph clearance rates were calculated at various applied pressures according to the method described above. Pressure was arbitrarily raised in 15 mmHg increments until lymph clearance was seen to disappear, indicating complete closure of the terminal lymphatics. This was noted when the measured decay rate equaled that of the radioactive half life

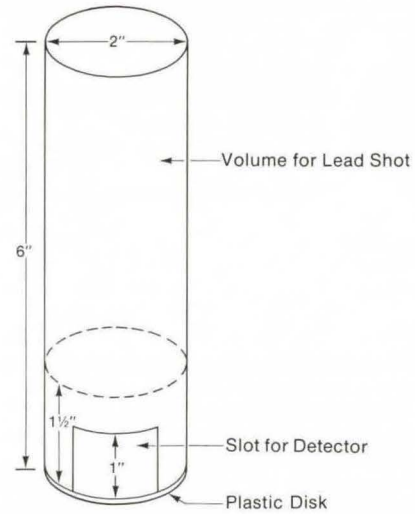

Fig. 1 Device for applying pressure to experimental site. Radioactive detector is placed in slot. Lead shot is inserted into chamber above to vary applied pressure

alone ($\frac{\ln 2}{T^1/_2}$). Several experiments were conducted at each pressure to verify the results.

Results

The continuous radioactive counts per minute over the injection site are plotted for all animals in Figs. 2A through 2E. Each curve may be plotted on a semilog basis to obtain ln N as a function of time. Such curves are approximately linear. The slope of each line, as computed by a least squares analysis of the data, represents the total decay ($\frac{F}{V} + \frac{\ln 2}{T^1/_2}$). Tabulated data and computed slopes for all animals are listed in Table 1. The latter term in the parenthesis above is 0.1155 hr^{-1} for a half life of six hours. The resulting lymph flow rate per tissue fluid volume can then be computed by subtracting this term from the total decay constant. The latter is obtained from the least squares fit of experimental data. The procedure for sample data is shown in Table 1.

Tab. 1 Specific lymph flow rate (ml/hr/ml) as a function of applied pressure. Experimental data for canine head hind limbs

Animal	Applied pressure (mmHg) 0	30	45	60	75
1	0.151	0.299	1.090	1.080	0.0227
2	0.166	0.424	0.413	0.190	0.0450
3	0.157	0.275	0.436	0.180	0.0385
4	0.296	0.533	0.367	0.980	
4	0.340	0.278	0.453	0.830	
6	0.223		0.256	0.250	
5	0.217		0.344		
8	0.785				
9	0.342				
10	0.560				
avg	0.324	0.362	0.480	0.585	0.0326

Least squares analysis of sample data

correlation	total decay (hr^{-1})	half life (hr^{-1})	Flow (ml/hr/ml)
0.993	0.251	0.1155	0.135
0.999	0.315	0.1155	0.199
0.992	0.257	0.1155	0.141
0.993	0.432	0.1155	0.316
0.991	0.438	0.1155	0.322

(Sample of preliminary data for unpressurized tissue)

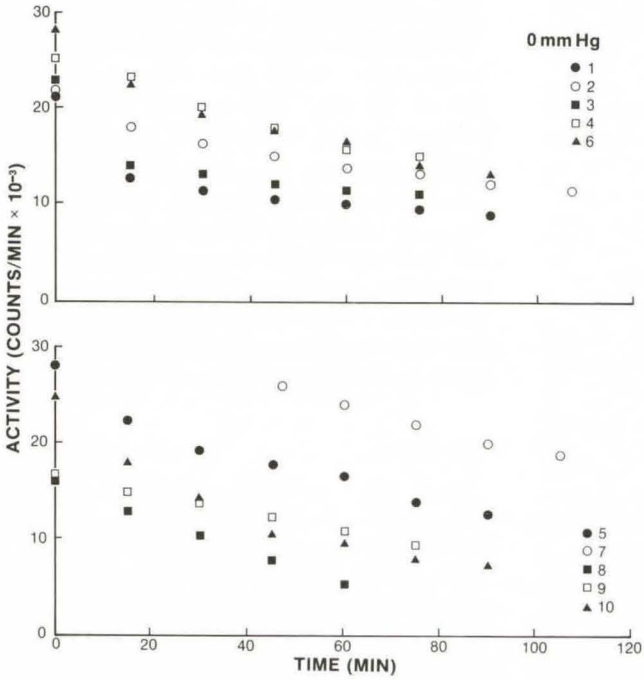

Fig. 2a P = 0 mmHg

Fig. 2 Total radioactive decay at injection site versus time for several externally applied pressures. Decay is due to half life decay and lymphatic clearance of tracer.

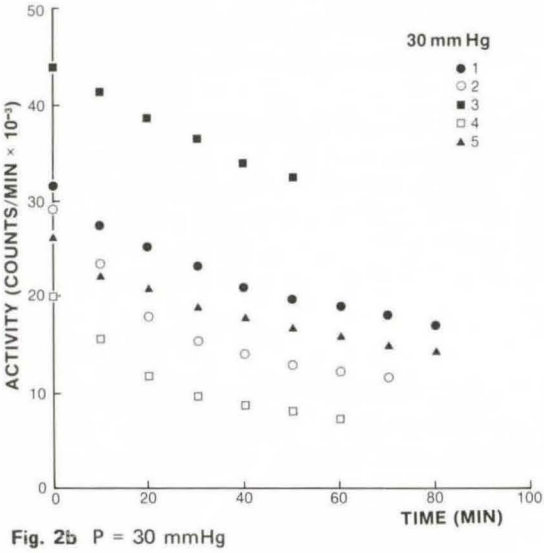

Fig. 2b P = 30 mmHg

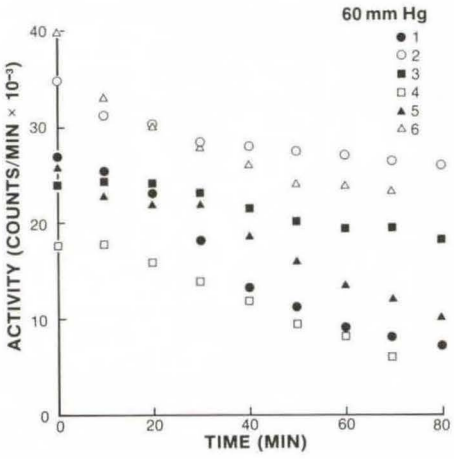

Fig. 2d P = 60 mmHg

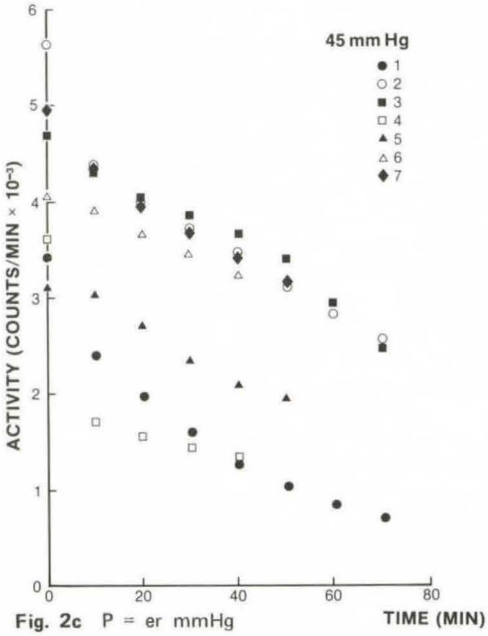

Fig. 2c P = er mmHg

Scintigrams of the radioactivity distribution in the vicinity of the injection site were taken at two hour intervals following injection. There is an initial slight amount of diffusion, due to the weight of the detector and dead weight device, but little bulk movement beyond that point. These results are similar to those seen earlier by *Miller* et al. (1). As with previous studies, a second scintillation detector placed over the carotid artery showed no increase in activity following injection of the tracer. A scintigram of the liver taken at the conclusion of the experiment showed no appreciable activity. Thus, no capillary clearance of the tracer was indicated.

Lymph clearance as a function of applied pressure is plotted in Fig. 3. There is a nonlinear rise in lymph flow with pressure until a critical closing pressure is reached. At this point, the flow ceases. The cessation of flow was noted on the raw experiment data by the total tracer decay equalling the half life decay with no lymph flow decay components.

Discussion

Lymph clearance flow rates per tissue fluid volume computed for the uncompressed animals varied from 0.252 ml/hr/ml to 0.785 ml/hr/ml with a mean flow rate of 0.324 ± 0.147 ml/hr/ml. Similar studies by *Zoltan* et al. (6) measured lymphatic clearance of a colloid tagged with ^{131}I that had been injected subcutaneously in the gluteal region. A mean lymph clearance flow rate per unit

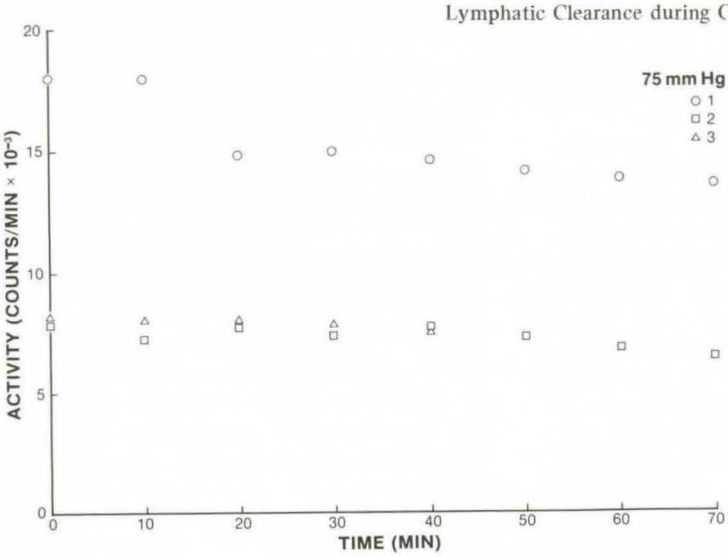

Fig. 2e P + 75 mmHg

volume was reported as 0.2195 ml/hr/ml which agrees with results presented in this study. Lymph clearance data at a pressure of 30 mmHg varied from 0.278 to 0.533 ml/hr/ml with an average of 0.362 ± 0.093 ml/hr/ml. Lymph clearance at an applied pressure of 45 mmHg varied from 0.256 to 1.09 ml/hr/ml with a mean value of 0.480 ± 0.174 ml/hr/ml.

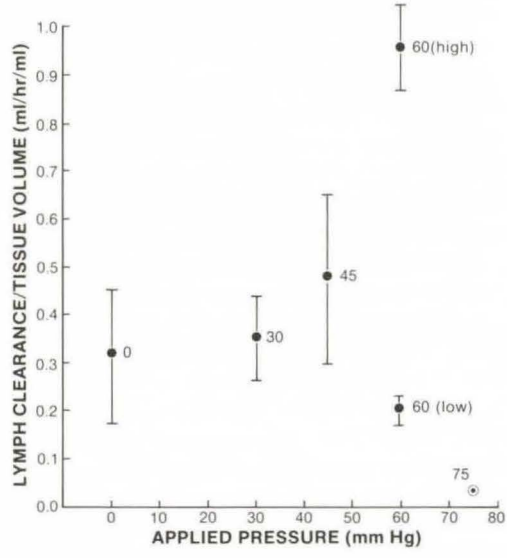

Fig. 3 Lymph flow per unit tissue volume as a function of applied pressure. Lymph flow increases with pressure until a critical closing pressure is reached. Some data at 60 mmHg shows vessel closure (low flow) and some shows enhanced flow, indicating that the critical closing pressure may be at or near 60 mmHg

Clearance data at an applied pressure of 60 mmHg varied from 0.180 to 1.08 ml/hr/ml with a mean value of 0.585 ± 0.378 ml/hr/ml. However, this group of data could obviously be divided into two groups. One group had relatively large clearance rates (1.08, 0.98, 0.83) and the other group had much smaller clearance rates (0.19, 0.18, 0.25). The average flow rate of the larger flow group is 0.96 ± 0.09 ml/hr/ml while that of the smaller group is 0.207 ± 0.029 ml/hr/ml. Thus, there is much better agreement of data by subgroup (large flow, small flow) than there was by observing the entire data set for all 60 mmHg experiments. Clearance data at an applied pressure 75 mmHg shows very little decay with an average value of 0.0326 ml/hr/ml.

As can be seen in Fig. 3, there is an increase in lymphatic clearance with increasing pressure. Terminal lymphatics are vessels with open pores. Increasing the external pressure would create a greater translymphatic pressure drop, thus promoting increasing flow into the lymphatics. As pressure increases, so does flow, until the external pressure collapes the vessel. The data in Fig. 3 shows a nonlinear and rapid rise in lymph flow per unit tissue volume as the applied pressure increases. At an applied pressure of 60 mmHg, some animals show relatively large clearance levels indicating enhanced lymph flow. Others show reduces lymph flow indicating the onset of vessel closure. At an applied pressure of 75 mmHg, all clearance

data is very small, suggesting almost complete vessel closure in all of these cases.

There are certain limitations to these results. The model does presume tissue fluid volumes to be constant. Also, changes in lymph clearance per unit tissue fluid volume as a function of pressure were attributed to changes in flow, not in volume. Obviously, a decrease in fluid volume would also increase the flow per volume (F/V) term which is calculated from experimental data. However, the level of pressure which would cause lymphatic vessel collapse would not be affected. Thus, the hypothesis that an externally applied pressure of 60 mmHg can initiate lymph vessel closure appears to be justified. It has not been proven that collecting lymphatics rather than terminal vessels collapse in response to the applied pressure. However, it is more likely that the terminal lymphatics would collapse before collecting vessels as they are smaller and closer to the site of pressure application. The role of diffusion of tissue fluid and the subsequent alteration of fluid volume would only affect the flow data, and not necessarily the onset of vessel closure. Furthermore, the level of tracer diffusion, as seen on the scintigrams previously described, is minimal (1). Thus, the role of diffusion and tissue fluid volume changes as a factor in the F/V relation to applied pressure appears to be minimal. Further studies are being undertaken to determine the relative effects of diffusion. The hypotheses of of the present study result from assumptions of negligable fluid volume changes.

The determination of a critical closing pressure for terminal lymphatic vessels is two fold. Many wheelchair pad evaluation techniques measure applied pressures in order to maintain peak pressures below a critical value. Due to the role of lymphatic function in decubitus ulcer formation, a critical closing pressure for lymphatics should be incorporated into critical values in pad evaluations. Secondly, patient therapy is designed to temporarily relieve external pressure in order to restore capillary function. This is often done with patient push ups. New therapy techniques may need to incorporate lymphatic function restoration into the protocol.

References

1 *Miller, G.E., J.B. Smathers, J.Seal, D. Hightower, D. Hood:* Lymphatic Clearance of Radioactive Sulfur Colloid. Lymphology 13 (1980) 24–29
2 *Olszewski, W.L.:* Collection and physiological measurements of peripheral lymph and interstitial fluid in man. Lymphology 10 (1977) 137–145
3 *Szabo, G.:* Pressure and flow in the lymphatic system. Progress in Lymphology, Hefner Publishing New York (1955) 365–367
4 *Joyner, W.L., R.D. Carter, E.M. Renkin:* Influence of lymph flow rate on concentrations of protein and dextran in dog leg lymph. Lymphology 6 (1973) 181–186
5 *Winkel, K., zum, P. Schenck:* Radioisotope investigation of the lymphatic system. Progress in Lymphology, Hefner Publishing, New York (1966) 84–85
6 *Zolton, O.T., J. Fischer, I. Juvancz, M. Foldi:* Studies on the absorption of ^{131}I-Albumin and K^{131}I from the subcutaneous tissues of the dog. Acta Physiol. 20 (1961) 361–372

G.E. Miller, Bioengineering Program Texas A&M University, College Station, Texas 77843