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Lymphatic Clearance of Radioactive Sulfur Colloid

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Summary

Lymphatic clearance of radioactive sulfur colloid is measured in the hind limb of five mongrel dogs. A solid state Si(Li) detector is placed onto the skin to continuously record activity over the site of subcutaneous injection. Decrease in activity follows an exponential decay which is modeled as a dual decay occurring from both the radioactive decay of the tracer and the lymph clearance of the tagged sulfur colloid. The calculated decay constants for lymph clearance flow per tissue volume result in a mean value of 0.233 ± 0.077 ml/hr/ml which is consistent with results of other investigators. Adjacent lymph nodes are monitored with a scintillation detector to show that the colloid is absorbed by the lymph vessels. The carotid artery and liver are similarly monitored to show that there is little or no capillary absorption of the tagged colloid.

Introduction

Studies of the etiology of decubitus ulcers have resulted in the hypotheses that secondary effects of compressive forces on the immobilized tissue of spinal cord injured patients are twofold: (1) a disruption of capillary blood flow followed by a loss of nutrient supply, particularly oxygen, to the surrounding tissue; and (2) obstruction of contractile lymph flow followed by an accumulation of metabolic waste products (1). Several investigators have shown that external pressure leads to vascular ischemia as a direct result of capillary restriction (2–5). This is then manifested by tissue necrosis. *Husain* (2) recorded that a threshold pressure of 100 mmHg had to exist for two hours to produce the first signs of typical decubitus tissue breakdown. He made reference to the lymphatic system, noting that during edema, poor lymph return and possible lymphatic clogging are associated with decubitus ulcer formation.

Pressure gradients maintain terminal lymphatic drainage into larger contractile lymphatics which have the capability of actively transporting fluid materials against pressures as high as 60 mmHg (6). The inward permeability of the terminal lymphatic to metabolites increases with mechanical or chemical irritation (6). Necrosis only occurs when surface pressures are applied that are sufficient to occlude contractile lymphatic drainage or to block oxygenation of lymphatic smooth muscle for periods exhausting their anaerobic capabilities (7). Under these circumstances, toxic amounts of metabolites probably occupy the intercellular space, and thus, the tissue poisons itself.

The correlation between obstructed lymphatic function and decubitus ulcer formation is further affirmed when one considers that of all forms of edema, it is only in lymphedema that there is a tendency to develop tissue necrosis similar to that seen in decubitus ulcers.

It therefore becomes necessary to assess the ability of normal terminal lymphatic vessels to remove local metabolic wastes before studying the factors involved in the restriction of lymph flow. Although previous studies have measured lymph flow in single vessels (8–10), the aim of this study is to determine the clearance of tagged substances in a small finite region so as to apply the results to future studies of restricted lymphatic function under compressive loading. The use of radioactive tagged colloids to assess lymphatic function has been previously demonstrated (11).

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Methods and Materials

The investigations were conducted with five mongrel dogs each weighing approximately 16 kg. Anesthesia was induced by injection of sodium pentabarbitol (25 $\frac{mg}{kg}$) in the cephalic vein. A bolus of sulfur colloid tagged with Technetium 99 mm (Tesuloid, ER Squibb & Sons) was injected subcutanously in the upper thigh of a hind limb with the animal in lateral recumbancy.

A 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 for a 6.4 mm diameter hole at the front face. A charged sensitive preamplifier and a spectroscopy amplifier were used for signal amplification. This signal then passed through a single channel analyzer with the lower level set to eliminate electronic noise. The signals were then routed to a count rate meter and the output of the count rate meter to a recorder for a permanent record of the data.

The Si(Li) detector was used because it was small enough to be taped directly to the dog with the 6.4 mm D. Hole directly over the injection site. Any minor movement of the dog under these conditions did not change the radioactive source-detector solid angle and thus the detection efficiency. Earlier efforts using a Na I(TL) probe set several centimeters from the dog suffered from solid angle changes with movement and thus gave erratic results. The problem of animal motion was further reduced with the use of anesthesia.

A similar detector was placed over the neck of the animal to monitor radioactivity above the carotid artery. An increase in radioactivity above background noise shortly after injection would indicate significant capillary clearance of the tagged sulfur colloid. The tagged colloid would be propelled through the circulation until it was detected at the carotid artery.

The injection area was also followed using a "gamma camera" scintillation detector (GE Portacamera II). The activity distributions were measured immediately after injection and at the end of the data recording. Because of the shielding properties of the Si(Li) detector, it was not possible to accurately measure distributions during the data recording time interval.

At the conclusion of the experiment, the activity distribution in the region of the liver was measured. Accumulation of radioactive sulfur colloid would appear in the liver if significant blood clearance of the tagged material were involved. A distinct outline of the liver would be noticeable.

The radioactivity of the injection site was assumed to decay from two sources: The natural decay of Technetium (with a half life of six hours), and the clearance of the tracer as the sulfur colloid traveled out of the injection area by way of the terminal lymphatics. The total decay was modeled by,

$$N(t) = N_o e^{-[\frac{F}{V} + \frac{1n\ 2}{T_{1/2}}]t}$$

where N is the number of counts at time t, N_o is the initial count rates, $\frac{F}{V}$ is the lymphatic clearance flow rate per unit tissue fluid volume, $T_{1/2}$ is the radioactive half life, and t is time. With the measurement of N(t), the lymphatic clearance flow rate per unit tissue fluid volume, as the only unknown, can then be calculated. This model assumes tissue fluid volume to be constant with time. Similar models have been utilized to assess radioactive tracer clearance in the microcirculation (12).

Results

The continuous radioactive counts per minute over the injection site are plotted for all five animals in Figure 1. Each curve may be plotted on a semilog basis to obtain 1n N as a function of time. Such curves are approximately linear. The slope of each line, as computed by a least square analysis of the data, represents the total decay ($\frac{F}{V} + \frac{1n\ 2}{T_{1/2}}$). The slopes for all five 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 can then be computed by subtracting this term from the total decay constant. The computed flow rates are listed in Table 1.

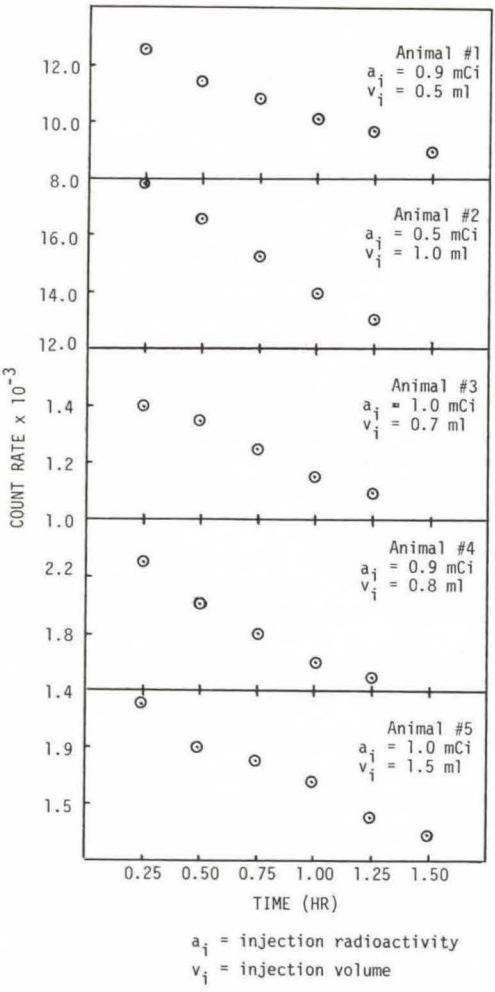

Fig. 1 Continuous radioactive counts per minute over the injection site for all animals

To confirm that the sulfur colloid is indeed removed by the lymph vessels, scintigrams of the radioactivity distribution in the vicinity of the injection site were taken at two hour intervals following injection. Figure 2 shows such a sequence of photographs. Figure 2a shows the activity over the injection site at the onset of the experiment. Figure 2b shows a slight uptake of the tagged sulfur colloid in neighboring lymph nodes two hours later, and Figure 2c shows a significant amount of tagged sulfur colloid in these nodes four hours after injection.

A similar scintigram of the liver taken four hours after injection shows no appreciable activity. Furthermore, a second scintillation probe placed over the head of an animal during the experiment shows little increase in activity. Both of these provide a qualitative measure of the slight level of capillary clearance of sulfur colloid.

Discussion

An assessment can be made of the validity of the decay model from the least squares analysis of the activity data listed in Table 1. All animal data shows a high correlation ($< .99$) to a linear relationship. The data relates ln N to time. Therefore, there is a high correlation to a true exponential relationship between the count rate, N and time. This verifies the form of the model.

The clearance flow rates per tissue fluid volume computed for each animal vary from 0.135 ml/hr/ml to 0.322 ml/hr/ml with a mean flow rate of 0.223 ± 0.077 ml/hr/ml. Similar

Table 1 Decay constants of least squares analysis

Animal	Total Decay (hr^{-1})	Half Life (hr^{-1})	F/V (ml/hr/ml)	Correlation Factor
1	0.251	0.1155	0.135	.993
2	0.315	0.1155	0.199	.999
3	0.257	0.1155	0.141	.992
4	0.432	0.1155	0.316	.993
5	0.438	0.1155	0.322	.991
Average	0.339 ± 0.077		0.233 ± 0.077	

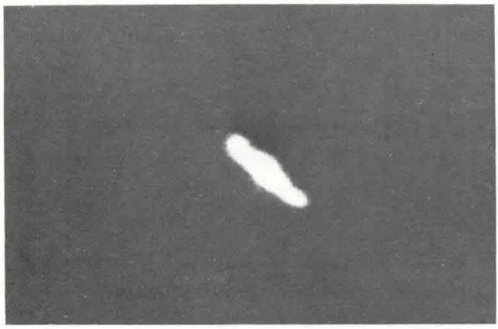

Fig. 2a Scintigram of injection site at onset of experiment. Diffusion causes spread of tagged colloid

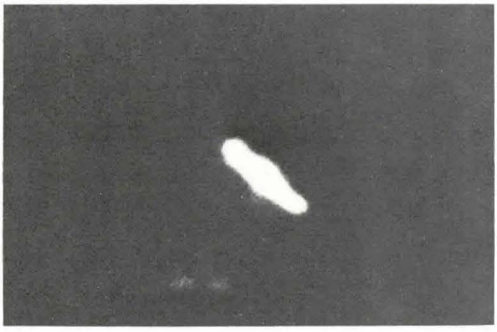

Fig. 2b Scintigram of injection site two hours after injection. Nearest lymph nodes begin to absorb radioactive colloid. Faint images of lymph nodes are below and slightly left of injection site

Fig. 2c Scintigram of injection area four hours after injection. Lymph nodes are clearly seen to have absorbed radioactive colloid

studies by *Zoltan* et al. (13) measured lymphatic clearance of a colloid tagged with ^{131}I that had been injected subcutaneously in the gluteal region. Lymph clearance per unit volume average 0.2195 ml/hr/ml which agrees with results presented in this study. Such results differ from those of *Jepson* et al. (14) and others who present higher values of lymph flow per unit volume (1.26 ml/hr/ml). However, *Foldi* (15) has analysed this descrepancy and has determined that higher values for lymph flow are artificially created by inaccurate measurement and analysis techniques. Therefore, it is assumed that the values for lymph clearance flow per unit tissue volume calculated in this study accurately represent the level of lymphatic clearance of macromolecules.

A comparison may be made between invasive and noninvasive techniques in measuring regional lymph flow. Although cannulation offers a direct means of measuring lymph flow, there is a possibility that the measurement system disturbs the flow of lymph. The noninvasive technique of scintillation counting of tagged particles should offer a more accurate quantization of flow, as the flow mechanisms are not affected by the measurement system. However, such a system cannot dissociate lymph flow rate from tissue fluid volume. Furthermore, there is no direct correlation between terminal lymphatic clearance of macromolecules and actual lymph flow. For this study, a measure of lymph clearance per unit volume is acceptable as the data is to be compared to similar results for compressively loaded tissue.

The effectiveness of sulfur colloid as an indicator of lymphatic function was seen in Figure 2, which showed the accumulation of Technitium tagged sulfur colloid in neighboring lymph nodes. This verifies that the injected material is absorbed by the surrounding lymph vessels. The appearance of this material in the nearest nodes several hours later supports the relatively small values for regional lymph flow. A measure of the relatively small level of capillary blood vessel absorption is seen in two figures. Figure 3 shows the liver four hours after injection. There is no discernable accumulation of tagged colloid, such as would occur with

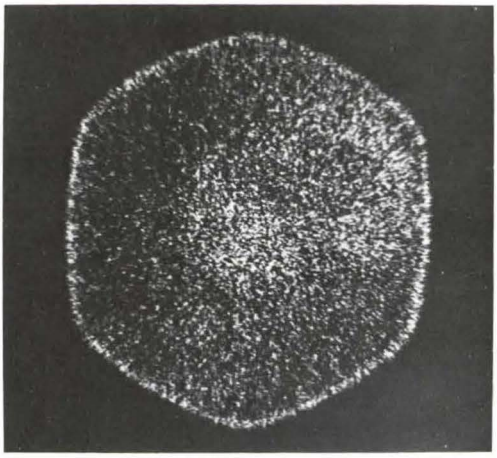

Fig. 3 Scintigram of liver four hours after injection. No discernable pattern is seen. Significant capillary clearance of sulfur colloid is thus doubtful

blood flow clearance following direct injection into a blood vessel. Furthermore, Figure 4 shows the continuous activity in the head following injection. There is no sudden increase in activity indicating blood flow involvement as would be the case for either direct venous injection or substantial capillary absorption of the colloid.

Thus the assessment of regional lymphatic clearance by scintillation counting of sulfur colloid tagged with Technition 99 m appears to be both applicable to lymphatic studies and an accurate method compared to invasive techniques. The full range of applications and limitations of such a system remains to be discovered.

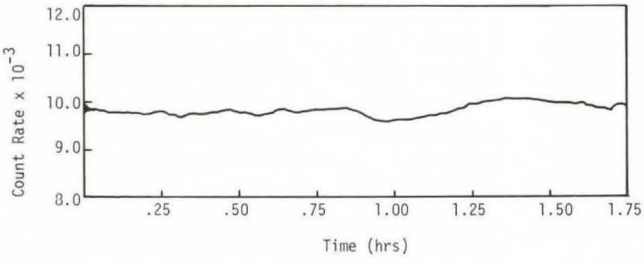

Fig. 4 Continuous radioactive counts per minute at the carotid artery following injection. No change in activity in blood indicated no appreciable capillary uptake of radioactive colloid at the injection site.

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