THE EFFECT OF LYMPHATIC FLUID PROTEIN CONCENTRATION ON LYMPHATIC RESISTANCE

A Senior Thesis

by

ELLEN MARIA WALKER

Submitted to the Office of Honors Programs
& Academic Scholarships
Texas A&M University
in partial fulfillment for the designation of

UNIVERSITY UNDERGRADUATE
RESEARCH FELLOWS

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ABSTRACT

The effect of lymphatic fluid protein concentration on lymphatic resistance.
(April 2001)

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Edema, increased tissue fluid volume, may be caused by impaired lymphatic system return. This edema formation inhibits organ function. We hypothesized that increased lymphatic fluid protein concentration would cause increased resistance to flow through lymphatic vessels. Mesenteric lymphatic vessels in anesthetized sheep were catheterized at both ends and connected to polyethylene tubing that functioned as inflow and outflow ports. Outflow pressure and, thus, the end-to-end pressure gradient were manipulated by altering the height of the outflow port. Two fluids – lactated Ringers solution and 6% albumin in lactated Ringers solution - were introduced alternately into the vessels. Flow through the vessel was determined for several pressure gradients. We calculated the resistance for each solution using the inverse of the slope of the flow-pressure gradient relationship. The calculated resistance for the Lactated-Ringers solution was 1.796 ± 0.213 mmHg·min/ml while the resistance for the 6% albumin solution was 1.911 ± 0.154 mmHg·min/ml. The resistance calculated for the 6% albumin solution was greater in all experiments, however the difference in resistance between the two solutions was not statistically

significant (P = .201). We conclude that the number of experiments included in this study was insufficient to resolve the nature of the relationship between protein concentration and resistance in lymphatic vessels.

ACKNOWLEDGMENTS

The author thanks Dr. Randolph Stewart for his computer skills and guidance in performing the experiments and writing the paper. I would also like to thank Dr. Glen Laine for the use of his laboratory and equipment.

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INTRODUCTION

Interstitial (tissue) fluid volume is regulated by the balance between microvascular filtration and fluid removal by the lymphatic system. Microvascular filtration is the flow of fluid from the capillaries into the interstitial space. Microvascular filtration occurs continuously as blood passes through the capillaries (Taylor 1991). The lymphatic system is a vascular system that serves to return interstitial fluid to circulation via the thoracic duct emptying into the great veins of the neck. Interstitial fluid that enters the lymphatic system is called lymph. Edema, increased interstitial fluid volume, may be caused by increased microvascular filtration or impaired removal of interstitial fluid by the lymphatic system.

Lymphatic vessels possess one-way valves that divide the vessels into segments termed lymphangions. Most lymphatic vessels have a layer of smooth muscle, so they are able to pump lymph through each lymphangion. As smooth muscle in the vessel relaxes, lymph enters one end of the lymphangion. Then, as the smooth muscle in the vessel constricts, the lymph is pumped into the next lymphangion.

The factors affecting lymph flow (Q_L) have been modeled on Ohm's Law:

$$Q_L = (P_i + P_p - P_o) / R_L$$

where P_i is inflow pressure, P_p is pump pressure, P_o is outflow pressure, and R_L is lymphatic resistance (Drake 1987).

A common cause of edema formation is increased capillary pressure. This

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This thesis follows the style and format of *American Journal of Physiology*.

occurs with heart failure, venous obstruction, and elevated pulmonary artery pressure. An increase in capillary pressure leads to an increase in microvascular filtration and a decrease in the protein concentration in the interstitial fluid. This occurs because the capillary membrane acts as an incomplete barrier to protein movement. As a result, the fluid filters through the capillaries into the interstitium leaving the protein behind in the blood vessels (Taylor 1991). This, in turn, causes a decrease in the protein concentration of the lymph that is returned to circulation. Another response to organ edema formation is a decrease in lymphatic resistance resulting in an increase in the rate of tissue fluid removal (Laine 1987).

A direct relationship exists between viscosity and resistance as shown by the Poiseuille equation:

$$R = (8L\eta)/(\pi r^4)$$

where R is the resistance, L is the vessel length, η is the viscosity of the fluid, r is the vessel radius, and π and 8 are constants (Sherwood 1997). Also, a direct relationship exists between protein concentration and viscosity (Allen 1984). We hypothesized that increased protein concentration would cause increased resistance to flow through lymphatic vessels. This hypothesis was tested by measuring the characteristics of flow through mesenteric (intestinal) lymphatic vessels in sheep using fluids with differing protein concentrations.

MATERIALS & METHODS

General anesthesia was induced in adult sheep (n = 3) with xylazine (0.05 mg/kg IV) and ketamine (2.0 mg/kg IV). Anesthesia was maintained with a pentobarbital drip (5-15 mg/kg/hr IV). The sheep were given butorphanol (0.1 mg/kg IV) to reduce pain. After induction, the trachea was intubated and the animal was mechanically ventilated. Following a laparotomy, a segment of small intestine was exteriorized and the mesenteric lymphatic vessels were identified after injecting Evans blue dye into the lymph nodes and observing the path of the dye through the vessels. The selected vessel was catheterized at two sites using 20-gauge catheters and connected to a fluid-filled polyethylene tubing apparatus that simultaneously measured flow through the vessel and pressure at each end of the vessel using pressure transducers (refer to Figure 1). Outflow pressure and, thus, the end-to-end pressure gradient were manipulated by altering the height of the outflow port. Heparin was administered through the inflow port to avoid clotting inside the vessels.

Lactated Ringers solution and 6% albumin in lactated Ringers solution were alternately introduced into the lymphatic vessels. These two fluids were introduced into the lymphatic vessels in no specific order. The rate of flow through the vessels was measured by collecting the fluid in a graduated cylinder from the outflow port during a timed interval. Flow was determined for many different pressure gradients for each solution.

Data analysis: Flow was plotted as a function of pressure gradient for each solution in each experiment. This relationship was analyzed by linear regression and resistance was

calculated as the inverse of the slope of the resulting regression line. The calculated resistance values for the two solutions were compared using a paired t-test. We performed data analysis with a computer executing SigmaStat (SSPS, Chicago, IL). A value of $P<0.05$ was considered significant. All data is presented as mean plus/minus standard error of the mean.

FIGURE 1

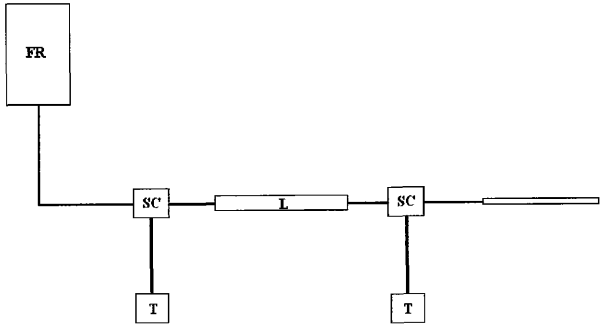


Figure 1. Apparatus for measurement of fluid flow and pressure. Fluid flowed from a fluid reservoir (FR) through a 3-way stopcock (SC), the lymphatic vessel (L) and a second stopcock (SC). Pressure within the stopcocks was measured using pressure transducers (T).

RESULTS

The calculated resistance values for all experiments are provided in Table 1. The resistance calculated for the lactated Ringers solution was 1.796 ± 0.213 mmHg·min/ml while the resistance for the 6% albumin solution was 1.911 ± 0.154 mmHg·min/ml. The difference in resistance between the two solutions was not statistically significant (P = .201). However, in all three experiments, the resistance of the 6% albumin solution was greater than that of the lactated Ringers solution. The power of the test was 0.164 and was below the desired power of 0.800.

TABLE 1

	Lactated Ringers Solution (mmHg·min/ml)	6% Albumin Solution (mmHg·min/ml)	Percent Change
Experiment 1	1.382	1.619	17.1%
Experiment 2	2.089	2.142	2.54%
Experiment 3	1.918	1.973	2.87%

Figures 2-4. Data from experiments 1, 2, and 3 are presented in figures 2, 3, and 4, respectively. Fluid flow through the lymphatic vessel is plotted as a function of pressure gradient. Individual data sets were analyzed by linear regression. (Lactated Ringers solution – open circles, 6% Albumin solution – closed circles)

FIGURE 2

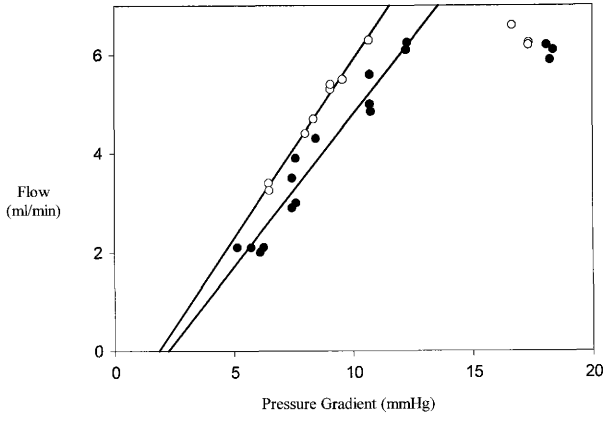


FIGURE 3

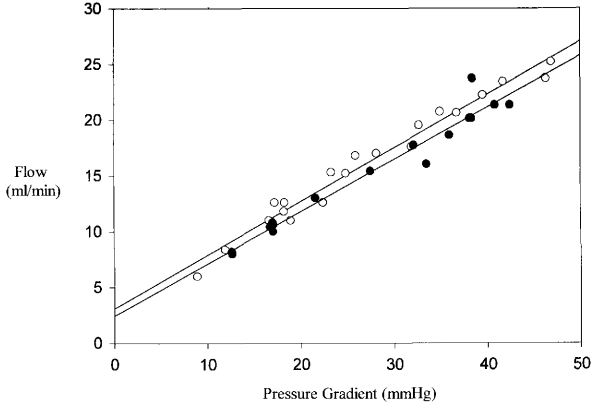
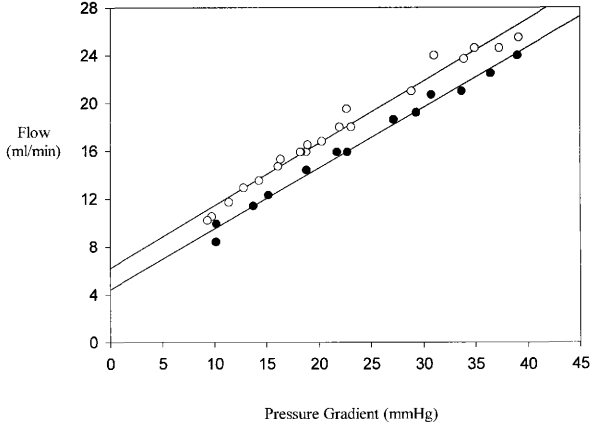

30
25
20
15
10
5
0
Flow
(ml/min)
0
10
20
30
40
50
Pressure Gradient (mmHg)

FIGURE 4


Flow
(ml/min)
28
24
20
16
12
8
4
0
0
5
10
15
20
25
30
35
40
45
Pressure Gradient (mmHg)

DISCUSSION

Our data demonstrate that there is a positive linear relationship between the pressure gradient and flow through a lymphatic vessel in accordance with Ohm's Law. A relationship between the protein concentration of lymph and resistance was not demonstrated by this study. In all three experiments, the resistance for the 6% albumin solution was greater than the resistance for the lactated Ringers solution. However, the difference in resistance between the two solutions was not shown to be statistically significant.

Figure 1 illustrates a collapsed flow system. Both solutions show that there is an increase in fluid flow as the pressure gradient increases; however, the fluid flow remained constant at a pressure gradient above 10 mmHg. The reason for this is that the outflow pressure decreases to a point where the external pressure (pressure on the walls of the lymphatic vessel) becomes greater than the outflow pressure, and the outflow port of the lymphatic vessel collapses. At this point, the external pressure becomes the effective outflow pressure. As a result, the real outflow pressure no longer affects the system and the pressure gradient and flow remain unchanged.

The protocol was adjusted for experiments 2 and 3 to avoid the collapsed flow system (see Figures 2 & 3). Experiments 2 & 3 predict positive flow at a pressure gradient of zero indicating lymphatic pumping. In both experiments, flow was greater for any pressure gradient with the lactated Ringers solution than with the 6% albumin solution. This indicates that there is a greater pumping pressure with the lactated Ringers solution suggesting that pumping activity is affected by protein concentration.

SOLUTION ALTERNATIVES

There are a few alternative ways to improve the setup and outcome of the experiment. First, the experiment could take place *in vitro* in an isolated bath by dissecting the lymphatic vessel. This would isolate the vessels so as to avoid any effects that are caused by factors other than those associated with the intrinsic characteristics of the lymphatic vessel. In this way, the variables would be limited, and the results would reflect only the effects due to the changing lymph viscosity.

A second alternative to the setup of the experiment would be to change the fluid viscosities. This could be accomplished by varying the type of protein used or by varying the concentration of albumin. This alternative would more accurately justify the results of the experiment if the same relationship was observed for each fluid viscosity.

A third way to modify and improve the experiment would be to repeat the experiment in different animals. The size and length of the lymphatic vessels for any given animal are different and unique. Therefore, if the same relationship between viscosity and resistance were obtained, the data would be more justified.

SUMMARY

We were unable to demonstrate a statistically significant difference in resistance between the lactated Ringers and 6% albumin solutions. However, resistance for the 6% albumin solution was greater in all three experiments. The low power of our statistical test suggests that our negative findings should be viewed with skepticism. We conclude that our experiments provide sufficient evidence that protein concentration affects resistance to warrant further experiments.

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