THE EFFECTS OF INTRAVENOUS INFUSION OF SOLUTIONS WITH DIFFERENT COLLOID OSMOTIC PRESSURES ON RAT MESENTERIC LYMPH PRESSURE

M. Shinozaki, M. Morio, H. Kikuchi, T. Yamanoue, K. Fujii, K. Kodama

Department of Anesthesiology and Division of Intensive Care Medicine (K.K.), Hiroshima University School of Medicine, Hiroshima Japan

ABSTRACT

The effects of intravenous infusion of solutions of varied colloid osmotic pressure on mesenteric lymph pressure were measured by a glass micropipette. The lymph pressure was 4.0 ± 1.6 cm H_2O between the second and third valve of rat mesenteric lymphatic vessels with diameter of 101 ± 25 μm (mean ± SD). Intravenous infusion of 1 ml/100g body weight (B.W.) of isotonic saline, 5% glucose solution, and 20% glucose solution increased the lymph pressure by 1.6 ± 0.5 cm H_2O, 1.5 ± 0.3 cm H_2O and 2.0 ± 1.0 cm H_2O, respectively. Infusion of 2 ml/100g B.W. of the same solutions increased the lymph pressure by 3.3 ± 1.3 cm H_2O, 3.3 ± 1.6 cm H_2O and 3.7 ± 0.8 cm H_2O, respectively. Infusion of hydroxyethyl starch solution, with colloid osmotic pressure of 25 mmHg, minimally altered the lymph pressure. One ml/100g B.W. infusion of low molecular weight dextran solution, with colloid osmotic pressure of 162 mmHg, decreased the lymph pressure by 1.3 ± 0.9 cm H_2O, and 2 ml/100g B.W. infusion of this solution decreased the lymph pressure by 2.0 ± 1.0 cm H_2O. The different effects on lymph pressure derive from the differences in colloid osmotic pressure of these solutions in conformity with the Starling hypothesis.

According to Starling's hypothesis fluid transport across capillaries is governed by the algebraic sum of effective hydrostatic and colloid osmotic pressures. The filtrated fluid returns to blood in two ways: via blood capillaries and via lymphatic capillaries (1). Although it is important to determine lymph pressure to understand better the movement of filtrated fluids through capillaries, little definitive information has been available until now because of difficulty in obtaining accurate measurements.

In this study, lymph pressure in rat mesenteric lymphatic vessels and the effect of intravenous infusion of solutions with various colloid osmotic pressures were directly measured by a glass micropipette.

MATERIALS AND METHODS

Experimental Preparation

Male Wister rats weighing 94g (SD=7, N=81) were anesthetized by intramuscular injection of sodium pentobarbital at a dose of 5 mg per 100g of body weight (B.W.). The femoral vein, femoral artery and jugular vein were cannulated to infuse solutions, to monitor systemic blood pressure and central venous pressure, respectively. The rat was placed on a microscope stage in a lateral position and was warmed with a warm bath

built into the microscope stage to maintain a rectal temperature of 37°C. About 7-10 cm of intestine was gently exteriorized through a midline incision in the lower abdomen and was placed on a transparent plastic block and unfolded. A warm (37°C) mammalian Ringer's solution composed of 146 mM NaCl, 4 mM KCl and 2 mM $CaCl_2$, and adjusted to pH 7.4 with $NaHCO_3$, was irrigated by an infusion pump over the unfolded intestine to keep the exteriorized intestine under physiological conditions.

We used a Nikon triocular light microscope (magnification: object lens x10, TV relay lens x1), attached to a TV camera connected to a video tape recorder, to observe the lymph circulation on a TV screen and to record the data. The time was indicated on the screen. The final magnification on the TV screen was 400 times. The diameter and automatic contraction rate of the lymphatic vessels were calculated from reproduced video-pictures.

To study the effects of intravenous infusion of solutions on lymph pressure in mesenteric lymphatic vessels, the rats were divided into 11 groups, namely: a control group given no infusion; 1 ml or 2 ml per 100g B.W. isotonic saline infusion group; 5% glucose solution infusion group; 20% glucose solution infusion group; hydroxyethyl starch solution infusion group; and low molecular weight dextran solution infusion group. Each rat was given only a single intravenous infusion with one kind of solution. The solutions were injected manually at a rate of 1 ml/15 seconds. All solutions employed were commercial products for parenteral use in humans. For the hydroxyethyl starch solution, 6-HES (Morishita Pharmaceutical Co., Ltd., Osaka, Japan) was used which contained 6g hydroxyethyl starch (MW 60,000) in 100 ml of isotonic saline, and the colloid osmotic pressure was 25 mmHg. For the low molecular weight dextran solution, Rheomacrodex (Green Cross Co., Ltd., Osaka, Japan) was used which contained 10g dextran (MW 25,000) in 100 ml of 5% glucose solution, and the colloid osmotic pressure was 162 mmHg.

Lymph pressure was measured for two minutes before infusion and for 20 minutes after infusion at about 20 second intervals, and averaged lymph pressure for every two minutes was calculated. Results were expressed as mean ± SD. Paired and unpaired "t" tests were used to evaluate the statistical significance of observed changes within the groups and the statistical significance of differences between the groups, respectively. If the P value was less than 0.05, then the changes and differences were regarded as statistically significant.

Procedure and Principle of Measuring Lymph Pressure

With aid of a manipulator under a microscope, a selected lymphatic vessel was punctured by glass micropipette and filled with 1 M NaCl solution, generally between the second and third valve. The other end of the glass micropipette was connected by a tube to a reservoir that contained 1M NaCl solution. The height of the reservoir was recorded together with electric resistance of the glass micropipette, as measured by a micropipette resistance meter (Nihonkohden S-4882 Nihonkohden Co., Ltd., Tokyo, Japan).

The electric resistance of a glass micropipette depends on the concentration of electrolyte within it. When the pressure in the micropipette is higher than the pressure in the lymphatic vessel, the 1M NaCl solution in the micropipette keeps flowing out into the lymphatic vessel. Therefore, the electrical resistance of the micropipette is stable, since the lymph cannot enter into the micropipette. On the other hand, when the pressure in the micropipette becomes lower than in the lymphatic vessel, the lymph in the lymphatic vessel begins to enter the micropipette, and the micropipette then contains both lymph and 1M NaCl. As a result, electrical resistance of the micropipette increases greatly and immediately (2-4). When an acute change of electrical resistance in the micropipette is observed, the height of the solution level in the reservoir above the tip of the micropipette is defined as lymph pressure. The accurate static response of our measuring device is shown in *Fig. 1*.

The tip of the glass micropipette was 16 ± 3.5μm (N=81) in diameter. Since the rat mesenteric lymphatic vessels contracted
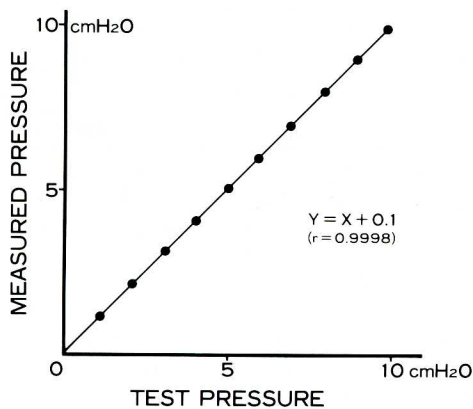
Fig. 1: The linear relationship between test pressure and measured pressure for our device.

rhythmically, lymphatic vessels that contracted at a slower rate were selected, and lymph pressure was measured during a stationary phase.

RESULTS

1) Lymph pressure in rat mesenteric lymphatic vessels

The lymphatic vessels, in which lymph pressure was measured, were 101 ± 25 μm in diameter. The lymph pressure was 4.0 ± 1.6 cm H_2O, as shown in *Fig. 2*. The lymph pressure in the control group that received no infusion was stable, as shown in *Fig. 3*.

2) The effects of intravenous infusion of solutions on lymph pressure in rat mesenteric lymphatic vessels *(Fig. 4)*

The rate of infusion was 14.3 ± 2.4 seconds (N=25) in the 1 ml/100g B.W. infusion groups and 29.6 ± 7.9 seconds (N=25) in the 2 ml/100g B.W. infusion groups. These rates of infusion minimally affected blood pressure and central venous pressure.

a) *Isotonic saline:* One ml/100g B.W. infusion of isotonic saline increased the lymph pressure by 1.6 ± 0.5 cm H_2O (N=5) and 2 ml/100g B.W. infusion increased the pressure by 3.3 ± 1.3 cm H_2O (N=5). The difference in increase of lymph pressure between these two infusions was significant. The increase of lymph pressure that resulted from infusion of 2 ml/100g B.W. was maximal at two to four minutes and was still
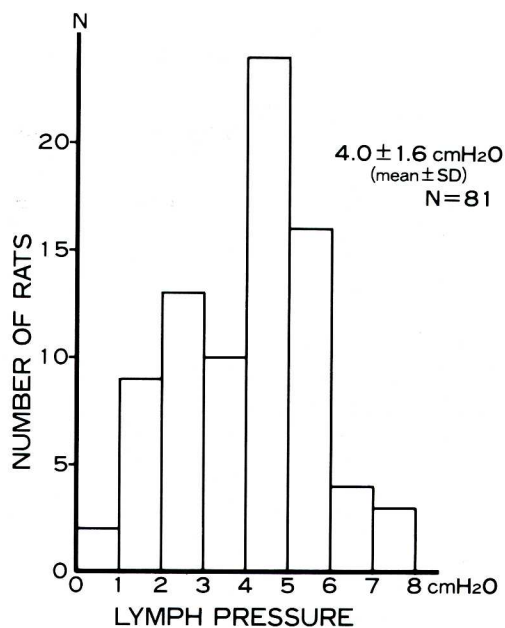

Fig. 2: Distribution of average lymph pressure in rat mesenteric lymphatic vessels. The results are based upon observations made over two minutes for each of 81 rats. Lymph pressure is 4.0 ± 1.6 cm H₂O (mean ± SD).

significant after 20 minutes. However, the increase that followed infusion of 1 ml/100g B.W. was no longer significant after 14 minutes.

b) *5% glucose and 20% glucose solution:* One ml/100g B.W. infusion of 5% glucose and 20% glucose solution increased the lymph pressure by 1.5 ± 0.3 cm H_2O (N=5) and by 2.0 ± 1.0 cm H_2O (N=5), respectively. Infusion of 2 ml/100g B.W. increased the pressure by 3.3 ± 1.6 cm H_2O (N=5) for 5% glucose and by 3.7 ± 0.8 cm H_2O (N=5) for 20% glucose. The difference in increase of lymph pressure between 1 ml/100g B.W. infusion and 2 ml/100g B.W. infusion of these two solutions was significant. There was, however, no significant difference in the increase of lymph pressure that followed 2 ml/100g B.W. infusions of isotonic saline, 5% glucose solution or 20% glucose solution. However, 1 ml/100g B.W. infusion of 20% glucose solution increased the lymph pressure significantly more than that of 5% glucose solution.

Table 1.
Change in Diameter of Lymphatic Vessels after Infusion

1 ml/100gB.W. iv group

NaCl		5% Glucose		20% Glucose		HES		LMWD	
a	b	a	b	a	b	a	c	a	d
85	88	100	100	50	50	100	100	88	88
130	130	125	125	95	113	75	78	63	63
140	150	103	100	105	125	75	75	88	88
100	100	125	125	163	163	148	130	83	88

2 ml/100gB.W. iv group

NaCl		5% Glucose		20% Glucose		HES		LMWD	
a	b	a	b	a	b	a	c	a	d
138	150	88	113	75	75	63	65	135	120
95	100	70	113	100	115	53	63	43	50
100	105	150	150	120	120	85	78	75	75
88	88	143	145	113	125	108	105	100	88

a: Diameter in μm before infusion
b: Diameter in μm at the maximum lymph pressure after infusion
c: Diameter in μm at 10 minutes after infusion
d: Diameter in μm at the minimum lymph pressure after infusion

NaCl, HES, and LMWD represent isotonic saline, hydroxyethyl starch solution and low molecular weight dextran solution, respectively.
The difference between pre- and post-infusion diameter of lymphatic vessels is statistically insignificant under each set of infusion by paired *t* test.

c) *Hydroxyethyl starch solution:* Infusion of this solution barely altered the lymph pressure. Two ml/100g B.W. infusion decreased the lymph pressure significantly for the first four minutes.

d) *Low molecular weight dextran solution:* One ml/100g B.W. infusion of dextran solution decreased the lymph pressure by 1.3 ± 0.9 cm H_2O (N=5), and 2 ml/100g B.W. infusion decreased the pressure by 2.0 ± 1.0 cm H_2O (N=5). The decrease of lymph pressure after 2 ml/100g B.W. infusion was greater than that after 1 ml/100g B.W. infusion, but the difference was not statistically significant. The decrease of lymph pressure was maximal after four minutes and persisted for 20 minutes.

3) The effect of intravenous infusion of solutions on the diameter and intrinsic contraction rate of rat mesenteric lymphatic vessels.

No significant change was found in the diameter of the lymphatic vessels, as shown in *Table 1*. The contraction rate of rat mesenteric lymphatic vessels was 4.8 ± 5.8

(N=50) during the two minutes immediately before infusion, and no significant change was found after infusion.

4) The relationship between the changes of lymph pressure, blood pressure and central venous pressure following infusion of solutions.

There was no relationship between changes in lymph pressure, blood pressure and central venous pressure, as shown in *Fig. 5*. The change of blood pressure was transient. When blood pressure returned to the preinfusion level, the change in lymph pressure was still observed.

DISCUSSION

Pressure recordings of lymphatic vessels have been obtained from wings of the bat (5,6), and in mesenteries of the cat and rat (7). Since it has been reported that lymph pressure rises upon passage through each valve (7), we measured lymph pressure mainly between the second and third valve.

Thoracic duct lymph is believed to be influenced by anesthesia (8), laparotomy (9), and the status of water and food intake of the animals (10). These factors may affect rat mesenteric lymph pressure, but our measurements of lymph pressure corresponded well to that reported by Zweifach and Prather (7).

Thoracic duct lymph is derived largely from the abdominal viscera, especially in an anesthetized animal (11), and is increased following infusion of isotonic saline (12,13). This increase of thoracic duct lymph is thought to arise from an increase of intestinal lymph (14). Our results show, however, that an infusion of isotonic saline increases mesenteric lymph pressure without a significant change in the diameter of the lymphatic vessels. Despite lack of lymphatic dilation, the increase in lymph pressure following infusion is likely brought about by the increase of lymph flow that originates in a shift of fluid from capillary to interstitial space. Indeed, the increase of lymph pressure occurs almost immediately after the infusion of isotonic saline. In conformity with this viewpoint, Sekizuka reports that a solution of 20% fluorescein sodium (MW 360),

injected into a rat femoral artery, appears about 60 seconds later in the mesenteric lymphatic vessels (15).

The increase in lymph pressure that followed 2 ml/100g B.W. infusion of isotonic saline reached a peak in two to four minutes, and lasted for 20 minutes. A similar increase of lymph flow in the thoracic duct after the infusion of isotonic saline was observed by Yamada et al (16). One ml/100g B.W. infusion of 20% glucose solution raised lymph pressure more than one ml/100g B.W. infusion of 5% glucose solution. Nakagawa also reported that 50% glucose solution increased the flow of thoracic duct lymph more than 5% glucose solution (17). Taken together these results strongly support the assumption that the change in lymph pressure that followed intravenous infusion of solutions derived from changes in lymph flow.

Because the colloid osmotic pressure of isotonic saline, 5% glucose solution and 20% glucose solution is zero, these solutions, upon infusion into a vessel, must pass through capillaries into the interstitial space to increase lymph pressure. The presence of hyperosmotic glucose solution in the inter-
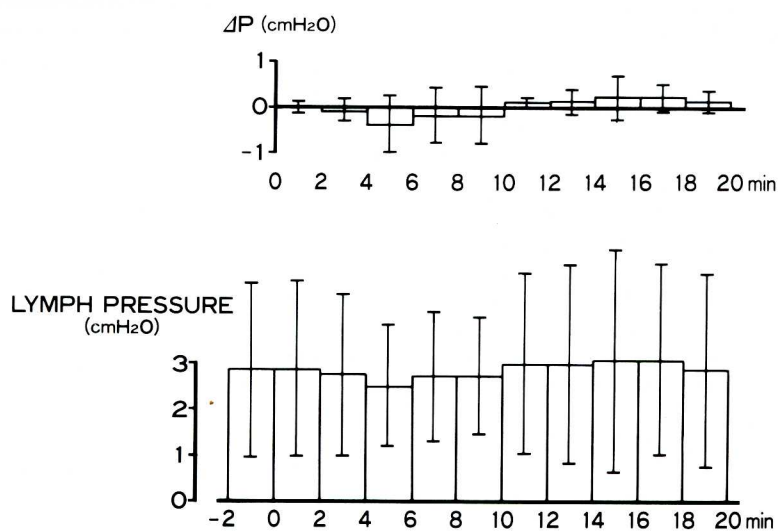
Fig. 3: Time course of variations in lymph pressure over 22 minutes in the control group given no infusion of solutions. The upper panel shows change of lymph pressure (ΔP). The results are means ± SD for five rats. The changes in lymph pressure are not statistically significant.
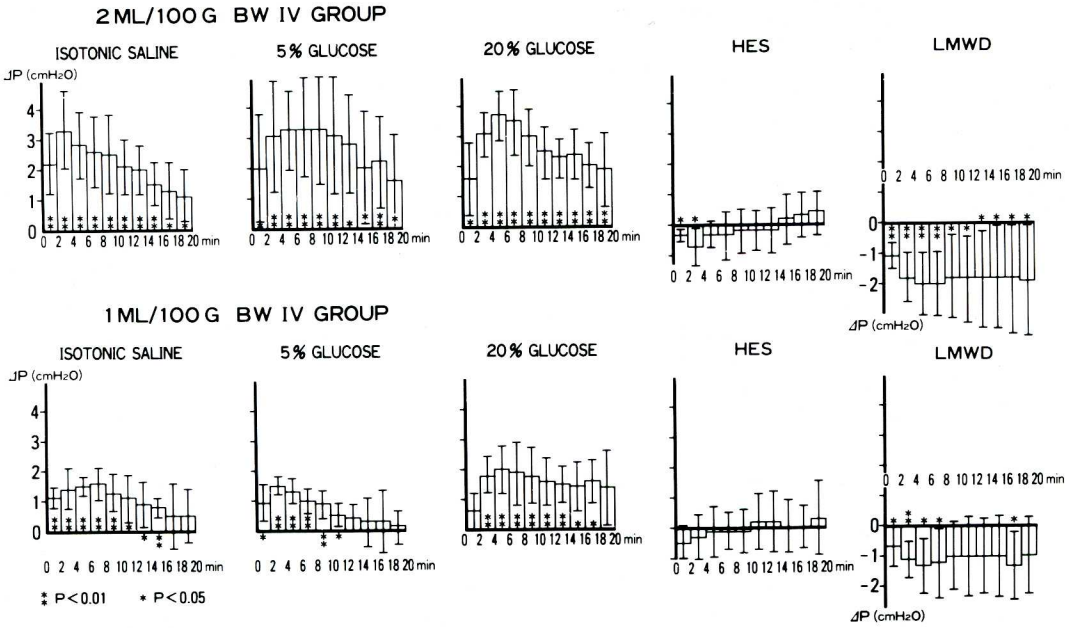
2 ML/100 G BW IV GROUP

Fig. 4: Effects of intravenous infusion of isotonic saline, 5% glucose, 20% glucose, hydroxyethyl starch (HES), and low molecular weight dextran (LMWD) solution on lymph pressure. One or two ml/100 g body weight of these solutions is injected at time 0.

The results are given as the mean ± SD for five rats under each set of conditions. Single asterisk and double asterisks indicate P < 0.05, and P < 0.01, respectively.
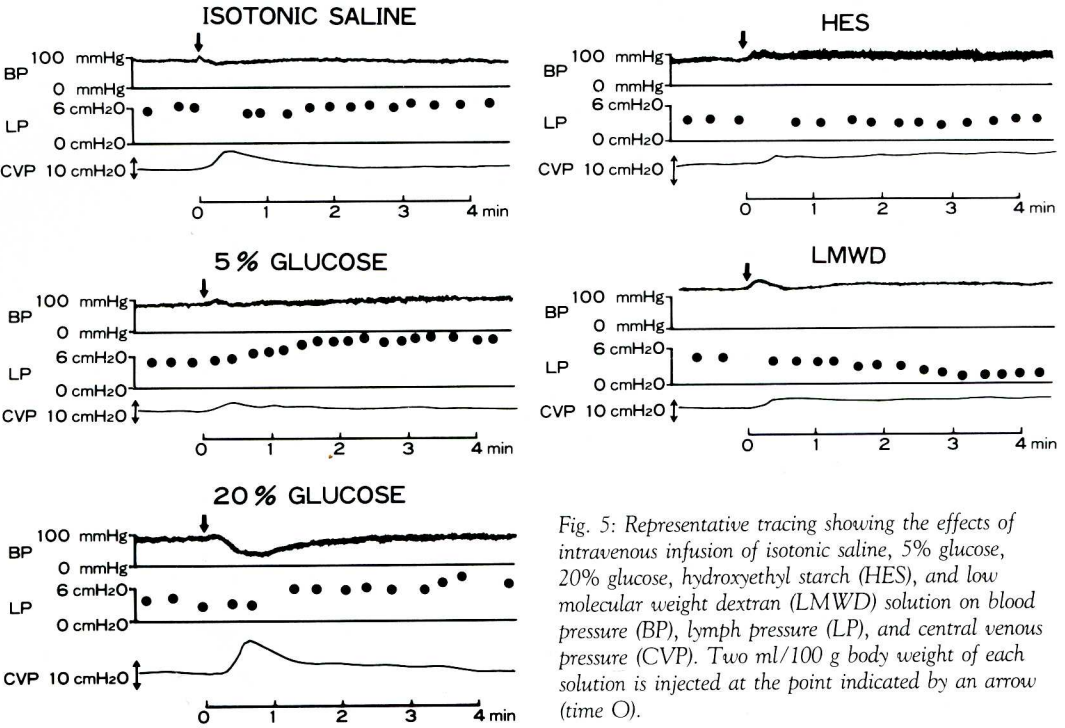

Fig. 5: Representative tracing showing the effects of intravenous infusion of isotonic saline, 5% glucose, 20% glucose, hydroxyethyl starch (HES), and low molecular weight dextran (LMWD) solution on blood pressure (BP), lymph pressure (LP), and central venous pressure (CVP). Two ml/100 g body weight of each solution is injected at the point indicated by an arrow (time O).

stitial space that results from the infusion of 20% glucose solution, causes a fluid shift from the intracellular space to the interstitium. This phenomenon probably accounts for the fact that 1 ml/100g B.W. infusion of 20% glucose solution increased lymph pressure more than that of 5% glucose solution.

In contrast, infusion of hydroxyethyl starch solution caused little or no change in lymph pressure. Infusion of Tayrod's solution that contained dextran (MW 60,000-90,000), with colloid osmotic pressure of 20 mmHg, did not affect thoracic duct lymph (18). Because these two solutions have a colloid osmotic pressure nearly equal to that of rat plasma (20 mmHg), these solutions after vascular infusion remained within the vessel and, consequently, minimally affected the lymph pressure.

Infusion of 25% serum albumin into cat jugular vein decreases intestinal lymph flow and mesenteric lymph pressure (19). Intravenous infusion of low molecular weight dextran solution with hyper-colloid osmotic pressure causes a fluid shift from interstitial space into blood (20). This shift probably explains why our infusion of low molecular weight dextran solution, with colloid osmotic pressure of 162 mmHg, decreased lymph pressure. Even 1 ml/100g B.W. infusion of the dextran solution was capable of reducing lymph pressure to almost zero; therefore, the difference in the decrease of lymph pressure between 2 ml/100g B.W. infusion and 1 ml/100g B.W. infusion of this solution was not significant.

An elevation of pressure in the jugular vein was reported to raise lymph pressure in abdominal great collecting trunks (21), but no correlation was found between central venous pressure and mesenteric lymph pressure in our study. The elevation of central venous pressure apparently·was not transmitted to peripheral mesenteric lymphatic vessels.

The rate of rhythmic contraction of rat mesenteric lymphatic vessels in our results was lower than that reported by Fujii et al (22), and Tanra (23). This inconsistency may be attributed to: 1) our dosage of anesthetic agent was larger; 2) the less vigorously con-

tracting lymphatic vessels were deliberately selected to measure lymph pressure; 3) our Ringer's solution used for irrigation of the exteriorized intestine did not contain 1% gelatin, which reportedly preserved vascular responsiveness (24).

Increase of lymph production or lymph pressure has been suggested to raise the contraction rate of lymphatic vessels (25-27). Although our results did not demonstrate this effect, contractility of lymphatic vessels may have been suppressed by "deeper" anesthesia, as mentioned by Hall (28).

The diameter of the lymphatic vessels was not affected by the changes in lymph pressure that followed intravenous infusion of solutions. Presumably, the changes of tissue pressure that occurred concomitantly with the changes in lymph pressure (29,30) counteracted an enlargement in diameter of lymphatic vessels.

In summary, our study has demonstrated that intraluminal pressure within rat mesenteric lymphatics is 4.0 ± 1.6 cm H_2O and that the response of lymph pressure to intravenous infusion of solutions with various colloid osmotic pressure conforms to Starling's hypothesis.

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Dr. Masamitu Shinozaki, M.D.
Department of Anesthesiology
Hiroshima University School of
** Medicine**
Kasumi 1-2-3, Minami-ku
Hiroshima 734, Japan