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The Influence of Absorption/Enterosorption and Partial Occlusion of the Portal Vein on the Quantity and Composition of the Intestinal Lymph

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Summary

What influence does the admixture of absorbed water exert on the rate of flow and composition of the lymph? To analyse this question rats were given twice distilled water 20 ml/kg intraduodenally. As a result of this fluid loading, lymph flow rose to approx. 400% of its initial value, and this lymphagogue reaction lasted some 30 min. There was a time-related decrease in PVP filtration coefficient and in the total density and the protein content of the lymph. However, the decrease in these values was less than would have been expected from the "dilution effect" caused by the absorption of the water.

After intraduodenal instillation of mannitol solution (six times isotonic in relation to blood) in a dose of 7 ml/kg there was enterosorption of water out of the blood into the intestinal lumen, or in other words this induced a reverse flow of water against the direction of intestinal absorption. As might be expected, the quantity of intestinal lymph diminished, while the PVP filtration coefficient and the density and protein content of the lymph rose, though once again less than would have been expected from the decrease in lymph flow.

After partial occlusion of the portal vein for 10 min lymph flow rose to an average of seven times its initial value. Under these conditions there was also a decrease in the PVP filtration coefficient and in the density and protein content of the lymph, which once again was less than might have been expected from the increase in lymph flow.

Intestinal lymph occupies a special position among the lymphs produced by the organs of the body in that it is derived from two sources: capillary filtration and absorbed water. In view of the behaviour of the PVP filtration coefficient and the density and protein content of the lymph under various conditions – absorption of protein-free water, enterosorption of fluid from the blood into the intestinal lumen, and partial occlusion of the portal vein (which raises capillary filtration pressure) – it seems implausible that the plasma-lymph barrier can be envisaged merely as a simple membrane with pores and leaks. The concept of a matrix-like layer seems to offer a much better explanation of the experimental results.

Introduction

The lymph produced by the gut, in particular the small intestine, occupies a special position among the lymphs produced by the organs of the body, in that it is derived from at least two (*Barrowman* (2)) and possibly even three sources (*Lee* (12)). Lymph is formed by filtration from the blood capillaries situated in the submucosa of the intestine, and to it is added absorbed fluid, the proportion of the latter depending on the intake of water and other liquids. The admixture of absorbed fluid must play some part even when there is no exogenous intake of water, because bile, pancreatic secretion and intestinal juice

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Dedicated to Dr. med. *Rolf Madaus* on his 60th birthday

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enter the lumen and are in part reabsorbed. When intestinal lymph is collected by puncturing the mesenteric duct just before it enters the cisterna chyli there is also some possibility of contamination by lymph from the liver, though this varies considerably from one individual to another, depending on local anatomical circumstances.

The presence of endogenous proteins in the intestinal lymph is mainly due to the fact that proteins escape from the capillaries into the interstitial space and pass from there into the central chyle channel of the intestinal villus. The limiting membrane which controls this protein outflow is undoubtedly the wall of the capillary, because the central lymphatic of the intestinal villus is full of leaks, and is indeed virtually fenestrated.

Two series of experiments were performed to investigate the significance of the absorbate (A) in relation to lymph flow and composition. This was done by inducing, first of all, fluid flow from the intestinal lumen into the blood (absorption) and secondly fluid flow in the opposite direction (enterosorption) (B). Next, by partial occlusion of the portal vein we produced a rise in the pressure in the capillaries with consequent distension of the vessels (C). If we accept the concept of the capillary wall as a membrane perforated by pores and leaks, it is clear that under conditions of increased intravascular pressure larger amounts of macromolecules must pass through the membrane and macromolecules of greater molecular mass will also find their way through. These experiments based on partial occlusion of the portal vein have provided evidence which seems to throw light on the structure of the plasma-lymph barrier.

A) Materials and Methods

The experiments carried out to evaluate the influence of water absorption on the flow and composition of the lymph were carried out in rats. The treatment, anaesthesia and operative procedure were the same as described previously (15). PVP of molecular weight 38,000 was used exclusively for the filtration coefficient determinations. The lymph collection periods were started 30 min after the beginning of the PVP infusion and at the end of the first lymph collection period 20 ml/kg of twice distilled water was instilled into the first part of the jejunum. PVP estimations were carried out as described by *Vogel* et al. (15), and in addition the protein content of the lymph and plasma samples was determined by the biuret method and their density was measured by the mechanical oscillation technique. The length of the lymph collection periods was governed by the time-course of the lymphagogue reaction, and blood was taken at intervals of 30 minutes throughout the duration of the experiment. As the density and protein content of the plasma remained practically unaltered throughout the experiment, it was not thought necessary to calculate the ratio $\frac{\text{lymph}}{\text{plasma}}$ for this parameter. Changes in the density of the lymph were confined to a range of 1–11 g (1001–1011). The calculations of the relative density changes $\Delta\%$ hence refer exclusively to that range.

The mechanical oscillation technique was first introduced by *Kenner* et al. (10, 11) for continuous measurements of blood density and was later used by *Hinghofer* (9) for measuring μ-amounts of blood and plasma. The method is based on the mass-spring principle; the material to be measured is introduced into a glass tube bent in the shape of a U. The tube is then made to oscillate electronically and the oscillation frequency (f) is measured. The frequency is governed by the elasticity and mass of the oscillating body, while the latter is made up of the mass Mo of the empty oscillator and the mass of the sample. As density is defined as mass per unit volume, the mass of the sample can be expressed as the product of density and volume (p x V),

$$\frac{1}{T} = \frac{1}{2\pi}\sqrt{\frac{c}{Mo + pV}}$$

in this formula T represents the duration of one oscillation and c the elasticity constant of the oscillating system. Conversion of the equation (1) gives the following expression

$$p = \frac{T^2 - b}{a} \qquad (2),$$

where a and b represent the calibration constants of the particular oscillator, the terms c, Mo and V being contained within them. The constants are determined by measuring the oscillation periods (T) after filling the oscillator with two media of known but different densities (air and water) and are then recorded for that particular apparatus (calibration). This does away with the need for volume measurements and weighing as in conventional density determinations, all that is required being continuous frequency measurements in a calibrated oscillating system. Continuous measurements can thus be carried out in the flow-through technique. The density measurements were performed by means of a DMA 602 MW measuring cell in combination with a DMA 55 computer unit (Fa. Paar KG, Graz, Austria). The measuring system was kept at a constant temperature of 37.00 ± 0.01 °C by means of an ultrathermostat (Heto 04 PT, Birkerød, Denmark). The accuracy, resolution and precision were within ± 0.01 g/l. The lymph or plasma samples were injected into the open end of the oscillator by means of tuberculin syringes. The volume of fluid required to fill the measuring unit was 0.06 ml. After each measurement the oscillator was cleansed with water and dried in air. An average of 10–20 density determinations can be performed in one hour.

A) Results

Instillation of 20 ml/kg of twice distilled water (Fig. 1) resulted in a rise in lymph flow from 8.6 to 30 µl/min for a duration of approx. 30 min. The filtration coefficient of PVP of MW 38,000 fell from 0.35 to 0.23, overall density decreased from 1008 to 1004 g/l and protein concentration from 32 to 17 g/l (Fig. 2). The time curves of the density and protein changes are somewhat straighter than the changes in the PVP filtration coefficients, but as expected the density and protein concentration run parallel. As will be seen from Figs. 7, 8 and 9,

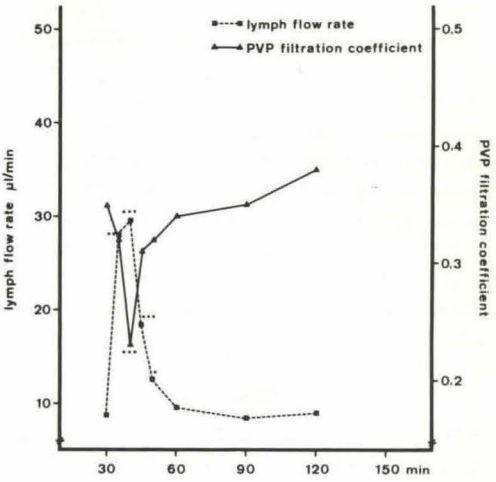

Fig. 1 Intestinal lymph flow and PVP filtration coefficient (MW 38 000) in rats during absorption of distilled water
• $P < 0.05$ •• $p < 0.01$ ••• $p < 0.001$
in comparison to the control period

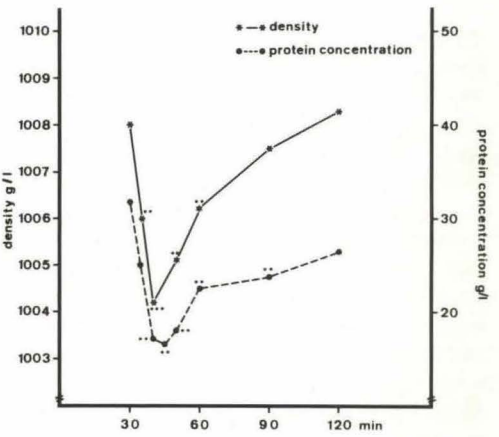

Fig. 2 Protein concentration and density of rat's intestinal lymph during absorption of distilled water
• $p < 0.05$ •• $p < 0.01$ ••• $p < 0.001$
in comparison to the control period

the changes in PVP filtration coefficient, density and protein concentration are not merely those which would be expected from a simple "dilution effect", for the percentage decreases in concentrations are considerably

lower than those that would be expected from the percentage increase in lymph flow.

A) Discussion

Data on the effects produced by absorption of water, sodium chloride or glucose solutions on the flow and composition of the intestinal lymph have been recorded by *Barrowmann* et al. (3) and *Gartemann* et al. (4). The technique used by these workers differed from ours in that they worked with conscious rats immobilized in Bollman cages. The experiments of *Barrowman* et al. (3) are most closely comparable to our own.

Whereas we found that the average increase in lymph flow produced by intraduodenal instillation of 20 ml/kg of twice distilled water amounted to 400%, and the simultaneous decrease in PVP filtration coefficient, protein concentration and lymph density was *not* as great as would have been expected from a "dilution effect", in the experiments of *Barrowman* et al. the decrease in the protein concentration of the lymph corresponded exactly to the increase in lymph flow. This is what would be expected if we assume that a completely proteinfree absorbate is added to the capillary filtrate. The results of our own experiments can best be explained by assuming that the ingestion of 20 ml/kg of water causes a transient hypervolaemia and hence a rise in capillary filtration pressure. The extra quantity of lymph produced after ingestion of water would then consist partly of an increase in protein-containing capillary filtrate and partly of an increase in protein-free absorbate. In addition, it is conceivable, as *Lee* (12) has pointed out, that the lymph in the mesenteric duct may contain an admixture of liver lymph, which contains protein, and that after instillation of water into the intestine lymph flow may also be increased by the absorption of water and its transport via the portal vein. It may also be noted that the movement of protein between plasma and interstitial tissue and vice versa during absorption appears to be considerably greater than would be expected on the basis of the transit of protein into the lymph (8).

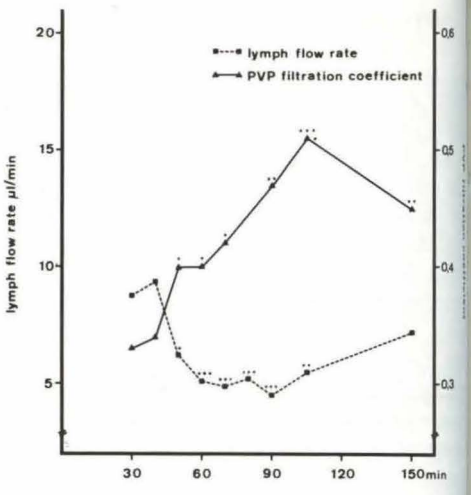

Fig. 3 Intestinal lymph flow and PVP filtration coefficient (MW 38 000) in rats during enterosorption of fluid by instillation of a hypertonic solution of mannitol
· $p < 0.05$ ··· $p < 0.01$ ··· $p < 0.001$
in comparison to the control period

B) Materials and Methods

The experimental conditions were the same as in Series A; 7 ml/kg of mannitol solution (six times isotonic in relation to blood) was instilled into the upper jejunum.

B) Results

After instillation of 7 ml/kg of mannitol solution (six times isotonic in relation to blood there was, as expected, a decrease in lymph flow; over the course of 60 min it dropped from 9 to less than 5 µl/min (Fig. 3). At the same time the PVP filtration coefficient rose from 0.33 to 0.50, the time characteristics this change corresponding to those of the fall in lymph flow. Density increased from 1008 to 1011 (Fig. 4), but the increase in protein concentration did not run parallel the rise in density, as density rose more sharply than protein concentration. In keeping with Figs. 7, 8 and 9 it will be seen that the percentage changes in PVP filtration coefficient, density and protein concentration do not correspond to a simple "concentration effect", as the relative decrease in lymph

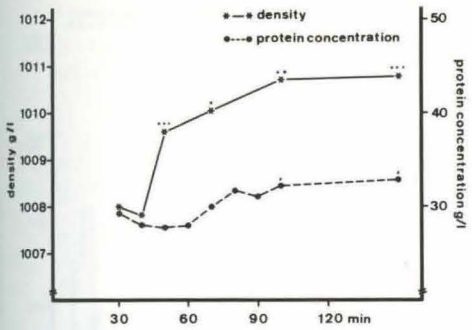

Fig. 4 Protein concentration and density of rat's intestinal lymph during enterosorption of fluid
• $p<0.05$ ••• $p<0.01$ ••• $p<0.001$
in comparison to the control period

flow is less than the relative increase in filtration coefficient and density. The protein concentration increases to a lesser extent than would be expected from the decrease in lymph flow.

B) Discussion

So far as we are aware, there have been no experiments by other workers on the significance of enterosorption of endogenous fluid from the blood and tissues into the intestinal lumen with regard to the flow and composition of intestinal lymph. In keeping with the "two source theory" of intestinal lymph, there was in our own experiments, as expected, a decrease in lymph flow together with a rise in PVP filtration coefficient, protein content and density. The rises in concentration did not correspond in degree with a simple "concentration effect", and furthermore protein content and density did not run parallel in all the experiments. It seemed possible that escape of mannitol from the bowel lumen into the lymph might explain the discrepancies between density and protein content which measurements of the osmolarity of the lymph, the plasma and the intestinal contents had revealed. However, the conditions existing during instillation of relatively concentrated mannitol solution into the intestinal lumen are by no means simple and cannot be explained by assuming that capillary filtrate is sucked out of the interstitial tissues of the intestine into the intestinal lumen, while the macromolecules remain in the interstitial tissues and are transported in increased concentration in the residual lymph. We did indeed select the volume of hypertonic mannitol solution in such a way that the small intestine was at most half filled, but as a result of enterosorption the bowel regularly became filled to capacity with a corresponding increase in the tension in the wall. It must therefore be assumed that the capillaries running in the intestinal wall are mechanically compressed, with the result that capillary filtration undergoes an overall decrease and that compression of the leaks hinders the escape of protein and other macromolecules out of the blood into the lymph. *Granger* et al. (6), working with cats, raised the intraluminal pressure in the small intestine step by step from 0 to 30 mmHg. Their findings under these experimental conditions were at variance with ours; lymph flow increased to roughly five times its initial value while the protein filtration coefficient diminished by 20%. So far as the influence of blood flow on capillary permeability is concerned, *Perry* and *Granger* (14) showed that as blood flow through the cat's ileum increased, its permeability for small molecules (inulin, raffinose) rose steeply, but its permeability for large molecules (β-lactoglobulin A) hardly increased at all.

C) Materials and Methods

The experimental conditions were the same as in Series A. The partial clamping of the portal vein lasted for 10 minutes and was carried out with a fine arterial clamp sheathed with PVC tubing.

C) Results

As a result of partial occlusion of the portal vein for 10 minutes lymph flow rose from 8.2 to 54 µl/min; after 30 min the reaction had almost completely subsided. At the same time the PVP filtration coefficient fell from 0.31 to 0.17 (Fig. 5), the time characteristic of the fall corresponding to that for the rise in lymph flow. The density of the lymph fell from 1007.5 to 1004.8 g/l and the protein

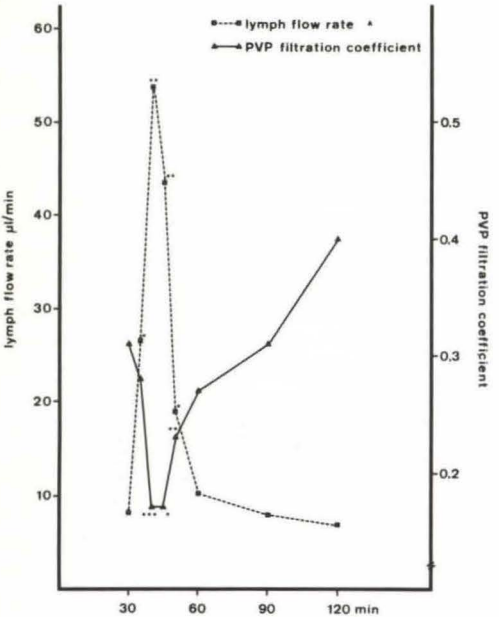

Fig. 5 Intestinal lymph flow and PVP filtration coefficient (MW 38 000) in rats during partial occlusion of the portal vein
· $p < 0.005$ ·· $p < 0.01$ ··· $p < 0.001$
in comparison to the control period

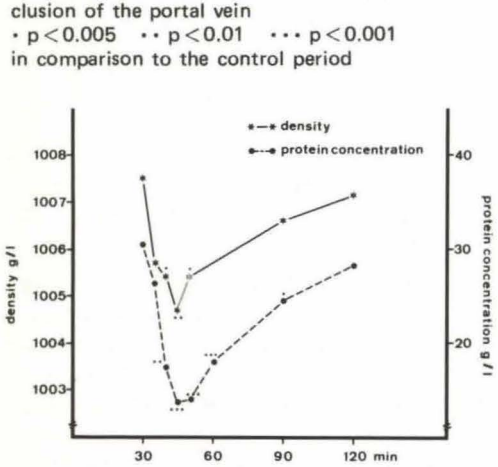

Fig. 6 Protein concentration and density of rat's intestinal lymph during partial occlusion of the portal vein
· $p < 0.05$ ·· $p < 0.01$ ··· $p < 0.001$
in comparison to the control period

concentration from 30.6 to 13.7 g/l (Fig. 6); the subsequent rises in density and protein concentration were somewhat slower than the rise in PVP filtration coefficient. Here

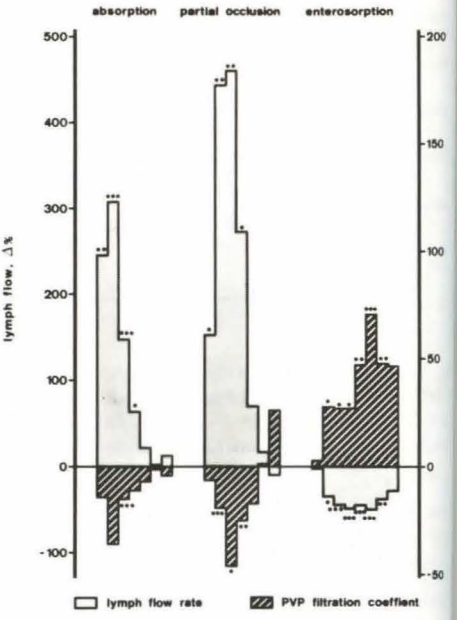

Fig. 7 The relative changes in intestinal lymph f and PVP filtration coefficient (MW 38 000) in ra during absorption, partial occlusion of the porta vein and enterosorption
· $p < 0.05$ ·· $p < 0.01$ ··· $p < 0.001$
in comparison to the control period

again, it was apparent that the percentage decreases in PVP filtration coefficient, den sity and protein concentration did *not* cor respond to those which would be expected from a simple "dilution reaction", as the relative increase in lymph flow was consid erably greater than the relative decrease in the concentrations (Fig. 7, 8, 9).

C) Discussion

In our own experiments partial occlusion the portal vein resulted in a very marked in intestinal lymph output, the flow rate increasing to up to 10 times the initial va the PVP filtration coefficient, the protein content of the lymph and its density dec ed, but not to such an extent as would co respond to a pure "dilution effect". Theo ically it would be expected – at least for limited rise in capillary filtration pressure – that there would be an increase in the

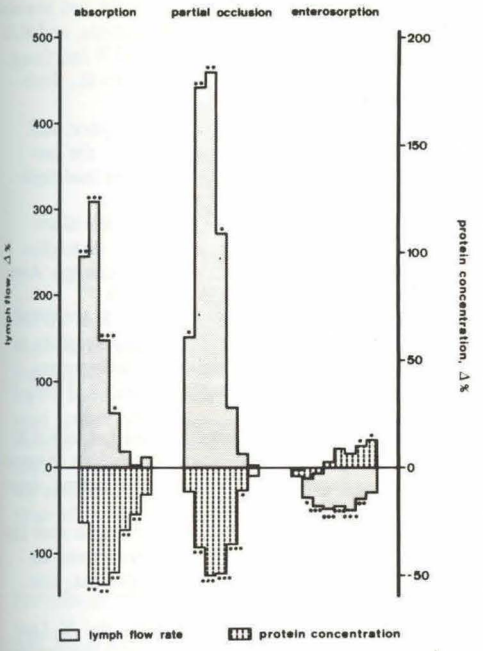

Fig. 8 The relative changes in intestinal lymph flow and protein concentration in rats during absorption, partial occlusion of the portal vein and enterosorption
· $p<0.05$ ·· $p<0.01$ ··· $p<0.001$
in comparison to the control period

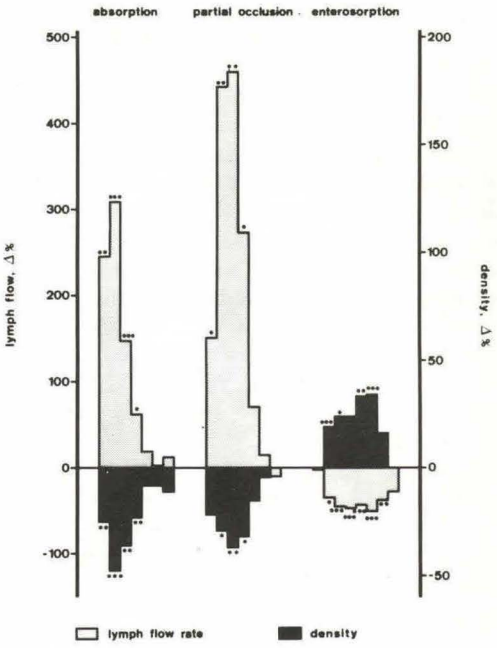

Fig. 9 The relative changes in intestinal lymph flow and density in rats during absorption, partial occlusion of the portal vein and enterosorption
· $p<0.05$ ··· $p<0.01$ ··· $p<0.001$
in comparison to the control period

ume of the capillaries and hence some enlargement of the pores and leaks in the capillary membrane. Under such conditions lymph flow would inevitably increase and the concentration of macromolecules in the lymph would either remain unchanged or would even increase, especially as regards those macromolecules which, simply because of their molecular weight, normally have difficulty in penetrating the membrane. In fact, however, we found that though lymph flow rose considerably there was a relatively slight decrease in the concentration of macromolecules.

It is well known that when venous pressure exceeds a certain critical level there is indeed an increase in lymph flow, but this is accompanied by escape of fluid from the blood into the intestinal lumen. It remains uncertain whether, in these circumstances, macromolecules can also pass through the interstitial tissue and the mucosa into the lumen of the intestine.

Some time ago *Arturson* et al. (1), working with dog heart-lung preparations, showed that the filtration coefficient for dextran under conditions of varying venous pressure depended on the molecular weight of the particular batch of dextran in use. For example, under conditions of raised venous pressure the filtration coefficient for low molecular dextran dropped from 1.0 to 0.7; while for dextrans of molecular size greater than 40,000 the height of the venous pressure had no influence on their filtration coefficient. *Granger* et al. (5), working with the cat's small intestine, measured lymph flow and lymph protein concentration at venous pressures of 10 and 30 mmHg. Whereas lymph flow generally rose by a factor of

15 or more, the protein concentration of the lymph decreased by only 20% of its initial value. Studies of the liver have given data which are at variance with this behaviour (7). As venous pressure rose there was indeed a pressure-proportional increase in lymph flow, but there was no simultaneous decrease in protein filtration coefficient – on the contrary there was an increase. In further experiments on the cat's small intestine, *Granger* et al. (6) showed than when venous pressure was raised from 0 to 30 mmHg lymph flow increased by more than three times, while the protein filtration coefficient diminished only slightly, from 0.58 to 0.39. Other investigations in the dog's lung (13) have shown that when pulmonary venous pressure (left atrial pressure) is raised the resulting increase in lymph flow is much greater than the corresponding decrease in the concentration of total proteins in the lymph.

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