Capillary Permeability to Macromolecules: Stretched Pore Phenomenon

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ABSTRACT

SHIRLEY, H. H., JR., C. G. WOLFRAM, K. WASSERMAN AND H. S. MAYERSON. Capillary permeability to macromolecules: stretched pore phenomenon. Am. J. Physiol. 190(2): 189-193. 1957.—Radioactive iodinated serum albumin and dextran fractions of average molecular weights of 51,000–255,000 are injected into nembutalized dogs, and concentration changes of these substances are followed in plasma and thoracic and right duct lymphs for 4–6 hours. At this time, when a 'steady state' has been established between plasma and lymph, the plasma volumes of the dogs are increased by the infusion of 40 ml/kg of 5% serum albumin in 0.9% saline solution. This results in a significant and striking increase in the concentration of the injected radioactive iodinated albumin and dextrans in right duct and thoracic duct lymph in spite of increased lymph flows. The increase in dextran lymph/plasma concentration ratios occurs with all molecular weight fractions. These results are interpreted as reaffirming our previously formulated concept that infusions producing plasma volume expansion decrease the resistance of the capillary wall to the passage of macromolecules or increase the size of the capillary 'pores.' The concept of capillary permeability as a function of 'pore' size must, therefore, be modified to include a labile capillary 'pore' size, subject to change with variations in plasma volume as well as other factors.

A previous report from this laboratory (1) described results of experiments in which solutions of eight separate dextran fractions of average molecular weights ranging from 10,600 to 412,000 were infused into anesthetized dogs. Their disappearance from the plasma was followed as well as their appearance in thoracic duct lymph. When small volumes (5 ml/kg) were infused, lymph/plasma dextran concentration ratios were inversely related to molecular weight. The lowest molecular weight fraction (average 10,600) showed a concentration ratio of approximately unity and the highest molecular weight fraction (average 412,000) showed a ratio of approximately 0.26. These results are in accord with those postulated by the molecular sieving concept of Pappenheimer (2). The latter suggested that, during ultrafiltration through isoporous membranes, the filtrate/filtrand ratio is not necessarily unity or zero but may be some intermediate value which depends on the size of the molecule relative to the size of the membrane pore and the filtration rate. Molecular sieving is thus conceived as due to steric hindrance and viscous drag of the large molecules during ultrafiltration. From theoretical considerations and experiments with collodion membranes, Pappenheimer shows that the filtrate/filtrand ratios decrease and the ratio will approach unity as the concentrations become more closely the same or the molecular weights become less.
(greater degree of molecular sieving) as the filtration rates increase due to the increased role of viscous drag during high rates of filtration.

In our experiments, infusion of small volumes, as indicated above, gave results which were in accord with Pappenheimer's concept. On the other hand, when we increased ultrafiltration by infusing large volumes (40 ml/kg) of 5% serum albumin or 6% dextran solutions, lymph/plasma concentration ratios for albumin and all molecular weight fractions of dextran increased and tended to approach unity. The changes in lymph/plasma concentration ratios were greatest for the high molecular weight fractions and occurred in spite of increased lymph flows. On the assumption that lymph is a filtrate of plasma, these findings of an increasing ratio of concentration in filtrate or filtrand with increasing filtration rate were contrary to the concept of molecular sieving. We interpreted these results of large infusions as due to increases in pore size so that large molecules encountered less resistance and passed through capillary membranes in higher concentration even though the filtration rate was increased.

Grotte (3), in a recent study, took exception to our conclusions because they were based on data derived from thoracic duct lymph. He points out that thoracic duct lymph in the anesthetized dog is mainly derived from the intestines and liver and that the great permeability of the liver may constitute a special outlet mechanism, i.e. after large infusions of colloidal solutions, the increase in macromolecular concentration measured in the thoracic duct lymph may be due to a relatively greater increase of lymph flow from the liver than from the other sources of thoracic duct lymph (mainly intestine) having a lower permeability to macromolecules. The object of the present experiments was to investigate the existence of the stretched pore phenomenon by using lymph from a more homogeneous source, the right duct. Drinker (4) believed that in the quiescent, anesthetized dog the amount of lymph collected from the right duct expresses the lymph delivery from the contracting heart and the moving lungs. Right duct lymph resembles cervical and leg lymph in that it has a low protein concentration (0.5-3.5 gm%) and, as will be shown later, right duct lymph/plasma concentration ratios for macromolecules are ordinarily low and similar to those found for cervical lymph.

METHODS AND PROCEDURES

The general pattern of the experiments is similar to that previously used (1). Dogs, fed evaporated milk 1 hour earlier, were anesthetized with Nembutal (30 mg/kg) and the right ducts isolated and catheterized with polyethylene tubing by a procedure slightly modified from that described by Drinker (4). The thoracic ducts were also isolated and catheterized. Lymph was collected continuously from both sources. Blood was sampled from the femoral artery through an indwelling Cournand needle and infusions were given into the femoral vein on the contralateral side. The simultaneous collection of lymph from the thoracic and right ducts provides an excellent opportunity to check on the presence or absence of communications between the two vessels. Since the animals are fed milk previous to the start of the experiments, thoracic duct lymph appears as a creamy white fluid in contrast to right duct lymph which is clear in the absence of communications. When the catheters are raised to a vertical position, the contained lymph rises to certain levels. If there are no communications between the two ducts, pressure on the abdomen raises thoracic duct lymph always flows faster than
right duct lymph. When anastomoses are present, the
flow from the right duct increases and appears chylous.

When it was demonstrated by the above methods
that anastomoses were absent between the thoracic
and right lymph ducts, 2 ml/kg of 12% solution of a
dextran fraction of specific molecular weight and 1 ml
of radioactive iodinated albumin were injected simul-
taneously. Four to six hours were allowed for a 'steady
state' to be achieved between blood and lymph. At the
end of this period, plasma volume was increased by the
infusion of 40 ml/kg of 5% serum albumin in 0.9% saline
and the changes in dextran and 1131 albumin
centration in blood, thoracic duct lymph and right
duct lymph followed for the subsequent 2–3 hours.
Radioactivity was measured with a Geiger counter,
albumin by the Howe method and dextran by the
method of Bloom and Wilcox (5) modified according
to a personal communication from R. J. Dimmler by
adding the anthrone to the carbohydrate solutions in
an ice bath followed by color development in a boiling
water bath for 10 minutes.

RESULTS AND DISCUSSION

A typical experiment is shown in figures 1 and 2. Two milliliters per kilogram of 12%
dextran of average molecular weight of 255,000
(NRC fraction 7) were injected simultaneously
with 1 ml radioactive iodinated albumin. Figure 1 illustrates the disappearance of
injected radioactive iodinated albumin from
the plasma and its appearance in thoracic and
right duct lymphs. Initially, lymph contains
a small amount of unlabeled albumin but, with
time, labeled albumin leaking from the capil-
-laries appears in the lymph and a 'steady
state' is reached in which the lymph/plasma
ratio remains essentially constant. The con-
centration of radioactive iodinated albumin in
right duct lymph is considerably less than in
thoracic duct lymph. In the experiment shown
in figure 1, the lymph/plasma albumin ratio for
right duct lymph was 0.077 whereas for
thoracic duct it was 0.508. These data suggest
that capillaries of the lung and heart are
much less permeable to albumin than are
capillaries of liver and intestine.

Four hours and forty minutes after the
original injection and after a 'steady state'
had been reached in thoracic and right duct
lymphs, a 5% serum albumin solution was
infused (40 ml/kg). There was an immediate
sharp drop in plasma radioactive iodinated
albumin concentration, a slight decrease in
thoracic duct lymph concentration and a
marked rise in right duct lymph concentration.
Significantly, the increased concentration in
the latter occurred with minimal decrease (or, in
other experiments, with an increase) in right duct
lymph flow (table 1). The right duct lymph/
plasma concentration ratio rose from 0.077
to 0.515. In the thoracic duct, lymph flow was
approximately double the preinfusion level
while the lymph/plasma concentration ratio
increased from 0.508 to 0.905. Thus, for both
right and left duct lymphs, the lymph/plasma
concentration ratios increased significantly
after the infusion. This can occur only if
capillary porosity has increased, for if capillary
porosity remained constant, lymph/plasma
centration ratios should decrease, since a)
the plasma concentration is reduced to ap-
proximately one-half of its original level and
b) molecular sieving would be enhanced due
to the higher filtration rate (2).

Human serum albumin was furnished by the American
National Red Cross through the courtesy of Dr.
Sam Gibson. 1131 was obtained from the Oak Ridge
National Laboratory.
The pattern of change for the high molecular weight dextran (fig. 2) is similar to that of radioactive iodinated albumin except that the changes are of greater magnitude. Control lymph/plasma dextran ratios were lower than for albumin, 0.031 and 0.266 for right and thoracic duct lymph, respectively, a reflection of the larger molecular size of the dextran as compared to albumin. Following infusion, plasma dextran concentration dropped from a preinfusion level of 820 mg% to 450 mg% and remained at approximately this level for the rest of the experiment. Thoracic duct lymph showed a marked increase in dextran concentration, rising from 220 mg% before the infusion to 280 mg% at the end of the experiment. The lymph/plasma ratio increased from 0.266 to 0.770. Particularly significant was the increase in dextran of right duct lymph, which was approximately 20 mg% before the infusion and rose to 120 mg% at the end of the experiment, approximately 2½ hours after the infusion. The curve of concentration was still rising at this time. The dextran lymph/plasma concentration ratio for right duct lymph rose from .031 to .288.

The magnitude of the changes in lymph/plasma ratios can be better appreciated by reference to figure 3 in which we have plotted the changes for radioactive iodinated albumin (fig. 3A) and for dextran (fig. 3B) when NRC dextran fractions 3, 4, 6 and 7 of average molecular weights of 51,300, 91,700, 194,000, and 255,000, respectively, were used. For these molecules, which have initial ratios of less than unity, there is always a significant and striking increase in albumin and dextran lymph/plasma concentration ratios after the volume is expanded by the 40 ml/kg albumin infusion. In our previous publication (1) we showed that changes in thoracic duct lymph/plasma ratios were greater for larger dextran molecules than for the smaller molecules. The data for right duct lymph show a similar trend. Thus in the experiment shown in figures 1 and 2, the 40 ml/kg serum albumin infusions were always followed by significantly increased lymph flows from the thoracic and right ducts which persisted during the post-infusion period when plasma volumes were falling. The increased concentrations of albumin and dextrans in lymph following the large infusions thus cannot be explained as the result of movement of water into the blood stream leaving the macromolecules more concentrated in the lymph.

With the exception of the experiment shown in figures 1 and 2, the 40 ml/kg serum albumin infusions were always followed by significantly increased lymph flows from the thoracic and right ducts which persisted during the post-infusion period when plasma volumes were falling. The increased concentrations of albumin and dextrans in lymph following the large infusions thus cannot be explained as the result of movement of water into the blood stream leaving the macromolecules more concentrated in the lymph.

These results extend and fully confirm our previous findings and are free from the objections raised by Grotte. As previously indicated, right duct lymph, as collected from the quiescent anesthetized dog, is relatively homogeneous, and in the experiments presented, contains no lymph from the abdominal viscera. Permeability of lung and heart capillaries to macromolecules is low, judged from right duct lymph analysis and compared with the permeability of intestinal and hepatic capillaries (to be published). The results leave little question of the correctness of our previously formulated concept that infusions producing plasma volume expansion cause capillary 'pores' to enlarge or stretch with a resultant increased leakage of large molecules. Our results reaffirm the necessity of modifying the current concept of capillary permeability to include changes in pore size (or in capillary wall resistance to the passage of macromolecules). Changes in pore size undoubtedly account for the significant
loss of plasma volume, plasma protein and macromolecules following large infusions of plasma, blood and blood substitutes (6, 7) and may also account for much of the edema formation in pathological conditions associated with high venous pressure (8).

REFERENCES