CAPILLARY PERMEABILITY TO NARROW-RANGE MACROMOLECULAR DEXTRANS AT NORMAL AND HYPOBARIC PRESSURES

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In view of its varied concepts and interpretations, and because of the discrepancies produced by the previous utilization of polydisperse dextran fractions, a study using extremely narrow-range molecular weight dextran fractions was initiated to reevaluate and consolidate some of the aspects of capillary permeability. A portion of the study was performed under decreased barometric pressure in order to clarify further some of the mechanisms involved in particulate transfer across the capillary endothelial membranes. Gel filtration procedures augmented the study as an assessment of the polydispersity effects of the dextran employed.

Mongrel dogs were chosen as the experimental animal. Their capillary permeability to dextran was facilitated by monitoring dextran concentrations in plasma, thoracic lymph, and leg lymph. Control and simulated altitude groups of animals were examined in four molecular weight fractions of dextran.

The concentrations of macromolecular, narrow-range dextran fractions increase in the thoracic lymph during
exposure to a simulated altitude of 28,000 feet (247 mm Hg). The increases are suggested to originate from a combination of changes in the capillary endothelial membranes, increased central blood volume, and changes in the gel reticulum and pressure balances of the interstitial compartment.

The lymphatic network of the popliteal fossa is found unsatisfactory for use in studying capillary permeability at decreased barometric pressures. A possible alternate method to monitor leg lymph would be the lymphatics of the inguinal fossa.

Gel filtration procedures reveal substantial polydispersity differences between the acquired, narrow-range dextran fractions and commercially prepared dextran. However, no significant differences are evident in their capillary permeability.

It is suggested that further investigations should be made to determine the pooling of dextran and the maximum molecular weight limits of dextran for capillary transport. An increase in both the control and treatment animals would also enhance statistical evaluation.
CAPILLARY PERMEABILITY TO NARROW-RANGE
MACROMOLECULAR DEXTRANS AT NORMAL
AND HYPOBARIC PRESSURES

THESIS

Presented to the Graduate Council of the North Texas State University in Partial Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

By

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CHAPTER I

INTRODUCTION

The capillary wall forms the barrier between the closed mammalian circulatory system and the tissues which it serves. This wall has the distinct characteristic of having a selectively restrictive permeability. With this unique characteristic the wall functions to retain the fluid volume of the blood, yet still allow the numerous components of the circulating plasma to pass across in amounts necessary to the physiological function of the system. This special permeability of the capillary barrier and the mechanisms by which materials are transported across the barrier have long been the subject of physiological investigation. The present work is submitted as an extension of that investigation.

Capillary Permeability

Theory

Rudiments of the study of capillary permeability and the related lymphatic system have been reported as early as the seventeenth century. The most significant developments, however, were brought forth in the middle of the nineteenth century. With the theory of a closed lymphatic system
gaining impetus, related questions concerning the mechanism of capillary permeability and lymph formation arose. Two schools of thought developed to answer these questions (20). The first group, those following the Heidenhain line of thought, claimed that lymph was formed by active secretion on the part of the lymphatic endothelial membrane. A second group, led by Carl Ludwig, adhered to the idea that lymph was formed by filtration. The latter line of reasoning gained experimental prominence, and in 1873 one of Ludwig's students, H. P. Bowditch (7), presented a paper describing some of this experimental evidence. In this paper the connections between the lymphatic vessels and what is now called interstitial spaces in connective tissue were established. It still remained, however, for Ernest Starling to set forth the underlying principles that regulated the dynamic process of capillary permeability.

Prior to Starling's basic pronouncement in 1896, experimentation had been primarily involved with injecting foreign substances into the previously described interstitial space and observing their absorption through natural diffusion into the blood vessels (52). With this in mind, Starling (53) set out in a series of experiments to explain the absorption effects of fluids having the same tonicity and same approximate constitution as the circulating plasma. From the results of these experiments he recognized the distinction
between total osmotic pressure and colloid osmotic pressure as the force regulating fluid balance across the capillary membrane. Thus he was able to establish the simplest description of capillary membrane selectivity: free permeability to crystalloids and water, and relative impermeability to colloids. With a few minor additions, the basic thrust of the Starling hypothesis has remained valid through present investigations.

For several years after Starling submitted his hypothesis to the scientific community, it remained untested. It was not until 1926, when Landis (32) introduced his micro-injection technique, that anyone had the capability to test the theory. His method permitted measurement of mean pressure fluctuations in successive periods without disturbance of blood flow in individual capillaries. Through the use of this technique, he was able to study and report several physiological parameters involving the capillary dynamics in frog mesentery (33, 34). In 1930 Landis (35) applied his technique to mammalian mesenteric capillaries, and his observations indicated a balance between the average capillary blood pressure and the osmotic pressure of the plasma proteins. He reported that the average arteriolar capillary blood pressure was above the colloid osmotic pressure, and the average venous
capillary pressure was below this value. These findings provided evidence in favor of Starling's hypothesis for fluid interchange in mammals.

At the same time that Landis was setting forth the results of his experiments, another group of investigators was discovering some facts that did not seem to coincide with some of Landis's findings. Churchill, Nakazawa, and Drinker (10) measured colloid osmotic pressure of blood serum and lymph from subcutaneous lymph spaces in frogs. With this procedure they were able to show physical and chemical forces acting to produce a normal movement of blood plasma, including a fraction of its protein, from the blood vessels into the lymphatic system. In a more elaborate set of experiments, Conklin (11) demonstrated the presence of serum proteins in lymph. She also discovered that an almost total depletion of protein from plasma and lymph occurred with injection of large amounts of Ringer's solution into the frog's circulatory system. In addition to this, foreign proteins, when injected into the circulatory system, were found to pass through the capillaries and to be recovered in the lymph. This latter phase of experimentation was thought to be somewhat dependent on molecular weight.

With this evidence of serum proteins in amphibian lymph, Drinker and Field (13) published a paper citing
references of lymph content from various regions of different mammals. In almost all instances, the lymph contained serum proteins, usually in concentrations above one per cent. This led Field and Drinker (14) to develop a series of experiments involving the quantitative detection of horse and dog serum in lymph. By ligating various lymphatic branches and by injecting one of the above sera into strategic locations, they were able to show a generalized though graded permeability to protein on the part of blood capillaries in different parts of the dog. This and previous findings modified but did not invalidate Starling's hypothesis. It was determined that the difference between the osmotic pressures of the intra- and extravascular fluids was the important factor. This meant that the variations between the capillary pressure and this "effective" osmotic pressure determined filtration and absorption by the capillary endothelial wall.

This theory was further refined in 1948 by Pappenheimer and Soto-Rivera (41). They developed a technique for perfusion of dog and cat hindlegs so that the protein osmotic pressure of the plasma could be independently adjusted to desired constant values. Included in this perfusion method was the delicate, individual control of capillary and venous pressures. The resulting rate of filtration of fluid from blood to tissues or the absorption
of fluid from tissues to blood was determined respectively as a gain or loss in weight of the leg. By manipulating each of the parameters individually or in pairs, these investigators determined that with any given protein osmotic pressure, there were an infinite number of pairs of values of arterial and venous pressures at which no net transfer of fluid occurs. Two or more pairs of such values were thus defined as the mean hydrostatic pressure in the capillaries. Using this method the two colleagues also established that the principal osmotic factor regulating the fluid exchange normally involves changes in the plasma protein concentration rather than changes in the composition of tissue fluid. As a result of these findings, a clarification and a delicate method of measurement were attained for capillary pressure and effective osmotic pressure described by Field and Drinker (14).

The study of the kinetic aspects of this effective osmotic pressure was greatly enhanced by the use of radio-isotopic tracers. In experiments using trace amounts of albumin and globulin labeled with radioactive iodine (\(^{131}\)I), Wasserman and Mayerson (57) were able to follow the pathway of these serum proteins as they traveled from the plasma to the lymph and back to the plasma. They discovered that both proteins passed through the capillary wall, albumin passing approximately 1.6 times faster than globulin.
After mixing with the pericapillary interstitial fluid, about two-thirds of these "leaked" proteins were returned to the circulatory system via the thoracic lymph duct. Newly metabolized proteins were not detected in the thoracic duct lymph.

In a later study using the same techniques, Wasserman, Joseph, and Mayerson (60) established that the extravascular albumin mass exists as a separate entity. Net movements from this mass occurred when the equilibrium between the vascular and extravascular masses was disturbed. With this last information, the importance of "effective" osmotic pressure, its role and its maintainance in capillary permeability, was essentially determined.

This meant that the only aspect of the Starling hypothesis left to be investigated was the effect of interstitial pressure on the net transcapillary pressure. Early attempts at actual measurement of this pressure centered around the needle or micropuncture technique. It involved inserting a very small needle into a tissue and then determining the minimum pressure required to make fluid flow from the needle into the tissue. A good example of this technique was given by Swann and co-workers (56) in 1950. A major criticism of the method, however, was the fact that at best the inserted needle was still some 300 times as large as the widths of the tissue spaces. As a result, distortion of these spaces and false measurements were thought to occur.
In order to bypass this criticism, Guyton (22) developed a technique using perforated capsules implanted in the tissue spaces. Two to three weeks after implantation, his histological examination of the capsules revealed that they were filled with interstitial fluid, tissue and blood vessels had begun to fill the capsule, and the intra- and extracapsular fluids communicated freely. In essence, he had created a comparatively large interstitial space into which he could insert a small needle and measure the pressure without distortion of the surrounding tissues.

Using this method the workers in Guyton's laboratory set out a series of experiments in 1963 to investigate the importance of interstitial fluid pressure on capillary permeability. Guyton (22) initially established that this pressure was negative and that it only became positive in cases of tissue edema. He also demonstrated that the pressure within the capsule was affected by the classic permeability mediators of arterial pressure, venous pressure, and effective osmotic pressure. A later study (23) depicted these effects in the form of pressure volume curves.

In a series of experiments that climaxed their studies in 1966, Guyton and several of his co-workers (24) used the implanted capsules to compare the effects of changes in interstitial fluid pressure with the effects of changes in venous and arterial pressures. Used in this manner the implanted capsules were described as internal plethysmo-
graphs, and the net movement of fluid through the capillary membranes was measured. As a result of this modification, they were able to demonstrate that a decrease in interstitial fluid pressure of 1 mm Hg increased the filtration out of the capillaries 1.20 times as much as did a 1 mm Hg increase in venous pressure. In addition, a filtration coefficient for fluid movement through the capillary wall per unit change in interstitial fluid pressure was calculated, and this value was compatible with the coefficient calculated by Pappenheimer and Soto-Rivera (41) following changes that they induced in capillary and osmotic pressures.

The primary significance of the experimentation cited to this point was that it unquestionably confirmed the principles of Starling's basic hypothesis. The net transfer of fluids through capillary endothelial membranes was governed by a delicate balance and interplay of two factors: net transcapillary pressure, which was determined by arterial pressure, venous pressure, and interstitial fluid pressure; and (2) effective colloid osmotic pressure, which was determined by plasma colloid osmotic pressure and interstitial fluid colloid osmotic pressure. A pictorial summation of these factors can be seen in Figure 1.
Fig. 1. Summation of physical forces governing net fluid movement in capillaries. Heavy arrows indicate main force affecting fluid movement and direction of fluid movement; C.P. = Capillary Pressure; I.C.O.P. = Interstitial Colloid Osmotic Pressure; I.F.P. = Interstitial Fluid Pressure; P.C.O.P. = Plasma Colloid Osmotic Pressure.

Mechanism

Although the basic principles for capillary permeability were established, the exact mechanisms and criteria for the endothelial membrane selectivity have been and still are a highly debatable subject. Numerous theories have been pro-
posed as an explanation, but as yet none have emerged as a completely acceptable answer.

The process of diffusion has been regarded by most as an integral part of the transfer mechanism, especially that of low molecular weight molecules. As early as 1926, Arnold and Mendel (3) studied the composition of total solids, chlorides, calcium, phosphorus, sugar, non-proteins, and proteins in serum and lymph of dogs. Applying some abnormal physiological concentrations, they concluded that interchanges occurred in response to alterations in concentration gradients. They also observed that diffusable substances passed easily and rapidly at all times between the blood, lymph and tissues. Renkin (48) extended this observation to include carbon dioxide and lipid-soluble molecules. Several other investigators have independently substantiated these findings.

In a comprehensive review of the capillaries presented in 1947, Chambers and Zweifach (9) cited some endothelial properties which were proposed to account for the diffusion observations. Using accepted histological staining techniques and the light microscope, they distinguished three types of cellular membranes on the basis of structural factors regulating the passage of materials. The first two types involved active secretory work by the endothelial cell constituents. This involved either total transport by secretion, as in the endothelial cells of renal proximal
tubules; or partial cell secretory action aided by passive transport through intercellular material, as in intestinal mucosa. The third type that they identified limited permeability and transport to the intercellular cement substance. In this instance the role of the cells was relegated to being building blocks and maintaining the intercellular cement. Thus the relatively undiscriminating nature of permeability in the blood capillary could be explained by this type of endothelium.

This apparent indiscrimination to some molecules on the part of the capillary led Pappenheimer, Renkin, and Borrero (42) to a direct, experimental comparison of them with artificial, porous membranes. The similarities were so evident that they proposed a system of endothelial pores to explain the capillary diffusion. In a 1953 review, Pappenheimer (43) attempted to clarify and summate the existing evidence for capillary endothelial pores. He proposed that uniform, cylindrical pores of radius 30-45 Å and a population density of 1-2 x 10^9 per cm^2 of capillary wall would account for the observed rates of passage of water and lipid insoluble molecules of various sizes. Using these "effective" dimensions, a total cross-sectional area of the pores would comprise less than 0.2 per cent of the histological surface of the capillaries. This fact indicated that the pores were limited to areas between endothelial cells as proposed by Chambers and Zweifach (9).
Pappenheimer made the additional distinction that most or all of the capillary endothelial surface was available for the passage of oxygen, carbon dioxide, and other lipid soluble molecules. These substances were not dependent on pores for transcapillary exchanges.

The transport of larger molecules proved to be a weak point in Pappenheimer's (43) reasoning. Previous investigators had reported decreased diffusion rates for the serum proteins and other large molecules. To explain these decreases, Pappenheimer developed the concept of restricted diffusion or molecular sieving. According to this theory the effective pore size in the capillary wall was sufficiently great to allow even the plasma protein molecules to penetrate the pores. The degree of molecular sieving of any given solute depended upon the ratio of its restricted diffusion coefficient to the filtration rate through the capillary wall. Thus the degree of transport for large molecules was dependent upon their restricted filtration rates through the pores. This explanation left many investigators in doubt, and as a result, experimentation and hypotheses became abundant in this area.

One group of investigators headed by H. S. Mayerson initiated some experimentation in 1955 that dominated the thought in the area of capillary permeability for the next few years. Prior to this time Wasserman and Mayerson (58) had studied the effects of the polysaccharide dextran on
plasma expansion and transfusion. Some of their results suggested that a biological fractionation occurred when the dextran was infused as a heterogeneous mixture of molecular sizes. This led them into a concentrated study of dextran and capillary permeability.

Wasserman, Loeb, and Mayerson (59) obtained eight "well-characterized" fractions of dextran and infused then as mixtures and as discrete fractions. Their results showed that all sizes of dextran fractions, including that with an average molecular weight of 412,000, penetrated the capillaries and entered the thoracic duct lymph. The possibility of large and small pores with unequal distribution in capillary beds was suggested to account for this observation. These studies also suggested the possibility that pore sizes stretched with increased blood volume. This possibility was later substantiated by Shirley and co-workers (50), and the concept of capillary permeability was modified to include a labile capillary pore size, subject to change with variations in plasma volume as well as other factors.

Wasserman and Mayerson's (59) explanation that pores might exist in different sizes and in different proportions in various capillary beds, led Grotte (20) into an attempt to characterize capillary membranes in the dog in terms of their permeability to dextran molecules. This characterization was accomplished by studying dextran in steady state
lymph/plasma ratios and by monitoring the rate of disappearance of dextran from the plasma. Using these procedures, he studied capillary permeability in three regions: the leg, the cervical region, and the liver.

In the leg region, Grotte calculated an approximate pore radius of 35-45 Å. The studies further revealed that larger molecules passed from plasma to lymph in bulk flow, indicating the presence of capillary leaks of at least 120 Å radius. Results for the cervical and liver capillaries were similar except that the number of "leaks" greatly increased with each region. In a subsequent study, Grotte, Juhlin, and Sandberg (21) used solid spherical particles of methylmethacrylate (radius 300-700 Å) to further characterize the capillary "leaks". The radii of the "leaks" were thought to be in the range of 120-300 Å in the leg, heart, and bronchial capillaries and above 700 Å in the liver.

At about this point in time, the morphologists and electron microscope began to have some bearing on the physiologists viewpoint of capillary permeability. Palade (40) had first reported the presence of vesicles in the endothelial cells of capillaries in 1953. He proposed then that they were the mechanism for capillary transport. His reports were largely ignored, however, because physiologists regarded his vesicles as artifacts in tissue preparation for the electron microscope. This remained the circumstance
even with increasing reports of vesicular presence in capillary endothelium.

In 1959, Bennet, Luft, and Hampton (6) presented the morphological features of blood capillaries from various vertebrate forms of organs. They attempted a simple classification using structural features as criteria. The most significant part of their work, however, was the fact that they could find no perforations or pores as described by Pappenheimer (43) in capillary endothelial cells. They termed this failure very significant and suggested that a reconsideration and reinvestigation of the concepts of capillary physiology was in order.

This reevaluation was begun by Mayerson and his colleagues (39). They used the same procedures as in a previous study (59), but the dextran fractions were monitored from three regions (cervical, intestinal, and hepatic) rather than from one as before. The data they obtained were somewhat similar to Grotte's (20) regional study. It indicated the presence of two discrete sets of "pores": (1) a set of "small pores" allowing passage of molecules not greater than 250,000 MW; (2) a set of "large pores" permitting passage of molecules of at least 412,000 MW. In view of the lack of favorable electron microscopic evidence, however, Mayerson and his co-workers proposed an alternate theory of transport for the molecules greater than 250,000 MW. The proposal was essentially vesicular
transport, but they called it "cytopempsis". This description was employed to convey the idea that substances were being transported through the cytoplasm rather than being engulfed for cell use as in "pinocytosis".

As the physiologists began to reconcile their position, the morphologists also had to change their views. With improved techniques in electron microscopy, Luft (37) reported some of the same observations as Chambers and Zweifach (9) had seen with a light microscope. He used ruthenium red with the electron microscope and confirmed for the first time the presence of the "endocapillary layer" and its continuation between the cells as the "intercellular cement". Luft further observed that the tight junction of the intercellular cement at the region of endothelial contact could still remain porous. In this case, Starling's hypothesis had a plausible foundation in structure without the need to invoke vesicular transport of fluid across the capillary wall. This fact did not account for the transport of large molecules, however.

A survey of the current literature does not resolve this question any more satisfactorily than earlier reports. Numerous vesicular transport models based on electron microscope studies are available. Shea and Karnovsky (49) seem to have the most prominent model. It employs the use of horseradish peroxidase, and it is based on Brownian movement of vesicles through the endothelial cells. The work of
Casley-Smith (8) in measuring, numbering, and calculating the rate of transport of these vesicles supports the model fairly well.

Garlick and Renkin (15) on the other hand, have attacked the question from an experimental aspect. Using classical infusion and monitoring techniques of dextran, they study the permeability characteristics of capillaries in the dog's paw. With the addition of mathematical calculations to their experimentation, they favor a system of small pores of 40 Å radius for transport of small molecules. In order to carry the larger molecules, Garlick and Renkin propose either a few large pores of approximately 800 Å radius or pinocytotic vesicles, of approximately 250 Å radius. It is obvious that much knowledge has been gained since Starling set forth his hypothesis in 1896, but it is also evident from the lack of decisive agreement of these and other investigators that much more knowledge still remains to be attained.

Altitude Studies

The effects of high altitude were probably first noticed by the early explorers as they scaled the mountains in search of various goals. Indeed, their reports of some of these effects stimulated some of the initial scientific expeditions. Douglas and his associates (12) reported one of these classical scientific adventures in 1913. They
traveled to the top of Pike's Peak and described how hypoxia or oxygen deficiency progressively stimulated their ventilation. As a result, their alveolar CO₂ tension fell as they ascended to the summit. Such reports relating to hypoxia and its associated effects have dominated the literature in the area of altitude physiology.

Alberto Hurtado (29), who with his research group in Lima, Peru, has been the most active investigator in this area, related the rationale behind the predominance of hypoxia-related reports. At reduced barometric pressure, he explained that the partial pressure of oxygen in the inspired air was low. As a result, the hemoglobin of the blood circulating through the lungs became less saturated with this gas. This fact, together with the decreased tension of the fraction physically dissolved in the plasma, made its diffusion and utilization at a tissue level more difficult. A variety of coordinated mechanisms compensated in an attempt to relieve the stress placed on the tissues.

Reports of these compensatory mechanisms, both long and short term, have covered a myriad of physiological phenomena. Shifts in oxygen-hemoglobin dissociation curves, changes in pH of body fluids, increases in hemopoietic activity, and various aspects of pulmonary edema are a few of the areas in which altitude research has flourished. The primary concern of the present study, however, involves
cardiovascular responses to short-term, simulated altitude with an attempt to eliminate the effects of hypoxia.

To eliminate the effects of hypoxia, the most natural answer was to administer a supply of oxygen to the experimental subject during the test period. Whitehorn, Edelman, and Hitchcock (62) examined this solution in 1946. Allowing their subjects to breathe 100 per cent oxygen at normal barometric pressure, they determined resulting cardiac outputs from ballistocardiograms. Their results indicated a maintainance of the blood pressure level in spite of reduced cardiac output. This was interpreted as evidence of an increase in the general peripheral vascular resistance. The investigators suggested that the effects be remembered in conditions of hyperoxygenation of the blood, such as denitrogenation, and that their presence may play a part in the physiological responses to changes in barometric pressure.

A direct application of these suggestions was presented by Girling and Maheux (16) in 1952. They studied the peripheral circulation of rabbits at 10,000, 20,000, and 30,000 feet simulated altitude. Animals taken above this level demonstrated fatal signs of anoxia, but animals supplied with oxygen could safely withstand the additional decreased pressure. The peripheral resistance exhibited the same response with or without the supplemental oxygen; small change in resistance to 20,000 feet, but an appreciable increase in resistance from ground to 30,000 feet. In
order to eliminate the possibility of nitrogen bubbles forming in the blood and increasing peripheral resistance, a subsequent study (17) by the same investigators employed preoxygenation periods of up to four hours. The results remained the same, however, and they concluded that the observed vasomotor response was due to reduced barometric pressure causing decreased critical closing pressures of peripheral blood vessels.

In an attempt to observe the effects of simulated altitudes of 60,000 and 80,000 feet, Sullivan and De Gennaro (54) studied the peripheral circulation in the web of a frog's foot. Their observations suggested that the extent of decrease in blood flow was dependent on the altitude, the length of time the animal was subjected to this altitude, and whether or not air was supplied to the animal during decompression. These results were thought to be primarily due to the hypoxia accompanying decompression, but the investigators also suggested that the initial, reduced venous flow was due to stasis following increased capillary endothelial permeability under the influence of hypoxia. At any rate, the effects could be alleviated by reducing hypoxia.

In 1966 Marotta and Boon (38) extended the studies of oxygenation at simulated altitudes to dogs. They monitored femoral arterial blood flow and related cardiopulmonary parameters during exposure to altitude (9,000, 17,000, and
25,000 feet simulated altitude) while breathing 100 per cent oxygen or a mixture of oxygen and nitrogen. One series of experimental animals were subjected to per-altitude denitrogenation with 100 per cent oxygen at ground level. A decrease in femoral arterial blood flow was observed in all animals except those denitrogenated prior to ascent. Initial and final blood flows in the latter animals, however, were all less than in the other animals. To this extent, denitrogenation exhibited some slowing effect in all of the animals examined. It thus appeared that increased peripheral resistance to blood flow was an unavoidable legacy in the attempt to overcome the more severe effects of hypoxia. Experimentation has thus proceeded with and without the aid of supplemental oxygen depending on the nature of the experiment involved.

Another cardiovascular response that interested investigators in recent years has been the fluid shifts and the blood component imbalances that developed with the encounter of low barometric pressure. A general plasma volume decrease had long been accepted as a part of the altitude acclimatization process, but it had been thought this loss was due to body dehydration. In 1969 Hannon, Chinn, and Shields (25) used a new, more reliable method for determining total body water. As a result they reported a decreased plasma volume with a static or slightly raised total body water. Further investigation (26) of the extra-
vascular compartments showed decreases in interstitial and extracellular spaces and an increase in intracellular space. This was thought to be caused by osmotic effects associated with a transfer of electrolytes, principally, sodium, chloride, and bicarbonate. Krzywicki and associates (31) recently reported, however, that their experimentation indicated that the loss in body weight was still due to loss in total body water. They maintained that this hypohydration was an adaptive mechanism to altitude. At this point in experimentation, the purpose and destination of fluid shifts in subjects maintained at altitude remains unresolved.

Another baffling question of recent years has been the shift in components of the blood with the advent of altitude. Surks (55) reported an initial albumin shift from intravascular to extravascular compartments with an increased degradation of albumin during initial stages of altitude. After six days at altitude, however, the shift was reversed from the extravascular to the intravascular compartments. This latter shift was accompanied by a decrease in the rate of albumin synthesis and net transfer. Westergaard and his associates (61) also observed the initial shift in albumin from intravascular to extravascular compartments. They suggested that this might be due to increased capillary permeability, but a direct measurement was not performed. Independent attempts were recently made by Parker (44) and
Reaves (47) to directly measure the capillary permeability of dogs at altitude. Their results both indicated an increase in capillary permeability possibly due to stretched pores. As in the case of fluid shifts, however, the exact purpose and mechanism of blood component shifts at high altitude levels still remains to be determined.

**Molecular Weight Determination by Gel Filtration**

The class of polysaccharides called dextrans consists of a great variety of α-polyglucosans produced by the coccus *Leuconostoc mesenteroides* and closely related bacteria under suitable environmental conditions. In their native state, the dextran chains are composed of about 200,000 glucose units, corresponding to a molecular weight of about 40 million. Partial hydrolysis of these chains yields smaller molecular weight molecules that exhibit desirable characteristics for experimental use. They appear to be electrically neutral, metabolically inert (no mammalian tissue extract is known to break them down), apparently non-toxic in high concentration, and can be assayed accurately and easily (51). Their one drawback in most experimental endeavors is that they are very difficult to obtain in narrow-range molecular weight fractions. Thus, experimentors employing their use must in some way lessen the effects of the molecular weight dispersity, or they must acknowledge the limitations placed
on the validity of their results by the use of the wide range of molecular weight molecules.

One of the methods by which the molecular weight range of dextrans can be narrowed, or at least estimated, is gel filtration. In this process, a mixture of molecules is passed through a column of porous gel granules. The molecules appear in the effluent in order of decreasing size. Fractionation is believed to occur when diffusion of the molecules into the pores is restricted but not prevented because of their size. As a result of this restriction, the molecules pass through the column at rates that are related inversely to the fluid volume accessible to them within the column. This molecular sieving is the basis by which different and somewhat narrow-range dextran fractions can be obtained. An estimation of their molecular weight may also be determined as a result of this basic principle.

An early application of gel filtration to large molecules was attempted by Lathe and Ruthven (36) in 1956. Using columns of starch grains, they were able to achieve some separation of polypeptides and proteins. They proposed that their method be used as a means of molecular weight determination, and they reported some interesting results in this connection. Their starch columns, however, did not effect enough separation between proteins for further development of the idea with this particular gel.
Numerous other types of porous gels were introduced, however, and the idea of molecular weight determination was applied to them. One of the more successful gels was a cross-linked dextran gel introduced by Porath (46) in 1960. He reported that highly cross-linked dextran gels separated amino acids from proteins and large peptides. Gels possessing a low degree of cross-linking even retained proteins for fractionation. This dextran gel was later used by Granath and Flodin (18) to fractionate low molecular weight dextrans. These two investigators were able to establish the limits of the molecular weight sizes of the gels involved. With further use and development a series of cross-linked dextran gels was marketed under the trade name of Sephadex.

By using G-75 and G-100 of the Sephadex series, Andrews (1) was able to correlate the elution volumes, $V_e$, and molecular weights of proteins of known molecular weight. Within the limits of certain ranges, the gels gave optimum separation of proteins according to molecular weight, but their useful working ranges extended only to about 70,000 and 150,000 respectively. The complete exclusion limits for proteins was estimated somewhat higher, however. As a final test, Andrews used his columns to estimate the molecular weights of various enzymes, and he found that these values were in agreement with those reported in the literature. He had essentially calibrated Sephadex columns within
prescribed limits for the determination of molecular weights of proteins.

In 1967 Granath and Kvist (19) were able to apply the same basic procedures to calibration of dextrans. This was more difficult considering the polydispersity characteristics of dextrans, and certain physical and mathematical modifications had to be employed. The results were comparable to those obtained for proteins. Thus, the calibration of porous gel columns for molecular weight determination of proteins and dextrans had been accomplished. They were only useful, however, for molecular weights up to approximately 800,000 for compact, globular molecules like proteins, or approximately 200,000 for randomly coiled chain molecules like dextran (30). The fractionation and molecular weight determination of larger molecules and particles required a gel filtration medium of much higher porosity.

In 1961, Polson (45) had described a method in which granulated agar was used for separating protein compounds of different molecular weights. The method demonstrated that molecules ranging in molecular weights from 13,000 to several millions could be distinguished. Hence, Polson proposed that his gel be used in conjunction with the linked dextran gels for a powerful fractionation of substances of very low to very high molecular weights. The use of granulated agar, however, was severely limited due to variable gelling and adsorption properties encountered. These
variations were due to the poorly defined composition of commercially available agar which varied from batch to batch.

Araki (2) reported that the unsatisfactory characteristics of granulated agar as a medium for gel filtration was due to the fact that it consisted of two different polysaccharides, agaropectin and agarose. It was determined that agaropectin was a typical pectic substance containing a high percentage of sulfate and carboxyl groups. These constituents seemed to impart the undesirable adsorption effects seen in gel filtration. Agarose, on the other hand, was classified as an unchanged polysaccharide that consisted of alternating D-galactose and 3,6-anhydro-L-galactose units. It had the same good gelling properties as agar and was thus a superior medium for gel filtration.

Even with this discovery, the use of agarose in gel filtration was not widespread until Hjertén (27) developed a simple technique to remove the agaropectin from agar solutions by precipitation with cetylpyridinium chloride. A further development by Hjertén (28) and Bengtsson and Philipson (5) established it as a bonafide gel filtration medium. These investigators independently developed procedures for bead-gelling of agarose solutions. Photomicrographs of these beads showed a granular structure, but the nature of the granules was not readily explainable (30). The beaded agarose gels have been marketed in series,
similar to the Sephadex series, under the trade name of Sepharose. Their fractionation characteristics are similar to those of Sephadex, but their molecular weight exclusion limits extend into the millions.

In a recent article, Arturson and Granath (4) used Sepharose 4B as the gel medium for molecular weight calibration of narrow-range, high molecular weight dextrans. Their method was similar to that used by Granath and Kvist (19), who used Sephadex as the gel medium. The procedure thus yielded the first gel filtration columns capable of fractionating and detecting extremely high molecular weight dextrans. Its use was demonstrated in the study of biological membranes, and it was evident that its future use in this area could aid in the explanations of capillary dynamics and permeability.

Statement of the Problem

In view of its varied concepts and interpretations, and because of the discrepancies produced by the previous utilization of polydispersed dextrans, a study using extremely narrow-range molecular weight dextran fractions was initiated to reevaluate and consolidate some of the aspects of capillary permeability. A portion of the study was performed under decreased barometric pressure in order to clarify further some of the mechanisms involved in particulate transfer across the capillary endothelial
membranes. Gel filtration procedures augmented the study as an assessment of the polydispersity effects of the dextrans employed.
CHAPTER BIBLIOGRAPHY


CHAPTER II

METHODS AND MATERIALS

Experimental Design

Dextran was chosen as the experimental molecule in the study of capillary permeability because of its use in previous studies (1, 2, 8), and because of its desirable physical and chemical properties (7). Through the courtesy of Dr. Kirsti Granath, Uppsala, Sweden, five extremely narrow-range molecular weight fractions of dextran were obtained. These fractions were much narrower than those used in previous studies, and therefore, the false indications of permeability due to the trailing smaller molecular weight molecules would be decreased significantly. Some lower molecular weight contamination could still bias the results, but the realization of this fact would eliminate the tendency to make erroneous permeability conclusions. With these limitations established, experimentation was initiated using these narrow-range fractions as test molecules. Their physical characteristics as determined by Dr. Granath can be seen in Table I.
TABLE I
PHYSICAL PROPERTIES OF DEXTRAN FRACTIONS
AS DETERMINED BY GRANATH

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>$\bar{M}_w^*$</th>
<th>$\eta$</th>
<th>$\bar{M}_w/\bar{M}_n^{**}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>720,000</td>
<td>0.622</td>
<td>Between</td>
</tr>
<tr>
<td>6</td>
<td>581,000</td>
<td>0.590</td>
<td>1.22-1.25</td>
</tr>
<tr>
<td>7</td>
<td>467,000</td>
<td>0.565</td>
<td>for all</td>
</tr>
<tr>
<td>8</td>
<td>409,000</td>
<td>0.530</td>
<td>fractions.</td>
</tr>
<tr>
<td>9</td>
<td>312,000</td>
<td>0.496</td>
<td></td>
</tr>
</tbody>
</table>

* Determined by light scattering

** Analyzed by gel chromatography on Sepharose 4B

A total of twenty mongrel dogs, with an average weight of 10 kg, were utilized to study the transport of selected fractions of these macromolecular dextrans across the capillary endothelial barrier. On these experimental animals, seven involved the use of Fraction 9 (312,000 mol wt); five involved the use of Fraction 8 (409,000 mol wt); and two involved the use of Fraction 5 (720,000 mol wt). The six remaining animals were examined using Dextran 500 which had a $\bar{M}_w$ of approximately 500,000 (Lot no. 4376 obtained from Pharmacia Fine Chemicals, Uppsala, Sweden). With these molecular weight divisions as identifying characteristics, the animals were classified respectively as Groups A, B, C, and D.

Two dogs were designated as ground level control animals within each experimental group. Those animals remaining in the groups were subjected to 28,000 feet
(247 mm Hg) simulated altitude. The period at altitude lasted 2 hr, and it was preceded by a 2½-hr equilibration period. A 1-hr, post-altitude stabilization period concluded the experimental run. The rate of ascent and descent during the run was 4,000 ft/min. The animals were denitrogenated by breathing 100% oxygen 30 min prior to altitude, and their respiration was continually supported by this oxygen supply until 30 min after the period at altitude. Ground level control animals were studied under the same experimental parameters, except they remained at ground level during the period designated for simulated altitude. These altitude studies were deleted in Group C.

Under these criteria established for experimental study, each individual animal was examined under identical surgical and analytical procedures. Every attempt was made to reduce the occurrence of procedural variations. This experimental format was thus designed to test capillary permeability under the individual and collective influences of dextran molecular weight polydispersity and decreased barometric pressure.

Preparation of the Animals

It was necessary to monitor both the circulatory system and the lymphatic system in order to accurately observe the permeability of the capillary walls. Since it has been established that the permeability of
capillaries may vary in different regions of the body (3) it was also desirable to monitor more than one lymphatic drainage region. The following surgery and cannulation procedures were developed to satisfy these monitoring requirements. These procedures involve the thoracic lymph duct, which collects lymph primarily from the liver, intestines, and spleen; the popliteal lymph "network," which collects lymph from the subcutaneous and superficial muscles of the lower leg; and the femoral artery, which was used as the common monitor of the circulatory system as a whole.

**Surgery Procedures**

Approximately 30 min before administration of anesthesia, the dog was fed 50 ml of condensed milk. The absorption of this fatty substance into the intestinal lymph subsequently rendered the thoracic lymph a milky white. As a result, the thoracic lymph duct could be easily identified in later dissection. The animal was anesthetized by an intravenous injection of sodium pentobarbital, 30 mg/kg body weight, in the cephalic vein. A glass endotracheal cannula was inserted in the trachea, and the proposed surgical areas were shaved.

A 3-inch incision, beginning at the base of the neck, was made directly above the left external jugular vein. The external jugular, subclavian, and transverse scapular veins were exposed by blunt dissection. The entrance of
the thoracic lymph duct into the venous system was generally located in an area under the external jugular and around the junction of this vein with the subclavian vein. Anatomical variations were observed due to developmental anomalies and the use of mongrel dogs. Once it was identified, however, approximately 2 inches of the duct were dissected free of adipose and connective tissue for cannulation. A small portion of the transverse scapular vein was also cleared for subsequent bypass cannulation. At this point the incision was packed with moist, saline pads, and the remaining surgery was performed.

After performing a left femoral cut down to expose the femoral artery, the animal was turned on its ventral side and arranged for maximum exposure of the right popliteal fossa. The popliteal lymph nodes were located in the fossa by palpation, and a 1½-inch incision was made immediately distal to the lymph nodes and medial to the clearly visible external saphenous vein. Extraneous bleeding from small skin vessels was eliminated by cauterization. Two larger lymphatics could be found by blunt dissection on either side of and adjacent to the external saphenous vein. The lymphatic usually chosen for cannulation, however, was on the medial side of the vein. The remaining lymphatic branches, which were sometimes very numerous, were ligated. A schematic diagram of the lymphatic drainage of the dog leg can be seen in Figure 2.
Fig. 2. Circulatory and lymphatic drainage of the dog's leg. Point of lymphatic cannulation is indicated. Labels: e.s. = external saphenous, f. = femoral vein, i.s. = internal saphenous, l. = lymphatic, p.n. = popliteal lymph node.
Cannulation Procedures

When all dissection was completed, the dog was heparinized by injecting sodium heparin, 500 USP units/kg body weight, into the left external saphenous vein. All cannulae were also filled with heparinized saline. The leg lymphatic selected for cannulation was then ligated and allowed to swell from lymph pressure increased by squeezing the paw. Using iris scissors, a small, diagonal slit was cut in the side of the duct. This created an open flap through which Clay Adams PE 10 Intramedic polyethylene tubing could be inserted. Since numerous valves were present in the duct, the tip of the cannula had to be positioned between the valves to insure continuous lymph flow. The cannula was then secured with two ligatures, and the incision was closed with three stay sutures.

After completion of the leg lymphatic cannulation, the animal was returned to his dorsal side, and the left femoral artery was cannulated proximally and distally using PE 280 polyethylene tubing. This created an arterial bypass for blood sampling and further injections.

The thoracic lymph duct was then cannulated by a procedure similar to that used for the leg. The natural thoracic lymph pressure, however, was sufficient to greatly enlarge the duct after initial ligation without additional external pressure. The diagonal cut was made, and PE 190 or PE 90 polyethylene tubing was inserted a distance of
2-3 cm. One ligature was made to secure the cannula in place. A second cannula of PE 205 polyethylene tubing was inserted proximally and secured in the transverse scapular vein. The distal end of this cannula was then joined to the thoracic duct cannula. A lymph-external jugular bypass was thus formed that only slightly modified the natural route of the lymph. This completed the surgery and cannulation procedures, and the dog was moved to the decompression chamber.

Decompression Chamber

The decompression chamber utilized in this study was designed and built by Dallas Radionics, Inc., Dallas, Texas. It consisted of a large steel cylinder with large side windows and end port of plexiglass allowing continual observation of the experimental animal. The chamber was equipped with a Welch Scientific Company vacuum pump, and the pump was connected to a Wallace and Tiernan model FA-193100 absolute pressure contractor. Through these connections, the barometric pressure could be regulated over a very narrow range. The rate of decompression was monitored by a Pioneer Company model 1636-6ABL rate of climb control mechanism also connected to the vacuum pump. The absolute pressure was continuously measured by a Wallace and Tiernan model FA 129 absolute pressure gauge. These gauges and controls allowed the intricate manipulation and observation of the pressure within the chamber.
The dog was placed in this decompression chamber for the experimental period. The animal's right foot, having been previously wrapped in a constant heat pad, was fitted in a sling attached to a rotary device. The toe of the dog's foot was then anchored so that a passive muscular action of the leg was achieved when the rotary device was set in motion (20-30 rpm). This setup was designed to produce a milking action on the lymphatics and hence, a constant flow of lymph. The temperature of the dog's foot was monitored at $38 \pm 2^\circ$ C by a Yellow Springs Instrument Company, Inc., Tele-thermometer, model 43 TD. It was equipped with a thermistor probe, model 402, and the temperature was controlled manually through a Powerstat, Superior Electric Company type 116, connected to the constant heat pad. Both the rotary device and the Powerstat were joined to an external power source in one wall of the chamber, and the power source could be externally controlled during decompression.

Additional apparatus was employed to obtain a complete monitoring picture of the dog's physiological parameters at altitude. The arterial and thoracic lymph bypasses were exteriorized through one side of the chamber and connected to three-way luer stopcocks to allow sampling. Lymph samples from the leg were collected continuously in a tube inside the chamber. A Statham differential pressure strain gauge, model PR 23-40-300, located inside the chamber was
used to monitor the femoral arterial blood pressure. Additional electrical outlets on the side of the chamber connected the strain gauge to a Brush Universal amplifier, model RD-5612-00, and ink writing oscillograph, model RD-2321-00. The 100% oxygen that was supplied to the dog was vented through a valve located on the floor of the chamber. Tygon tubing was used to connect the valve to a T-tube on the endotracheal cannula. The open arm of the T-tube had a flutter valve to aid in regulating the oxygen flow and to assure that the animal did not inhale chamber air. Experimentation was ready to proceed when these procedures were completed.

**Sampling Procedures**

Initial samples of 4 ml of blood, 1½ ml of thoracic lymph, and approximately 0.5 ml of leg lymph were obtained. An initial blood pressure reading was also observed. After these preliminary observations were made, a 12% dextran solution containing a measured dosage of 20 mg dextran/kg body weight was injected into the femoral artery bypass. This injection also contained minute portions (ca. 0.2 mg/ml) of sodium azide for the fractions other than Dextran 500. From this point until termination, blood pressure, readings, blood samples, and thoracic lymph samples were taken at 15-min intervals. The volume was maintained at a constant level by immediate replacement of sample volume with
isotonic saline. The leg lymph samples were collected as flow would allow, preferably at 30- or 45-min intervals.

After the samples were acquired, several preparatory steps occurred before analysis could proceed. Hematocrits were obtained from each fresh blood sample using an Adams Autocrit centrifuge, model CT 2905. The remainder of each blood sample and the lymph samples were then centrifuged in an International Equipment Company clinical centrifuge, model CL 46417M-6, for 10 min at 3,000 rpm. The plasma and cell-free lymph thus obtained were pipetted to sterile plastic refrigerator tubes. The samples were then stored at 4° C until analysis procedures could be performed.

Dextran Analysis

The detection and quantitation of dextran in plasma and lymph was accomplished using the combined, modified procedures of Semple (5) and Scott and Melvin (6). A 1-ml portion of each experimental sample was deproteinized with 5 ml of 5% trichloroacetic acid (J. T. Baker Chemical Co.). Each was mixed on a Vortex shaker and allowed to stand for 10 min. They were then centrifuged for 20 min at 3,000 rpm in the IEC centrifuge. Volumetric portions of 4 ml of the resulting deproteinized supernatants were placed in 3/4-inch dialysis bags. The bags had been previously soaked in distilled water for 12 hr, washed thoroughly, tied at one end, and fitted with a short piece of tygon tubing which was held tightly in place by a rubber band. The open end
of the tygon tubing was securely plugged with a glass hook, and the whole assemblage was tied to a metal dialysis rack. When all of the deproteinized plasma or lymph samples from one dog were tied on the rack, they were dialyzed overnight against distilled, deionized water to remove the blood glucose. Dialysis was carried out for an initial minimum period of 2 hr at 40° C in a 17-liter water bath equipped with a Precision Scientific Company heater and circulator, model 66590. During this period, an additional 21 liters of distilled, deionized water was passed through the bath by continuous flow. The samples were transferred to 5 ml volumetric flasks when dialysis was completed, and the flasks were filled to mark with distilled, deionized water.

Volumetric samples of 2 ml were removed from the flasks and placed in test tubes in an ice water bath. Anthrone reagent (200 mg anthrone/100 ml of concentrated Reagent ACS sulfuric acid) was added to each tube in 4-ml quantities. A truncated 4-ml pipette was used for fast, uniform reagent addition. The tubes were then mixed by the Vortex shaker, topped with a marble, and placed in an Electric Hotpack Company water bath, model 106. The samples were thus incubated at 90 ± 2° C for 16 min to develop the color. Immediately after removal from the hot water bath, the samples were returned to the ice water bath. They were removed from the ice water bath after a short period of time and allowed to return to room temperature.
Each sample was then transferred to a quartz cuvette and read in a Perkin-Elmer double beam spectrophotometer, Coleman model 124. The reference cell contained a distilled water-anthrone blank, and the wavelength was set at 625 nm. The optical density readings for the samples were converted to dextran concentration in mg/100 ml according to the following formula:

$$C_u = \frac{\text{OD}_u - \text{OD}_c}{\text{OD}_s} \times C_s \times 6f \times k,$$

where \(C_u\) = concentration of dextran in unknown sample mg/100 ml; \(\text{OD}_u\) and \(\text{OD}_c\) = the optical densities of extracts from unknown and control (initial) samples respectively; \(\text{OD}_s\) = optical density of the standard; \(C_s\) = concentration of the standard (always 5 mg/100 ml); \(6f\) = the dilution factor resulting from deproteinization and dilution to 5 ml (\(f = 1.25\)); and \(k\) = a hydrolysis constant (1.0 for dextran standards). Calculated in this manner, the converted concentrations were then presented as data for evaluation and statistical analysis.

**Molecular Weight Determination by Gel Filtration**

The gel filtration procedures employed in this study were used to compare the polydispersity of the individual dextran fractions. The column used for the study was an
Ace Glass, Inc. stacked column, composed of two 600 x 25 mm columns joined by a column connector. An 18-inch flow adaptor was placed on the lower end of the column, and the column was packed with Sepharose 6B (Sigma Chemical Company) to a height of 93 cm. A double-vented plug was inserted into the top of the column to allow sample addition and fluid flow from the reservoir. The reservoir was filled with 0.85% sodium chloride for the liquid phase. In addition, 0.02% sodium azide was added as a bacteriostatic agent. A bubble trap was also incorporated between the reservoir and the top of the column. The column was allowed to run by descending flow for a few days until the gel bed had stabilized at a height of 91.5 cm. At that time, a 2.5 cm diameter filter paper disc was placed on top of the gel bed, and the void volume was determined.

Determination of void volume ($V_0$) was accomplished using Blue Dextran 2000 (10 mg/ml in 0.2 M NaCl) was layered on the filter paper disc. The vents were closed, and the column was run at a hydrostatic pressure of 10-20 cm H$_2$O. The samples were collected by an Instrumentation Specialties Company fraction collector, model 327. Equipped with an ISCO drop counter, model 600, the fraction collector was programed to allow 150 drops/collection tube. The volume was checked by graduated cylinder measurement. As the Blue Dextran began to appear in the collection tubes, the samples were transferred to cuvettes and read in the double beam
spectrophotometer at 623 nm. A plot of the optical density vs. volume yielded the void volume as the extrapolated high point of the curve.

The dextran fractions described in Table I were individually applied to the column. The procedure employed was identical to that used for determining $V_o$, except $2{1\over 2}$ ml were applied to the column instead of 3 ml. After a volume equal to $V_o$ had been collected, the collections were saved and diluted 1:4 with distilled water. A 2-ml sample of each dilution was analyzed using the anthrone reaction described previously. The optical density vs. volume curve was plotted for each fraction, and the extrapolated high point was determined to be the elution volume ($V_e$) for that particular fraction.

The total volume ($V_t$) of the column was determined in the same manner as $V_o$ and $V_e$. L-Tryptophan ($1{1\over 2}$ ml at 1 mg/ml in 0.2 M NaCl) was the molecule used to determine $V_t$, and the optical density was read at 280 nm. The extrapolated high point of the optical density vs. volume curve was used as the determining point of $V_t$. After this value was established the void volume was rerun to see if any shifts had occurred in the gel bed.

Calibration of the Sepharose 6B column was completed by calculating the partition coefficient ($K_{av}$) between the liquid phase and the gel phase for each Dextran fraction used. This value was obtained by using the following formula:
\[ K_{av} = \frac{V_e - V_0}{V_t - V_0} \]

The \( K_{av} \) for each fraction was then plotted against its logarithmic molecular weight. The line resulting from this plot represented the calibration curve for the column (4).

The molecular weight of an unknown dextran fraction was then examined on the calibrated Sepharose 6B column. The unknown fraction was composed of a combined collection of highly concentrated aliquots obtained from gel filtration of Dextran 500. The column used for this additional gel filtration study was a Pharmacia K 15/90 (I.D. = 1.5 cm, length = 90 cm) packed with Sephadex G-200 (Sigma Chemical Company). Operation of the filtration procedure was similar to that of the Sepharose 6B column, except that 2 ml of sample (5 mg/ml in 0.2 M NaCl) was layered on the bed surface. The aliquots containing the most dextran were determined by the anthrone reaction. These samples were then pooled, dialyzed by procedures previously described, and lyophilized. The resulting dextran flakes were dissolved in 0.2 M NaCl at a concentration of 10 mg/ml. A sample of 2½ ml was layered on the Sepharose 6B column, and the \( V_e \) was determined. \( K_{av} \) for the unknown dextran was then calculated and compared to the calibration curve previously plotted. In this manner, an estimate of the degree of polydispersity comparison of Dextran 500 was made with the narrow-range dextran fractions described in Table I.
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CHAPTER III

RESULTS

Capillary Permeability

The movement of macromolecular dextran fractions across the capillary endothelial membrane was monitored by periodic concentration determinations of dextran in plasma and lymph. These determinations were organized and averaged according to their specific molecular weight group and barometric pressure treatment. The dextran concentrations in thoracic and leg lymph were separately compared to the dextran concentration in plasma.

Plasma vs. Thoracic Lymph Concentrations

A constant decrease in plasma dextran was observed in all animals. This decline was somewhat enhanced by decreased barometric pressure in some groups, but no definite dextran loss was observed with the change in pressure. The dextran levels gradually approached zero as the experiments proceeded to termination.

Small concentrations of dextran generally appeared in the thoracic lymph within 15 min. This concentration gradually rose to a constant plateau after 1 hr, and then it began to decline slowly until termination of the experiments. Administration of decreased barometric pressure halted this
decline and significantly increased the dextran concentrations in thoracic lymph. These concentrations decreased to values that approached zero when the barometric pressure was returned to normal.

The general trends cited above for dextran concentration in plasma and thoracic lymph can be seen in the combined group means. These graphs are presented in Figures 3 and 4.

**Group A (321,000 mol. wt.).**--The general decline in plasma concentration of dextran was observed in both the ground level control and the altitude experimental runs. A rather erratic pattern was exhibited by the plasma means of the control animals. This was contrasted by the relatively smooth decline of the altitude mean plasma concentrations. The control plasma values reached zero after 4½ hr, and the altitude values were leveled off at 10 mg/100 ml at the end of experimentation.

Only a small amount of dextran was identified in the thoracic lymph of the control animals. A slight rise in concentration was observed later in those experimental animals, but the dextran concentration was essentially zero after 3 3/4 hr. On the other hand, the mean thoracic lymph concentrations of the altitude runs demonstrated the general characteristic pattern described for them. The concentration rise with decreased barometric pressure was not as dramatic as observed in some of the other groups, but it was
Fig. 3. Dextran concentration means in plasma and thoracic lymph of combined control groups (A, B, C, and D). Each point is the mean of eight animals. SEM is indicated by vertical bars.
Fig. 4. Dextran concentration means in plasma and thoracic lymph of combined altitude groups (A, B, and D). Each point is the average of twelve animals. SEM is indicated by vertical bars.
still significant. The mean concentration values for control and altitude runs can be seen in Figures 5 and 6.

**Group B (409,000 mol. wt.).**—Mean plasma dextran concentrations for both the control and altitude groups were similar. Each of them exhibited the same slow decline, and they both had a final dextran concentration around 2.5 mg/100 ml. The average dextran concentrations of the altitude animals, however, were initially much higher than the averages of the control animals. This resulted in a more rapid rate of decline during the first few hours for the altitude runs. Administration of oxygen and decompression created an erratic pattern in these animals that eventually developed into significant decreases in mean plasma dextran concentrations.

The dextran concentrations in thoracic lymph demonstrated exaggerated rises in the initial dextran concentrations of both the ground level control and the altitude runs. These values decreased, however, to constant levels which, in the case of the control concentration means, stayed around 5 mg/100 ml. The dextran concentrations of thoracic lymph were increased when the animals were subjected to decompression. To a limited degree, these increased concentrations were mirrored by the decreases in the plasma dextran concentrations. The thoracic lymph dextran decreased to zero after the barometric pressure was returned to normal. Graphs of these results can be seen in Figures 7 and 8.
Fig. 5. Dextran concentration means in plasma and thoracic lymph of Group A, ground level control animals. Each point is the average of two animals. SEM is indicated by vertical bars.
Fig. 6. Dextran concentration means in plasma and thoracic lymph of Group A, altitude animals. Each point is the average of five animals. SEM is indicated by vertical bars.
Fig. 7. Dextran concentration means in plasma and thoracic lymph of Group B, ground level control animals. Each point is the average of two animals. SEM is indicated by vertical bars.
Fig. 8. Dextran concentration means in plasma and thoracic lymph of Group B, altitude animals. Each point is the average of three animals. SEM is indicated by vertical bars.
Group C (720,000 mol. wt.).—The ground level control animals were the only animals examined in this molecular weight group. Although the initial plasma dextran concentration did not reach that of the other groups, it did show the same general decline. After $3\frac{3}{4}$ hr the mean plasma value was zero. Dextran of this molecular weight did appear in the lymph, but it was present in only small quantities. The mean concentration of dextran in thoracic lymph was rarely above 2 mg/100 ml after $1\frac{1}{4}$ hr of experimentation. Graphic analysis of these results can be seen in Figure 9.

Group D (Dextran 500).—The results obtained in this group were similar to the general trend already set forth. Plasma dextran concentrations in both control and altitude groups demonstrated a slow decline. The values reached zero in the control means and 6 mg/100 ml in the altitude means. No noticeable change in altitude concentrations was present in the plasma.

Dextran mean values presented in the thoracic lymph also appeared similar to those expressed in the general trend. The main exception, however, comes in the last samples of the control thoracic lymph values. They show a definite increase over both the previous thoracic lymph values and the compared values for plasma dextran. The differences in the concentrations of dextran in plasma and thoracic lymph at this point seem to be significant. Another less obvious exception to the general trend was the failure of the thoracic
Fig. 9. Dextran concentration means in plasma and thoracic lymph of Group C, ground level control animals. Each point is the average of two animals. SEM is indicated by vertical bars.
dextran means to fall drastically towards zero after the period at decreased barometric pressure. Otherwise the results were similar to those already expressed. Graphs of these results can be seen in Figures 10 and 11.

**Plasma vs. Leg Lymph Concentrations**

Efforts to obtain leg lymph in usable quantities encountered several physiological and mechanical difficulties. The rate of flow was normally very slow at the beginning of the experiment, and this flow was decreased with time to a complete stoppage in most animals. If the lymph was flowing at the time decompression was started, it stopped after a slight increase in rate. Problems with lymph duct consistency in size and number also hindered the attainment of significant lymph flow rates. Because of these problems, leg lymph samples were obtained from relatively few animals. Most of the samples that were taken suffered from lack of sufficient volume for valid analysis.

A total of nine dogs yielded leg lymph samples for dextran analysis. Representatives of each molecular weight group and barometric pressure treatment were contained within these animals. Using analysis procedures modified by dilution techniques, only two of the animals were judged to have concentrations of dextran in their leg lymph samples. A Group D (Dextran 500), ground level control animal exhibited slight increases in dextran concentration that reached 3 mg/100 ml at the termination of the experiment.
Fig. 10. Dextran concentration means in plasma and thoracic lymph of Group D, ground level control animals. Each point is the average of two animals. SEM is indicated by vertical bars.
Fig. 11. Dextran concentration means in plasma and thoracic lymph of Group D, altitude animals. Each point is the average of four animals. SEM is indicated by vertical bars.
Also, a Group A (321,000 mol. wt.), altitude animal showed inconsistent increases in dextran concentration, especially in the last sample collected after decreased barometric pressure. The remaining animals revealed no measurable amounts of dextran in leg lymph. Because of this lack, no comparison could be made between leg lymph concentrations and plasma concentrations of dextran.

**Statistical Significance**

Statistical analysis was applied to the plasma vs. thoracic lymph results to determine their significance. Standard errors of the mean (SEM) were calculated for each sample of every experimental run. The results of these calculations were presented as vertical bars on the graphs in Figures 3-11. Loss of significant differences between plasma and thoracic lymph concentration means were readily observed by the overlap of the SEM vertical bars. This overlap occurred in the combined means after 4 hr for the control animals and after 3½ hr for the altitude animals.

A further statistical evaluation was necessary to establish the significance of lymph changes and plasma changes occurring between ground level control and altitude animals. A calculated Student's *t* was obtained for the thoracic lymph vs. thoracic lymph and the plasma vs. plasma values of each barometric pressure treatment. These calculations were then compared to a table of *t* values (*t*<sub>0.05</sub>) to determine probability (P). The combined control runs were
compared to individual group altitude runs for this analysis. In addition, combined control runs were compared to combined altitude runs. Table II gives the results of these compared calculations with their levels of significance.

The calculated t values obtained for the combined control and altitude groups gave a general picture of the overall significance of the experimental runs when they were compared to the tabled t values. The thoracic lymph comparisons that were determined during the decompression period revealed significant differences in dextran concentrations. The 2:45- through 3:45-hr samples were significant at the 0.01 percent level, and the 4:00- through 4:30-hr samples were significant at the 0.05 percent level. These values and two other initial values in the plasma were the only comparisons that indicated any significant differences in the combined control vs. altitude groups.

Group A (321,000 mol. wt.).—In both the ground level controls and the altitude runs, the dextran concentration differences between plasma and thoracic lymph became insignificant after 3½ hr. The intercomparison of lymph and plasma values under different barometric pressure treatments yielded values of a 0.05 percent level of significance for all comparisons involving thoracic lymph at altitude. This significance level was extended to one sample of thoracic lymph in the post-altitude stabilization period. Other
### TABLE II
INTERCOMPARISON OF PLASMA VS. PLASMA AND THORACIC LYMPH VS. THORACIC LYMPH AT NORMAL AND HYPOBARIC PRESSURES

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Group A</th>
<th>Group B</th>
<th>Group D</th>
<th>Combined Groups</th>
</tr>
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<tr>
<td></td>
<td>Plasma</td>
<td>T. Lymph</td>
<td>Plasma</td>
<td>T. Lymph</td>
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<td>0:00</td>
<td>...</td>
<td>...</td>
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<td>...</td>
</tr>
<tr>
<td>0:15</td>
<td>1.3328</td>
<td>1.8493</td>
<td>2.7602*</td>
<td>1.8423</td>
</tr>
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<td>2.4072</td>
<td>2.1397</td>
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<tr>
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<td>1.7423</td>
<td>1.5615</td>
<td>3.0151*</td>
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<tr>
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<td>2.0332</td>
<td>1.5629</td>
<td>1.4964</td>
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<td>1.7055</td>
<td>3.3833**</td>
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<td>2.8961*</td>
<td>1.9563</td>
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<td>1.8854</td>
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<tr>
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<tr>
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<td>1.5413</td>
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<td>1.4000</td>
<td>2.2695*</td>
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<td>1.4611</td>
<td>0.5148</td>
<td>1.6044</td>
<td>-1.1190</td>
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</tbody>
</table>

* P < 0.05   ** P < 0.01   *** P < 0.001
comparisons in this group were not significant at the prescribed level.

Group B (409,000 mol. wt.).—Insignificant differences between plasma and thoracic lymph concentrations of dextran became evident after 4 hr in the control animals and after 3½ hr in the altitude animals. The plasma differences and thoracic lymph differences demonstrated a varied pattern of significance. The thoracic lymph samples taken at altitude showed significant levels of from 0.001 to no significance. Additional levels of significance (0.05) were found in the thoracic lymph at the 0:45-hr and 1:00-hr samples. A significant value was also found in the plasma at the 0:15-hr sample. Other comparisons did not give any significant values at this level.

Group C (700,000 mol. wt.).—In this molecular weight group, differences between plasma and lymph were not significant after 2½ hr. No plasma and thoracic lymph intercomparisons could be made since there were no altitude runs.

Group D (Dextran 500).—The concentration means of dextran in plasma and thoracic lymph showed some patterns that varied from the general trend, especially toward the end of the control animals. Insignificant differences between the two, however, could be established after 4 hr in the control animals and after 2 3/4 hr in the altitude animals.
In the comparisons between the thoracic lymph values of control and altitude animals, all but one of the samples involving altitude exhibited significant levels of 0.05 percent or better. The 3:30-hr sample was significant at a 0.001 percent level. Plasma comparisons demonstrated significance in the 4:30-hr, 4:45-hr, and 5:00-hr samples. None of the other calculations was significant at the prescribed level.

Physiological Parameters

**Pulse Pressure**

The pulse pressure of the experimental animals in Groups A, B, and C were affected by the presence of minute quantities of sodium azide (ca. 0.02 percent NaN₃) present in the injected dextran solution. These animals experienced a mean pulse pressure drop of 30 mm Hg immediately after injection. The pulse pressure then slowly rose until it was approximately normal after 3 hrs. Animals in Group D did not have sodium azide in their dextran injections, and they did not demonstrate the drastic drop in pulse pressure. A 0.02% sodium azide concentration was added to the dextran injection of one animal in Group D. The pulse pressure of this animal responded exactly like those pulse pressures of animals in Group A, B, and C. Animals not having sodium azide in the dextran injection showed a slight decrease in pulse pressure throughout the experimental run. The use of oxygen and decreased barometric pressure did not have drastic
effects on the pulse pressures. Comparisons of the pulse pressures of azide and non-azide animals can be seen in Figure 12.

Hematocrit

Average hematocrit values of the experimental animals changed very little during the course of experimentation. A maximum range of from 41 to 45 percent was observed, and the tendency was for the hematocrit to rise slightly as the experimentation period progressed. Some individual animals showed small decreases after injection of dextran, and other individual animals showed slight increases with the introduction of decompression. No definite trends could be established from these animals, however. The hematocrit means for all animals can be seen in Figure 13.

Molecular Weight Determination by Gel Filtration

Column Calibration

Calibration of the Sepharose 6B column was accomplished by determining the elution volumes \( V_e \) for each molecular weight fraction and the void volume \( V_0 \) and internal volume \( V_i \) of the column. Using these values, the partition coefficient \( K_{av} \) was calculated. The void volume and the internal volume of the column were 158.5 ml and 518.3 ml respectively. Elution patterns of the dextran fractions were symmetrical, and they allowed easy estimation of
Fig. 12. Comparison of the effects of sodium azide on the mean pulse pressure. Ten animals were averaged for the azide mean values, and four dogs were averaged for the non-azide mean values. SEM is indicated by vertical bars.
Fig. 13. Mean hematocrit values of all experimental animals. Each point is the average of eighteen animals.
elution volumes. The elution patterns of the respective molecules can be seen in Figure 14, and their individual elution volumes and partition coefficients can be seen in Table III. Semilogarithmic plots of partition coefficient vs. molecular weight established the calibration curve of the column and can be seen in Figure 15.

TABLE III

<table>
<thead>
<tr>
<th>Molecular Weight</th>
<th>$V_e$ (ml)</th>
<th>$K_{av}$</th>
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</thead>
<tbody>
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<td>720,000</td>
<td>199.9</td>
<td>0.115</td>
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<tr>
<td>581,000</td>
<td>208.2</td>
<td>0.138</td>
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<td>467,000</td>
<td>215.0</td>
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<td>409,000</td>
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<td>321,000</td>
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<tr>
<td>Unknown</td>
<td>227.9</td>
<td>0.193</td>
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</table>

Examination of Unknown Molecular Weight

The combination and lyophilization of selected samples, fractionated on Sephadex G-200, yielded 34.0 mg of dextran. The appropriate solution of this dextran was applied to the Sepharose 6B column. A rather broad distribution was exhibited with the elution of the dextran down the column. This elution pattern did have an exaggerated peak at the point of elution volume determination, but it was otherwise very flat and widespread. The partition coefficient was calculated to be 0.193, and this value corresponded to a molecular weight
Fig. 14. Comparison of elution patterns of dextran fractions on Sepharose 6B.
Fig. 15. Calibration curve for Sepharose 6B column.
of 330,000 on the calibration curve (Figure 15). The elution pattern of this dextran fraction can be compared to the elution patterns of the previous dextran fractions in Figure 14. The column characteristics of the unknown can also be compared in Table III.
CHAPTER IV

DISCUSSION

Capillary Permeability

The Starling hypothesis depicts the direction and rate of fluid transfer between plasma and tissue fluids as being determined by three factors: (a) the hydrostatic pressures on each side of the capillary membranes, (b) the colloid osmotic pressures of plasma and tissue fluids acting across the capillary membranes, and (c) the physical, chemical, and histological properties of the capillary membranes themselves (8, 37, 42). If an inert, lipid-insoluble molecular species is suddenly added to arterial blood, a disturbance is created in the osmotic balance that initiates two related processes to reestablish the Starling equilibrium (36). The first process involves the diffusion of the added molecule from the plasma across the capillary membrane to the interstitial fluid, and the second process involves the withdrawal of fluid from the interstitial compartment into the capillary blood due to the concentration gradient formed by the added molecules. Both processes continue until an equilibrium is attained according to the new osmotic pressures. The rate of these two processes and the time required for them to reach equilibrium are dependent on the
hindrance to diffusion at the endothelial wall. This membrane hinderance is primarily related to molecular size, and through mathematical applications of the progressive plasma and lymph concentrations, the larger molecular species can be used to observe and describe some of the characteristics unique to the capillary endothelial wall (13, 35, 38). Likewise, changes in the hydrostatic pressures can induce shifts in the transcapillary fluid flow and/or in the osmotically active molecules to return the system to the equilibrium portrayed by the Starling hypothesis. Monitoring the shifts of the large, membrane-selected molecular species can reflect additional properties of the capillary endothelial wall and the surrounding interstitial compartment (25, 26). In essence, all three of the factors expressed in the Starling hypothesis must be considered in discussing the properties of capillary permeability.

The macromolecular dextran fractions utilized in this study were chosen because of their similarities with the above hypothetical molecular species, both in physiological inertness (41) and in biological fractionation according to molecular size (21, 45, 46). The capillary permeability characteristics of the fractions were studied both during the initial equilibrium adjustments and during barometric pressure changes after equilibrium had been reached. These characteristics studies were facilitated by monitoring the
concentrations of dextran disappearing from the plasma and appearing in the lymph obtained from two different lymphatic drainage areas. This was in response to the reports of varied histological (3) and permeability (31) characteristics of capillary endothelial membranes in different anatomical regions. Within the limits of certain qualifications, the results obtained from the two different regions substantiated the reported variations in capillary permeability.

**Plasma vs. Thoracic Lymph Concentrations**

**Molecular Weight Effects**—The size of the molecular species, as reflected by molecular weight, has been pointed out as one of the primary considerations in capillary membrane selectivity. This fact was especially evident in the studies evaluating dextran as a plasma expander (4, 41, 45). In those studies, increased efficiency in rapidity and duration of plasma expansion was noted with increased molecular weights of dextran. Larger molecular weight dextrans were simply retained longer by the restrictive endothelial barrier. The increasing molecular weights in the present study, however, did not appreciably alter the permeability characteristics as they were monitored in the thoracic duct drainage regions. With two exceptions that will be explained later, there were no significant deviations in the progressive dextran concentration patterns in either
the plasma or the thoracic lymph. This observation can be explained by several factors.

The first explanatory factor involved, and probably the most important one, is the region from which the thoracic lymph is primarily derived, the spleen, intestine, and liver. Several investigators (13, 21, 31, 46) have shown the presence of dextran in thoracic lymph, and their reported molecular weight average is within the range of Groups A, B, and D. These workers have suggested the presence of large "leaks", especially in the liver, to account for this presence of macromolecular dextran. The estimated size of these "leaks" is 250 to 300 A, and electron microscope studies by Bennett and co-workers (3) have suggested their existence. Other workers (34, 39) have indicated that macromolecular transport may be by vesicles, but no evidence has been offered to support the vesicular transport of non-proteinaceous, lipid-insoluble molecules like dextran. At this point, the endothelial "leaks" attributed to the liver appear to be the most acceptable theory of macromolecular transfer of dextran to the thoracic lymph. The "leaks" must be larger than the effective molecular size of dextrans in Groups A, B, and D, because there are no selective differences between them in their thoracic lymph concentrations.

A second factor that must be considered is related to the dextran molecule itself. It has already been pointed
out that its molecular weight polydispersity has been a major drawback in its use in capillary permeability studies. Even though the dextran fractions used in this study were much less polydispersed than those used in previous studies, some lower molecular weight contamination could still be expected. An example of contamination can be seen where the thoracic lymph concentrations of dextran become greater than the plasma concentrations in the latter samples of Group D. This group is much more polydispersed than the other three groups. In addition to the polydispersity effects, the dextran molecule has been described as a flexible, linear coil that can be subject to shear deformation in smaller passageways (2, 22). These two molecular characteristics of dextran indicate that some limited transfer could occur within the proposed small pore system (13, 31, 35) or at least through smaller "leaks". This indication is especially significant when the comparatively small concentration dosages are considered. The effects of the increasing molecular weight of dextran may have been partially hidden by the false permeability characteristics of the dextran molecules.

Either one or combinations of both of the above factors can explain the lack of observed concentration changes with the use of increased molecular weight dextran fractions of Groups A, B, and D. Molecular weight averages within these groups present no drastic change. The molecular weight
average of Group C, however, is much greater than the averages of dextrans used in previous studies. Although only two ground level control animals are examined in Group C, the results obtained indicate that the dextran transfer is restricted. The average plasma concentrations approach zero much sooner than the other groups, and very small amounts of dextran appear in the thoracic lymph. Conclusions based on two experimental runs are questionable, but the tendency of the endothelial membranes in the thoracic duct drainage regions to restrict dextrans of much higher molecular weight averages seems to be present. Renkin (38) has said that it would take much higher molecular weight dextrans to establish the maximum permeability limits of the capillary endothelial membranes, and this may be true if the above tendency proves to be correct with further experimentation.

Since the ground level control runs were somewhat similar, their dextran concentrations were combined and averaged for comparison with the altitude runs. This is justified in the light of the above explanations for their similarities. The statistical significance of the later comparisons is also increased by doing this.

**Loss of Dextran**—One of the more puzzling aspects of this study centers around the constant decline of dextran concentration in both the plasma and the thoracic lymph of all groups. At first glance, elimination by the kidneys
might be suspected for the decrease, but this is difficult to support after further examination. Wasserman and his associates (46) have shown that dextran molecular weights greater than 40,000 are generally retained by the kidney and returned to the circulation. This limit is far below any of the average molecular weights used in this study, and all of the fractions except Group D were sufficiently narrow to believe that few if any molecules of this size were present. Even Group D showed no change in concentration patterns that would lead one to suspect significant kidney elimination of dextran.

Metabolism could also be suspected for the decline of dextran concentrations. Gray (19) reported that dextran was broken down by the animal, incorporated into the carbon pool, and distributed throughout the body in the form of normal biochemical constituents. In this instance, the experimental animals were mich, and the metabolic half-life of the carbon in dextran was approximately 6.1 days. This is contrasted to a metabolic half-life on dogs of 27 hr as reported by Wasserman and Mayerson (44). The latter investigators also calculated the slope of exponential disappearance to be -0.00043. When the 5½-hr period of experimentation for the present studies is considered, it is unlikely that metabolism alone could have caused the dextran decreases that were observed.
The factors involved in establishing an osmotic equilibrium with the low concentration dosage is one of the more probable causes for the decline in dextran concentration. Previous reports (40, 43, 44, 46) give conflicting periods of time (ranging 1-13 hr) that are required to establish an osmotic balance, and the time periods involved depend somewhat on whether a true equilibrium or a steady state condition is acquired. All of these studies, however, used dextran concentrations that were many times greater than the concentrations used in the present investigation, and some of the studies (40, 43) used the additional somotic effector of radioactive iodinated albumen. This means that the small quantities of dextran fractions used in this investigation should have reached a steady state condition much sooner than these previous studies. When the lymph to plasma concentration ratios are examined, it can be seen that they do indeed become somewhat static after one hour of experimentation, and even with the constant decline in respective concentrations, this ratio remains almost constant until the values begin to approach zero and become invalid indicators. These facts suggest that the plasma and lymph concentrations of the dextran fractions are basically in a steady state. However, there is either an additional steady state equilibrium that must be satisfied for constant concentration values, or, as hinted previously, the metabolic activity is accentuated by the low concentration values present.
The accentuated metabolic activity with small dextran concentrations is a distinct possibility, because dextran disappearance would be more noticeable in smaller total quantities. In light of the reappearance of dextran in the thoracic lymph with the advent of decreased barometric pressure, however, metabolism seems highly unlikely. The anabolic processes required under these circumstances are physically impossible. Thus, metabolism must again be discounted as a major cause for the dextran decline, and other dextran-consuming processes or secondary equilibria must be examined.

There are three mechanisms by which dextran could be consumed or in which a secondary equilibrium might be established. The first of these involves certain organs that perform specific functions for the animal's circulatory system. In this instance, the organs are the liver and the spleen. Both of these organs have storage and phagocytic properties that could account for the disappearance of dextran. Reports (16, 20) concerning these properties do not indicate that dextran concentrations would be altered from those seen in the plasma and thoracic lymph, but no real evaluations are presented. The sinusoidal spaces and phagocytic characteristics of one or both organs could cause the disappearance of dextran that was observed.
The second mechanism deals with a peculiar erythrocyte reaction in the presence of dextran. Bull and his collaborators (6) observed formation of rouleaux in smears of blood previously infused with dextran. Formation was enhanced by concentration and molecular weight increases. Although the concentrations used in the present study were below those reported, the molecular weights were much higher. There is no way to know now if significant quantities of rouleaux were formed or not, but if they were, they too might be an explanation for the dextran decline, especially in conjunction with the phagocytic properties suggested in the first mechanism.

The third mechanism encompasses the theories of the tissue spaces within the interstitial compartment. Histologists, biochemists, and physiologists (12, 14, 32) have all pointed out that almost all normal tissue spaces are filled with a gel or gel-like substance in addition to free interstitial fluid. The gel is composed primarily of mucopolysaccharides, and in most tissues, the most important one is hyaluronic acid. This substance is present in the form of long coiled filaments, and it exists in concentrations usually between 0.3 and 1.2 percent (33). Since the coiled filaments are oriented in all directions, the resulting network has been described by Ogston and Sherman (33) as a "brush pile" that, with its entrapment effects, impedes bulk movements of fluid and solutes within the fluid.
Guyton and co-workers (25) have shown that the degree of this impedence by this gel reticulum is inversely proportional to the quantity of free fluid that is also in the interstitial spaces. Under these conditions, it is conceivable that dextran could be in a quasi-equilibrium between the plasma and free fluid while still undergoing diffusion or entrapment within the gel reticulum. This mechanism could easily explain the disappearing pattern of the dextran fractions.

Any one of the three mechanisms cited above could have had a part in the decline in dextran concentrations. Interaction between the mechanisms could also have had some part. In view of the later results showing a reappearance of dextran in the thoracic lymph, the latter mechanism seems to be the most favorable. The first two mechanisms involve a somewhat irretrievable loss in dextran that would not allow for the observed increases in thoracic lymph dextran at altitude. These increases can be rationalized by gel-free fluid features present in the third mechanism. It must be pointed out, nonetheless, that no definite answer can be realized with the present data. Until further experimentation involving radioisotopically tagged dextran is performed, no conclusive statements about the disappearance of dextran can be made.

Effects of Pressure Changes--The changes observed in the thoracic lymph concentration of dextran at reduced barometric pressures were the most significant results of
the entire study. The thoracic concentrations of the ground level control animals continued their slow decline toward zero throughout the experimentation period, but the thoracic concentrations of the altitude animals demonstrated a dramatic increase in dextran concentration for the period of reduced barometric pressure. In both instances, however, the plasma dextran concentration sustained its slow decrease without significant change during the periods at altitude. These results could be due to one or combinations of all of the following physiological explanations: 1) an actual increase in the permeability characteristics of the capillary endothelial membrane changes, 2) an increase in central blood pressure that alters the dynamics of the Starling equilibrium, and/or 3) a change in the gel consistency and pressures in the interstitial compartment that shifts the steady state dynamics and increases the thoracic lymph dextran concentrations.

The stretched pore phenomenon elaborated by Shirley and co-workers (40) is an example of a change that could take place in the endothelial membrane itself. In their illustration of the phenomenon, pore distortion was created by enormous volumes of normal saline infused into the experimental animals after a large quantity of dextran had reached steady state conditions. The drastically increased plasma volume supposedly stretched the small pore system to stimulate transport of increased dextran molecular weight
sizes. Variations of this theory come from Grotte's system of large pore "leaks" (21) and from Arturson and Granath's "safety valve" system of increased large pore radii (1). The present study, however, did not allow for the possibility of increased pore size or leaks because of plasma volume expansion. On the contrary, dextran concentration dosages were intentionally kept low and no more than sample-replacement volumes of normal saline were added to avoid significant plasma expansion in the animals. Blood pressure was also decreased substantially by the addition of minute quantities of sodium azide. In this manner, any membrane changes that occurred, such as the stretched pores or "leaks", had to be related to the decreased barometric pressure.

Under these experimental conditions, membrane changes like those cited above are difficult to support for the capillary endothelial system as a whole. Decreased barometric pressure, however, does have some profound effects on the hemodynamics of experimental animals, and they may be related to the present observations. Investigators have identified increases in peripheral tonicity and resistance (10, 15) and decreases in venous compliance (47) with exposure to high altitude. The general conclusion derived from these observations is that the central blood volume is increased and the extreme peripheral circulation receives a limited blood flow (10, 47). With these facts in mind the question in the present study involves the
degree to which the central blood volume and peripheral resistance is increased. If these increases are sufficient to raise the capillary hydrostatic pressures significantly, then stretched pores or "leaks" may indeed have caused the rise in thoracic lymph concentration of dextran. The liver and spleen would again play an important part in this supposition. Because of the large amount of supposition imposed at this point, the stretched pore hypothesis must remain plausible but not fully substantiated by the present facts of this study.

The hydrostatic forces that purportedly allow the stretched pore phenomena to operate come from inside the capillaries. Since the conditions established in the experimental design would affect the forces surrounding the capillaries, the interstitial compartment and its related forces must be examined. Guyton (25) has described the interstitial compartment as being composed of the following three constituents: 1) the interstitial fluid within the interstitial spaces, 2) the cells and fibers of the stroma, and 3) the mucopolysaccharide gel. The first constituent forms the interstitial fluid pressure, and the last two make up the solid tissue pressure. Accordingly, the total tissue pressure is determined by the algebraic averages of the interstitial fluid and solid tissue pressures that occur on the surfaces of the capillaries.
Interstitial fluid pressure is commonly reported as a negative pressure (23) that pulls the surface of the skin toward the cell surfaces, as well as pulling the adjacent cell surfaces together. In turn, the solid fibers in the spaces are holding the surfaces apart. Hence, the solid tissue pressures exerted by the solid fibers would counteract the pressures exerted by the negative interstitial fluid pressure, and the total tissue pressure would be zero or slightly positive (25). This reasoning involves a simple application of physics that says that "at any single plane in space in a nonaccelerating system, the forces acting in one direction must be exactly opposed by forces acting in the opposite direction." Within the limits of fiber strength, skin turgor, and additional structural forces, this law should apply both to increasing and decreasing interstitial fluid pressures (25).

Assuming that the skin would be flaccid enough to transmit pressures faithfully, the total tissue pressure on the under surface of the skin is equal to zero with respect to atmospheric pressure. A decreased atmospheric pressure, like that presented in the present study, would cause a decreased pressure under the skin. This same amount of pressure is transmitted through the fluid and solid structural to the surface of the cells and capillaries because of the "equal but opposite" physical principal stated above. The total pressure in this instance would
still be around zero with respect to the reduced atmospheric pressure, but the fluid pressure in the space between the capillary and the surrounding tissues could be very negative (23, 24). Thus, bulk movement and diffusion of fluid and solute molecules would be increased in an outward direction through the capillary pores which, in effect, is the stretched pore phenomena in reverse. The solid forces involved in this mechanism would reach a point of compaction in the peripheral and cutaneous regions, and the blood vessels supplying these regions would be closed or severely restricted (7). This is in accord with the increased peripheral resistance and decreased venous compliance observations noted earlier (10, 15, 47). In deeper and more centrally located tissues, the compaction tendency would not be as great, and the forces causing bulk fluid and solute movement would predominate.

The influx of fluid into the interstitial spaces increases the lymphatic flow (29) which in turn would remove the dextran and other osmotic effectors that were present in the tissue spaces. The dextrans would be returned to the circulatory system in an attempt to reestablish the balance of the Starling equilibrium, and this fact could explain the lack of noticiable decline in plasma dextran concentration with concomitant increases in thoracic lymph dextran. The mechanism just explained is very similar to the "safety valve" system proposed by Arturson and Granath.
for the large pore system. Although their system was set forth to explain the mechanism involved in the relief of increased venous pressure, it also fits very well into the explanation for the relief of unbalanced forces coming from the interstitial compartment and decreased barometric pressure.

In light of the above discussion, it appears that all of the explanations initially presented combine to give a whole picture of the mechanism involved. This could have been expected because of the number of physiological variables that are inherent to capillary dynamics and capillary permeability. Certainly additional work needs to be done to establish the trends and reasoning presented in this study.

**Plasma vs. Leg Lymph Concentrations**

The lack of sufficient data severely hinders the evaluation of capillary permeability as monitored in the leg. The surgical procedures involving the leg lymphatics were aborted only once due to impossible morphological formation of the ducts, and in the remaining experimental runs, successful cannulation was performed with lymph flow observed at the onset of experimentation. However, only a few of these leg cannulations continued to flow for a significant period of time. Those cannulations which did continue to flow for the entire experimental period were handicapped by
volumes insufficient for accurate analysis, especially near the end of experimentation. Any discussion of the observations and general tendencies must be made with the realization that much additional work must be done to substantiate and explain them.

Initial responses of the leg lymph flow coincided with those reported by Irisawa and Rushmer (29). The steady state pressures appeared to be relatively low, but they could be raised to extremely high levels by squeezing the paw or otherwise manipulating the region distal to the lymphatic cannulation. Although these initial results were favorable to the success of the procedure, the subsequent lymph flow exhibited a general decrease in rate as the experiment progressed. The flow completely stopped in some instances shortly after the experimentation period had begun. These decreases and stoppages occurred in spite of warming the paw and passively rotating the leg to stimulate lymph flow. Similar difficulties have been indicated by other investigators (11, 13, 48), and they are thought to be primarily due to the overall inactivity of the anesthetized animal. In addition, fibrin clots were formed in the collecting tubes of some of the experimental animals in the present study, and this may have been a factor in the flow stoppage of some animals, even though thorough precautions were taken to eliminate clotting problems. Other additional physiological phenomena that could have slowed or stopped
the flow rate were as follows: 1) washout of the interstitial osmotic effectors causing increased fluid reabsorption (11, 25), and 2) compaction of the cutaneous region and occlusion of peripheral blood and lymph vessels with altitude (7, 15, 25). All of these factors and probably some that were undetected could have stopped or contributed to the slowing of the leg lymph flow.

It was additionally difficult to analyze and evaluate the results of the few experimental animals that did yield samples for the entire period. The dilution procedures necessary to analyze the samples demanded that only qualitative results could possibly be considered valid. In this light, only two dogs revealed the presence of dextran in the leg lymph, and one of these seemed dubious. A ground level control animal (Group D) showed the definite presence of dextran and an altitude animal (Group A) showed sporadic appearances of the test molecule. Previous reports (13, 21) indicate that dextran molecules of this size have passed through the capillary endothelial membranes, but the present study cannot lend any evidence to support these reports.

The results obtained from this portion of the study indicate that it may be of little value to continue to use leg lymphatics located in the popliteal fossa. It is very difficult to obtain significant flow rates from this area, especially with decreased barometric pressure studies. The
lymph obtained from these ducts is primarily derived from subcutaneous areas (9, 30) that are subject to compaction and closing pressures of vessels (7, 25). As an alternate position for the monitoring of leg lymph, the lymphatics in the inguinal fossa should yield more satisfactory results. Their lymph is derived from the deep muscles of the leg, and as a result most of the problems involving consistent flow rate and decreased barometric pressure should be diminished (9, 30).

**Statistical Significance**

The standard error of the mean (SEM) was calculated for the average of each sample of both barometric pressure treatments within each molecular weight group. These calculations presented rather wide standard errors of the mean, especially in the ground level control averages, that could have been reduced somewhat by an increased number of runs. Additional variation from the mean could have come from the fact that the experimental animals utilized were mongrel dogs. Bulgin and associates (5) attributed variations in protein concentrations in purebred beagle dogs to age differences, nutritional deficiencies, and/or disease. Although an attempt was made to uniformly select the animals, there was no real way of judging the above conditions in mongrels. Consequently the variation had to be increased by these factors.
Calculated Student's $t$ values demonstrated significant variations primarily in the thoracic lymph comparisons during the altitude period. The significance of the thoracic lymph increases in dextran is more consistently expressed in the combined group comparisons. This is because more experimental animals were evaluated, and a true expression of the statistical tool could be expressed. Individual group comparisons in the thoracic lymph showed essentially the same period of significance, but the level of significance variation within the individual groups could be diminished by increased numbers of animals. A general trend toward decreasing levels of significance during the latter samples of the altitude period could possibly be interpreted as the beginning stages of a steady state equilibrium at the new barometric pressure. No explanation other than the mongrel variations and the small number of experimental animals can be given for the spot appearances of significant $t$ values in initial samples. The obvious need is for more experiments to be run, but the important trends can be seen from the experimental runs presented in this study.

Physiological Parameters

**Pulse Pressure**

The pulse pressure drop observed in the experimental animals of Groups A, B, and C were due to the presence of minute quantities of sodium azide (ca. 0.02 percent Na$_3$N).
Graham (17) has described this chemical as a neutral salt of hydrazoic acid that acts peripherally in small concentration dosages to relax the smooth muscle of blood vessels and bronchi while increasing the force of cardiac contraction. As was seen in Figure 12, the result of this action was a drastic drop in systemic blood pressure. Graham also reported other physiological reactions, including death, could occur with further increases in azide concentration. These reactions, however, required much higher concentrations than were employed in this experiment.

Other than the hemodynamic effects discussed above, there appeared to be no significant differences in the results of the animals with or without the sodium azide. No apparent difference was present in the dextran concentrations monitored. The general trend in animals not injected with sodium azide was to show a slight decrease in pulse pressure throughout the experiment, and the treatment of oxygen and decreased barometric pressure had no drastic effects on the pulse pressure. Including the effects of sodium azide, the pulse pressure averages demonstrated no phenomena that would suggest physiological abnormalities in the capillary permeability studies.

**Hematocrit**

Average hematocrit values demonstrated no significant changes during the period of experimentation. The individual
variations could have been responses to fluid shifts between the vascular and extravascular compartments with the introduction of dextran and decreased barometric pressure. These changes were not uniform, however, and were therefore not significant. Because of the relatively short experimental period involved, no attempts were made to thoroughly examine fluid shifts and hemopoietic activity reported in longer stays under the influence of reduced atmospheric pressure (27, 28). The hematocrit, as used in this study, was simply employed as an indicator of the physiological status of the experimental animals, and this status remained constant throughout the period of experimentation.

Molecular Weight Determination by Gel Filtration

The method employed to calibrate the Sepharose 6B column in this study was sufficient to reveal the molecular weight characteristics pertinent to the involved dextran fractions. The semilogarithmic plot revealed a fairly straight line for molecular weight comparisons. If more exact molecular weight determinations had been desired, however, the polydispersity effects of the dextran calibration fractions would have had to be considered using additional methods of calibration (1, 18).

In examining the unknown molecular weight, the most significant result has to be the comparison of elution patterns seen in Figure 14. The patterns of the calibrating dextran fractions are symmetrical and somewhat concentrated
over a comparatively narrow volume range. The unknown dextran demonstrated a broad, flat elution pattern. Comparison of these two patterns suggests a much higher degree of polydispersity for the unknown dextran fraction, even though it had been previously fractionated on Sephadex columns. Since the steps to consider the polydispersity of the calibrating fractions were not taken, the molecular weight determined for the unknown dextran cannot be viewed with a great deal of exactness. It does appear, however, that the average molecular weight for Dextran 500 is less than 500,000 and that it is extremely polydispersed in this commercial form.

Summary

The outstanding aspects and conclusions derived from this study can be summarized as follows:

1. The concentrations of macromolecular, narrow-range dextran fractions were increased during exposure to a simulated altitude of 28,000 feet. The increases were suggested to originate from a combination of changes in the capillary endothelial membranes, increased central blood volume, and changes in the gel reticulum and pressure balances of the interstitial compartment.

2. Further investigations should be made to determine the pooling places of dextran and the maximum molecular weight limits of dextran for capillary transport.
3. The lymphatic network of the popliteal fossa is unsatisfactory for use in studying capillary permeability at decreased barometric pressure. Future studies should examine the possibility of using the lymphatics in the inguinal fossa for monitoring leg lymph.

4. Gel filtration procedures reveal substantial polydispersity differences between the acquired, narrow-range dextran fractions and commercially prepared dextran. However, no significant differences are evident in their capillary permeability.

5. Increased significance can be achieved by increasing the number of experimental animals in both the control and treatment areas.
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