Dynamics of Transcapillary Fluid Exchange

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ABSTRACT Fluid balance at the capillary level has been simulated with an analogue computer program, based on experimental data on regional differences in capillary permeability, surface areas, and hydrostatic pressures. The program takes into account fluid and protein fluxes into and out of the interstitial space. Solutions are obtained for tissue hydrostatic pressure, tissue fluid osmotic pressure, interstitial space volume, and lymph flow. Simulation of a variety of physiological experiments and clinical disease states has yielded reasonable agreement between experimental data and data obtained by computer analysis. Dilution of the interstitial plasma protein pool with a consequent reduction of its oncotic pressure appears to be a major factor, which prevents edema unless plasma oncotic pressures are reduced by 10–15 mm Hg or, alternatively, venous pressures are elevated by a similar amount. The computer analysis in all instances yields positive values for tissue pressure, in agreement with experimental data obtained by needle puncture. The negative tissue pressures observed in subcutaneous capsules can be reproduced in the computer program, if the interface between the capsule and the surrounding interstitial space is assumed to have the properties of a semipermeable membrane.

Partition of body fluids between the circulation, on the one hand, and the interstitial and intracellular spaces, on the other, is normally maintained within narrow limits. The mechanism maintaining this precise partition is still not well understood, but a balance of capillary hydrostatic and colloid osmotic pressures is generally acknowledged to play an important role, as originally pointed out by Starling (1). Current views on capillary water balance are summarized in Fig. 1, which is based on experimental data of Starling (1) and Landis (2). The graphs illustrate the relationship between hydrostatic pressures along the length of a capillary and the colloid osmotic pressure of plasma proteins.

The oncotic pressure of plasma (π_{PL}) averages 25 mm Hg (3), corresponding to a plasma protein concentration of 7%. The hdyrostatic capillary pressures are averages based on extensive series of pressure recordings in skin capillaries of man by Landis (2). In arterial capillaries (Fig. 1 *A*, left), the pressures averaged 32 mm Hg (P_A). The hydrostatic pressures in the

venous capillaries (Fig. 1 *A*, right) averaged 15 mm Hg (P_V). The transcapillary flux of fluid is governed by the difference in the hydrostatic and colloid osmotic pressure of the plasma; thus fluid is filtered from the capillary into the tissues in the arterial end of the capillary, and it is reabsorbed from the tissues into the capillary at the venous end. The hydrostatic pressures in the arterial and venous ends of the capillary bracket the colloid osmotic pressure, and the amount of fluid filtered at the arterial end is similar to the volume reabsorbed at the venous end.

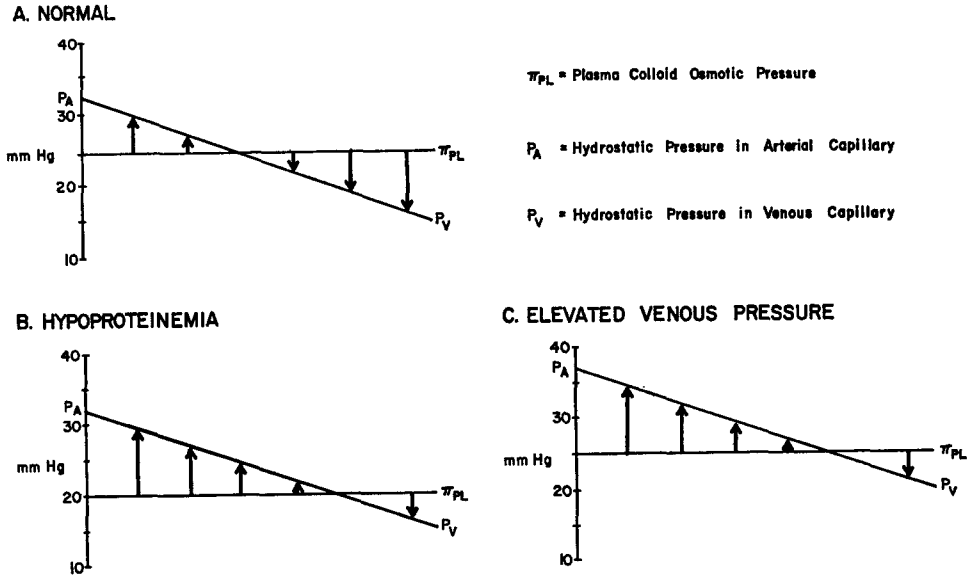

FIGURE 1. Diagram of hydrostatic and colloid osmotic pressures along a capillary. *A*, normal values; *B*, hypoproteinemia; *C*, elevated venous and arterial capillary pressure.

This equilibrium of filtration and reabsorption has been considered an essential feature of the mechanism which prevents excessive shifts of fluid between the blood and the interstitial space. Such an equilibrium would be unstable, however. If the colloid omsotic pressure of the plasma is reduced in hypoproteinemia, the equilibrium would be disturbed, as shown in Fig. 1 *B*. If the plasma osmotic pressure is reduced to 20 mm Hg, filtration occurs along almost the entire length of the capillary, and excessive fluid accumulation should occur in the interstitial space. This finding is contrary to clinical experience, however; edema does not occur until the plasma protein concentration has fallen to almost half its normal value (4–6). A similar disturbance of equilibrium would result if venous and arterial capillary pressures were elevated, as in heart failure or venous obstruction (Fig. 1 *C*). Increasing hydrostatic pressures in the arterial and venous ends of the capillary by 5 mm Hg

produces filtration along virtually the whole length of the capillary. Experimental data indicate, however, that edema does not develop until venous pressures are elevated by at least 10–15 mm Hg (7–9). Thus protective mechanisms appear to exist which limit the shift of fluids over a range of ±10 mm Hg around the normal hydrostatic and colloid osmotic pressures found in capillaries. The traditional capillary model fails to explain this feature of fluid balance at the capillary level.

Since the formulation of this model, much quantitative information relating to capillary function and structure has accumulated. This information indicates that the mechanism maintaining the partition of fluid between the circulation and the interstitial space is considerably more complex than indicated by the traditional capillary model. An equilibrium of fluid exchange in the traditional model implies three unstated assumptions: (*a*) the permeability to water and solutes is uniform throughout the capillary; (*b*) the surface areas of the arterial and venous ends of the capillary networks are identical; and (*c*) the hydrostatic and colloid osmotic pressures of tissue fluid are negligible compared to the corresponding blood values. The objectives of this report are, first, to examine the validity of these assumptions, and, second, to expand the traditional model of the capillary to include experimental data on regional differences in permeability, capillary surface area, permeability to protein, and lymph flow. Finally, these factors will be incorporated into equations describing fluid exchange at the capillary level; these equations will be subjected to computer analysis. Data obtained from the computer analysis will be compared with experimental data.

REGIONAL DIFFERENCES IN CAPILLARY PERMEABILITY

Substantial experimental evidence indicates that different regions of the capillary network in many different peripheral vascular beds are not uniformly permeable to solutes of intermediate molecular weight. The classical studies of Rous et al. (10) indicated that water-soluble dyes generally escaped more rapidly from venous capillaries and venules than from arterial capillaries in the mammalian skin. Similar studies by Mori et al. (11) demonstrated similar differences in permeability in the capillary networks of frog and rabbit mesenteries, and also in rabbit ear chambers.[1] Landis also showed that plasma albumin tagged with Evans blue dye escaped eventually through spotty leaks in venous capillaries and venules (12). Regional differences in permeability to a large dye molecule do not necessarily imply similar differences in permeability to water.

For this reason, a series of experimental studies were undertaken which were

[1] K. Mori. Personal communication.

designed to yield a quantitative measurement of the permeability to water in arterial and venous capillaries in the frog mesentery. The filtration rates in capillary segments in the frog mesentery were measured with an indirect densitometric technique, which has been described elsewhere (13). The experimental data indicated that permeability to water was approximately similar in arterial capillaries, midcapillaries, and venules. Venous capillaries, however, were approximately twice as permeable to water as the arterial capillaries. A similar set of data was also gathered in an independent study by Intaglietta (14), in which capillary filtration rates were measured by the Landis micro-occlusion technique. His data indicated that the permeability of venous capillaries in the frog mesentery averages 60% higher than that of the arterial capillaries. The assumption of uniform permeability of the capillary network thus is not justified in all cases. The elevated permeability to water at the venous end of the capillary tends to favor reabsorption, and thus leads to a relative dehydration of the tissues.

REGIONAL DIFFERENCES IN CAPILLARY SURFACE AREA

The second assumption in the traditional capillary model, that the surface areas available for filtration and reabsorption are similar, may also be challenged. That the subpapillary capillary loops in human skin are asymmetrical is well known. Characteristically, the arterial limb of the capillary is considerably smaller than the venous limb. Davis and Lawler measured the diameter of many skin capillaries, finding on the average that the venous limb is 50% larger than the arterial limb (15). The apex of the loop has generally the same dimension as the descending venous limb. Landis' measurements in the capillaries also indicated that the hydrostatic pressure at the apex of the capillary loop was lower than the plasma colloid osmotic pressure, and thus is presumably the site of reabsorption (2). The apex of the capillary loop should thus be included as a part of the venous limb for functional as well as morphological reasons. However, the surface area of the two capillary segments is difficult to estimate, as their lengths were not measured separately. The subpapillary venular plexus in man possesses a considerably larger surface than the capillaries. Wetzel and Zotterman (16) measured the surface area of the subpapillary venular plexus and of the skin capillaries. The objective of their studies was to evaluate the relative contribution of venules and capillaries to skin color, and their surface area values refer to a projection of these vessels on the skin surface. The capillary loops run perpendicular to the skin, in contrast with the venular plexus, which lies parallel to the skin. The total capillary surface area, however, can be calculated by assigning 200 μ (15) as an average value for the length of the capillary loops. The surface area values recalculated in this manner have been listed in a previous publication (13). The ratio

of the surface areas of the venular plexus and of capillaries ranged from a low of 0.82 in the skin of the knuckle to a high of 5.7 in the skin of the cheek. The average ratio for skin in six different regions was 2.0. The surface area of the venules is thus about twice that of the capillaries. The estimate of capillary surface area, however, includes both arterial and venous capillary segments. The area available for reabsorption in venous capillaries and venules, then, is at least 6 times as large as that available for filtration at the arterial end of the capillary. In a recent study, Wiedeman (17) measured dimensions and counted the vessels in the different categories of the microvascular bed in the bat wing. Recalculation of her data indicated that the surface area of venous capillaries was about 6 times larger than that of the arterial capillary (13). The relatively larger surface area of the venous capillaries tends to favor the reabsorption process, and thus lead to a relative dehydration of the tissues.

PLASMA PROTEIN CONCENTRATION IN INTERSTITIAL FLUID AND TISSUE PRESSURE

With few exceptions, the capillaries in peripheral vascular beds are somewhat permeable to plasma protein. It is generally conceded that the plasma protein passes through the capillaries through a system of large pores with sizes ranging from 250 to 350 A. Microscopic evidence indicates that these leaks or large pores are most frequent in the walls of venous capillaries and venules (12). Landis perfused capillaries in the frog mesentery with albumin solution tagged with Evans blue dye. About 10–12 sec after this perfusion was begun, the dye escaped in spotty leaks predominantly around venules and venous capillaries.

The relative order of magnitude of the plasma leakage through the large-pore system may be estimated from data in studies by Grotte (18). Grotte injected solutions of dextran of varying molecular radius into the circulation of dogs and subsequently measured plasma and lymph concentration of dextran. He found, as would be expected, that the concentration of dextran in the lymph decreased with increasing molecular radius. Even the largest dextran molecules, however, were found in lymph, in a concentration about 10% of the plasma concentration. Grotte estimated that for 34,000 small pores of 35 A radius only one of the large leaks with an estimated radius of 250 A was present. This value may represent an overestimate, however, since his derivation was based on the net filtration in the capillary bed, and the concentrating effect of reabsorption was not included.

In some of Grotte's experiments, however, the lymph concentration of dextran was found to be 5% of the plasma concentration under conditions of venous congestion (venous pressure = 50 mm Hg). Under these circumstances, filtration occurs along the whole length of the capillary. Recalculation of his data, with this value for dextran concentration and with the revised figure for the radius of the small-pore system (42 A) obtained by Landis and

Pappenheimer (3), yields a value of one large leak for every 24,000 small pores. It may be deduced from Grotte's data that, for every milliliter of plasma filtered through the large-pore system, 19 ml of fluid is filtered through the small-pore system during venous congestion.

Our best estimates of interstitial fluid protein concentration are based on tracer techniques, in which tagged plasma proteins were injected into the circulation. When the tagged proteins had equilibrated with the extravascular protein pool, partition of the proteins between the circulation and the extravascular compartment could be estimated. Several studies on the distribution of various plasma protein fractions have been reported from different laboratories (19–22). These studies indicate that 24–50% of the total plasma protein pool is found in the interstitial space. Three of the four studies indicate that 50% of the plasma proteins were located extravascularly. Since the interstitial space occupies approximately 3.3 times the plasma volume in mammals, the corresponding protein concentration in tissue fluid would amount to 2.1%. This is in the same general range as the protein concentration in lymph from extremities, which has been reported by several authors to range from 1.1 to 2.4% (23–25). A protein concentration of 2.1% is equivalent to an osmotic pressure of approximately 5 mm Hg, which would be sufficient to disturb seriously the equilibrium of filtration and reabsorption.

The magnitude of hydrostatic pressures in tissue fluid, i.e. the tissue pressure, has been subject to controversy. McMaster and other investigators inserted small hypodermic needles (25–30 gauge) into the tissue (26). A small volume of fluid was then injected into the tissues, and the equilibrium pressure was measured. This technique has yielded values of 1–5 mm Hg for tissue pressure. The method has been criticized, however, on the grounds that fluid injected into the tissue distorts tissue elements, and pressure recorded in this manner may simply represent elastic rebound phenomena. Furthermore, insertion of the needle might be associated with injury to capillaries and lymphatics along the needle track, possibly leading to increased local transudation of fluid and artificially elevated pressures. Recently, tissue pressures have been measured in the Microcirculation Laboratory with a pressure-recording technique which utilizes ultramicroscopic glass micropipettes as pressure transducers. Details of the technique have been described elsewhere (27). Pressures recorded in the subcutaneous tissues of the bat wing range from 0 to 4 mm Hg in virtually all locations of the wing web.[2] The tips of the micropipettes are less than 1 μ in diameter, and minimal interference with normal tissue structure is thus ensured.

An alternative method for recording tissue pressure was suggested by Guyton (28), who implanted perforated spherical capsules subcutaneously in

[2] C. A. Wiederhielm. Unpublished observations.

a large group of animals and 4 wk later recorded the hydrostatic pressures in the free fluid space within the capsule. Hydrostatic pressures in the capsules were always negative, averaging −6.3 mm Hg. Guyton felt that the intracapsular pressure was representative of tissue pressure, since, when Evans blue dye was injected into the capsule under positive pressure, the dye could be traced into the tissues surrounding the capsule. The formation of edema, either by infusion of large volumes of physiological saline or by venous compression, was associated with an elevation of the pressure within the capsule into the positive range. A good correlation was then obtained with tissue pressures recorded by insertion of needles into the connective tissue. The presence of negative pressures in the tissue space creates additional problems, however. A direct consequence would be increased capillary filtration, which would add to the effect of the high colloid osmotic pressure of tissue fluid. Furthermore, the transport of tissue fluid into lymphatics against a hydrostatic pressure gradient is difficult to visualize, particularly since electron micrographs of lymphatic capillaries indicate large spaces between endothelial cells, similar to those seen in liver sinusoids (29). The following general conclusions may be drawn from the studies quoted in the first section of the paper. (*a*) Capillary permeability to water is not uniform along the length of the capillary, but may be 50–100% larger in the venous capillary than in the arterial capillary. (*b*) The surface area of the arterial capillaries is not identical with that of the venous capillaries in all tissues, but may be only one-sixth to one-fourth of the surface area of the venous capillary. (*c*) The average protein concentration in interstitial fluid is not negligible, but may be as high as 2–3%, with corresponding colloid osmotic pressures of 5–8 mm Hg. (*d*) Measurements of hydrostatic tissue pressure have yielded contradictory results. Measurements obtained by direct puncture, either with hypodermic needles or with ultramicroscopic glass micropipettes, yield tissue pressures ranging from 0 to +5 mm Hg, whereas pressures recorded in subcutaneous capsules are negative and average −6 mm Hg.

In the following section of this report, these deviations from the traditional model have been incorporated into a revised model.

COMPUTER ANALYSIS OF CAPILLARY WATER BALANCE

The basic considerations on which the computer analysis is based are illustrated in Fig. 2 *A* and *B*. In the simulation program, fluid and protein fluxes into and out of the interstitial space and the compliance of the interstitial space were considered. The volume of the interstitial space is dependent on four separate fluid fluxes into and out of the space (Fig. 2 *A*). Filtration occurs from the capillary at the arterial end of the network; also plasma leaks into the interstitium through the large-pore system in the venous capillaries. At

equilibrium these two inward fluxes are balanced by reabsorption of fluid into the venous capillaries and smallest venules, and also by removal of tissue fluid through lymphatics. The equations describing the magnitude of these fluid fluxes are as follows.

$$\text{Filtration: } F = K_A A_A (P_A - P_T - \pi_{PL} + \pi_T) \quad (1)$$

where K_A represents the permeability of the arterial capillary to water, A_A the surface area of the capillary, P_A the hydrostatic pressure in the capillary,

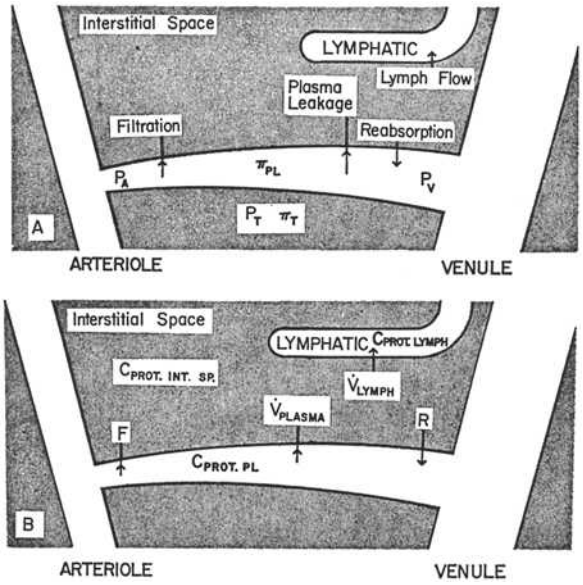

FIGURE 2. Fluid and protein fluxes in interstitial space. *A*, Fluid fluxes: P_A = arterial capillary pressure; P_V = venous capillary pressure; π_{PL} = plasma oncotic pressure; P_T = tissue pressure; π_T = tissue fluid oncotic pressure. *B*, Protein fluxes in interstitial space: $\dot{V}_{plasma}$ = plasma leak through large-pore system; $\dot{V}_{lymph}$ = lymph flow; $C_{prot.\ pl}$ = protein concentration of plasma; $C_{prot.\ lymph}$ = protein concentration in lymph.

P_T the hydrostatic pressure in the tissue of the interstitial space, π_{PL} the colloid osmotic pressure of plasma, and π_T the colloid osmotic pressure of tissue fluid. A similar equation describes reabsorption.

$$R = K_V A_V (P_V - P_T - \pi_{PL} + \pi_T) \quad (2)$$

where K_V and A_V represent the permeability and surface area of the venous capillaries, respectively, and P_V is the hydrostatic pressure in the venous capillary. The plasma leakage through the large-pore system is a bulk flow, and is mainly determined by the hydrostatic pressure gradient across the

venous capillary wall. Thus

$$\text{Plasma leakage: } \dot{V}_{\text{plasma}} = K_1(P_V - P_T) \tag{3}$$

When the tissue pressure exceeds the venous capillary pressure, plasma leakage is assumed to be zero. The lymph flow is assumed to be a linear function of the tissue pressure. Thus

$$\text{Lymph flow: } \dot{V}_{\text{lymph}} = K_2 P_T \tag{4}$$

Lymph flow is also assumed to be zero for negative tissue pressures. In equilibrium, the algebraic sum of the fluxes into and out of the interstitial space must be zero. If an input variable, e.g. venous capillary pressure or colloid osmotic pressure of plasma, is changed, net flux changes transiently, leading to a change in interstitial space volume before a new equilibrium is established. The change in interstitial space volume is

$$\Delta V = \int_0^t (\mathrm{F} + \dot{V}_{\text{plasma}} + R - \dot{V}_{\text{lymph}})\, dt \tag{5}$$

As a first approximation, the hydrostatic pressure of tissue fluid was assumed to be a linear function of the change in tissue volume.

$$\text{Tissue pressure: } P_T = K_4 \Delta V \tag{6}$$

where K_4 represents the compliance of the interstitial space. At a given volume of the interstitial space, which might be considered a reference volume (V_0), tissue pressure is zero. The total interstitial space volume under varying input conditions, then, is

$$V = V_0 + \Delta V \tag{7}$$

A similar set of expressions may also be derived for protein fluxes into and out of the interstitial space (Fig. 2 *B*). The protein flux into the interstitial space through the large-pore system is

$$\dot{Q}_{\text{prot. leak}} = \dot{V}_{\text{plasma}}\, C_{\text{prot. pl}}, \tag{8}$$

where $C_{\text{prot. pl.}}$ is the concentration of protein in the plasma. Similarly, the protein flux removed from the interstitial space by the lymphatics is

$$\dot{Q}_{\text{prot. lymph}} = \dot{V}_{\text{lymph}}\, C_{\text{prot. lymph}} \tag{9}$$

where $C_{\text{prot. lymph}}$ is the concentration of protein in the lymph. The protein content of lymph and tissue fluid is assumed to be identical. Thus

$$C_{\text{prot. lymph}} = C_{\text{prot. int. sp.}} \tag{10}$$

The quantity of protein in the interstitial space may be represented by a time integral of the differences of these two protein fluxes. Thus

$$Q_{\text{prot.}} = \int_0^t (\dot{Q}_{\text{prot. leak}} - \dot{Q}_{\text{prot. lymph}})\, dt \qquad (11)$$

The concentration of protein in the tissue fluid may then be determined.

$$C_{\text{prot. int. sp.}} = \frac{Q_{\text{prot.}}}{V} \qquad (12)$$

Colloid osmotic pressure in the tissue fluid was assumed as a first approximation to be a linear function of the protein concentration. Thus

$$\pi_T = K_3 C_{\text{prot. int. sp.}} \qquad (13)$$

TABLE I
ASSUMED NORMAL VALUES FOR PARAMETERS USED IN COMPUTER ANALYSIS

Parameter	Value
Arterial capillary pressure (P_A)	35 mm Hg
Venous capillary pressure (P_V)	15 mm Hg
Plasma oncotic pressure (π_{PL})	25 mm Hg
K_A/K_V	0.6
A_A/A_V	0.25
$K_A A_A/K_V A_V$	0.16
Plasma leakage	5% of total exchange
Interstitial space compliance	60%/mm Hg

It should be emphasized that the equilibrium is established by osmotic pressures, and any errors introduced by the assumption that the plasma proteins obey Van't Hoff's law will be reflected in values for the protein concentration rather than in the osmotic pressures.

The majority of the terms in the preceding equations represent experimentally determined input variables as listed in Table 1. Solutions are sought for steady-state values of the tissue fluid osmotic pressure (π_T) and hydrostatic tissue pressure (P_T). The solution for these two parameters in turn determines changes of interstitial volume (ΔV) and lymph flow ($\dot{Q}_{\text{lymph}}$).

A schematic of the analogue computer program is shown in Fig. 3. The two operational amplifiers in the upper left-hand corner are connected in an adder-subtracter configuration, and yield values for the net pressures for filtration and reabsorption. After multiplication by the coefficients $K_A A_A$ and $K_V A_V$, a measure of filtration and reabsorption is obtained. The additional two operational amplifiers on the left side of the figure, with their coefficient

potentiometers, generate the remaining two fluxes, the plasma leak and lymph flow. The diodes in the output circuits impose unidirectional flow through the large-pore system and through the lymphatics. The coefficient potentiometer K_5 establishes the relationship between plasma and protein leakage. The four fluid fluxes are added algebraically, yielding a measure of net flux, which after integration yields a measure of changes in the interstitial volume (ΔV). The coefficient potentiometer K_4 represents interstitial compliance, and relates tissue pressure to the change in interstitial volume. The net flux

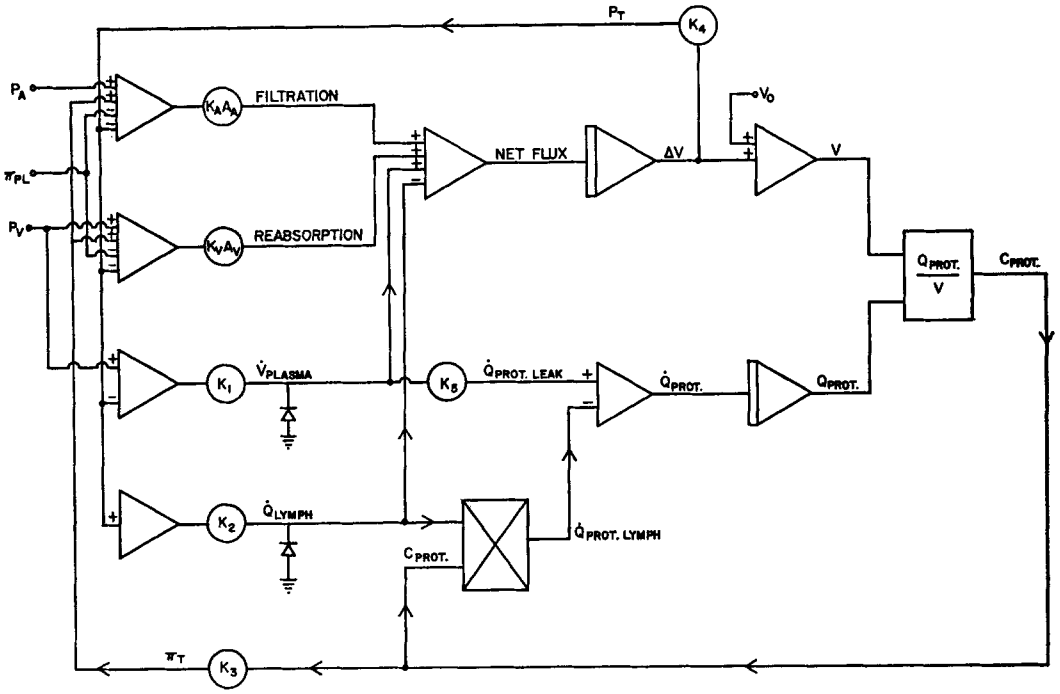

FIGURE 3. Diagram of analogue computer program. For a description, see the text.

of protein into the interstitial space is also derived from the output of the two operational amplifiers (Fig. 3, lower left). The net flux of protein ($\dot{Q}_{prot.}$) represents the difference of the protein flux through the large pore system and the lymph. By integrating the net flux, the quantity of protein ($Q_{prot.}$) in the interstitial space is derived. Dividing this quantity by the interstitial volume (V) yields a value for the protein concentration in the interstitial fluid. The coefficient K_3 relates this concentration to the colloid osmotic pressure of the tissue fluid.

Time scaling was not attempted in this first generation of the simulation program; only steady-state solutions were sought. A rigorous solution for transient events would require inclusion of nonlinearities in the relationship

between protein concentration and colloid osmotic pressure of plasma proteins, and also the interstitial compliance. These were not included at this time because of analogue computer limitations.

RESULTS

Normal Values and Simulation of Lymphedema and Inflammation

Ten input and output variables were recorded on an eight-channel strip-chart recorder, as shown in Fig. 4. Recordings of colloid osmotic pressure for plasma and tissue fluid were multiplexed on one channel, and arterial and venous capillary pressures were multiplexed on another. Fig. 4 *A* shows a recording obtained in experimental simulation of lymphedema. The first 5 mm of the recording represent the normal state. Interstitial volume in the normal state was considered to be 100%, and changes in interstitial volume were subsequently shown as percentages of the initial volume. The tissue pressure was approximately +1 mm Hg. Colloid osmotic pressures of plasma and tissue fluid were 24 and 10 mm Hg, respectively. Filtration under these conditions amounted to 15 AU, and reabsorption, to 9 AU. Plasma leakage, as expected, amounted to only a small fraction of the sum of filtration and reabsorption, on the order of 0.4 AU. Lymph flow was 5 AU; arterial and venous capillary pressures were 35 and 14 mm Hg, respectively. Lymph flow amounted to approximately 30% of the filtration. The computed tissue pressure value of 1 mm Hg agrees with McMaster's observations (26) and also with results obtained in this laboratory.

Lymphedema was simulated by reducing lymph flow to zero and recording subsequent changes in the different parameters. The most striking feature is a dramatic increase in interstitial volume, amounting to over 900%. If the interstitial space is assumed to represent 20% of the tissue volume, this increase in interstitial space would correspond approximately to a doubling of the tissue volume, which is not uncommonly observed in severe lymphedema. Associated with the drastic increase of interstitial volume, tissue pressure is elevated to about 13 mm Hg, which approximates the pressure in the venous capillary. The oncotic pressure of tissue fluid rises to levels approaching the plasma colloid osmotic pressure. Relatively small changes were observed in both filtration and reabsorption, mainly because of the parallel increases in tissue hydrostatic and oncotic pressures, which cancel each other. Plasma leakage decreases toward zero as the hydrostatic pressure gradient across the venous capillary is progressively reduced with increasing tissue pressure. Many clinical features of lymphedema thus can be simulated by the computer program.

Increased capillary permeability to protein, associated with an inflammatory reaction, may also be conveniently simulated with the computer (Fig. 4 *B*). In this computer run, inflammation was simulated by increasing the

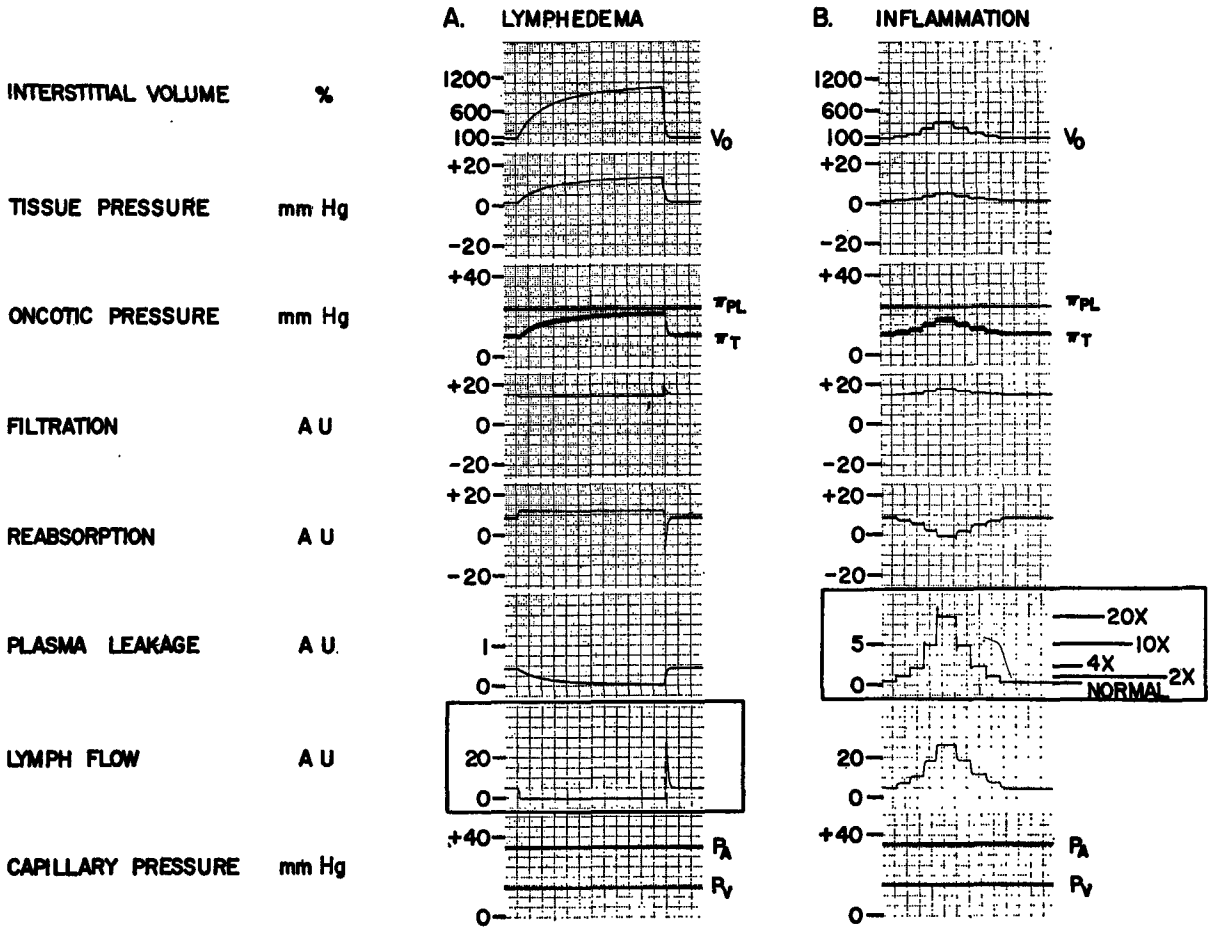

FIGURE 4. Simulation of lymphedema and inflammation. The eight recorder channels show recordings of 10 input and output variables. Oncotic pressures of plasma and tissue fluid are multiplexed on the third recorder channel. Arterial and venous capillary pressures are multiplexed on the eighth recorder channel. The altered input parameter is indicated by a frame.

plasma leakage from normal to 2, 4, 10, and 20 times normal. Edema, reflected in the increased interstitial volume, was less pronounced than in the case of lymphedema. With a 20-fold increase in permeability to protein, interstitial volume increased only 3-fold. Tissue pressure showed a moderate elevation to +5 mm Hg. Colloid osmotic pressure of tissue fluid increased from a normal of 10 mm Hg to a peak of approximately 16 mm Hg. There was a slight increase in filtration, and a marked depression of reabsorption, presumably because of the elevated tissue fluid protein concentration. Lymph flow increased dramatically from a normal of 5 AU to a maximum of 26 AU. The higher removal rate of tissue fluid by means of lymphatics probably accounts for the relatively small accumulation of fluid in the interstitial space.

Even though it is reassuring that pathological processes may be reproduced in some detail by means of a computer program, its greatest value rests in the ability to elucidate basic physiological mechanisms, as described in the next section.

Effects of Changing Plasma Oncotic Pressure and Venous Pressure

As pointed out in the first section of this report, a protective factor appears to exist which limits major shifts of fluid from the circulation into the interstitial space, unless changes in either plasma oncotic pressure or venous pressure are larger than 10–15 mm Hg. In order to elucidate the nature of this protective mechanism, the plasma oncotic pressure or, alternatively, the venous pressure was changed in separate computer runs (Figs. 5 and 6). Fig. 5 illustrates an experiment in which the plasma oncotic pressure was changed from 40 to 0 mm Hg and then returned. With hypertonic pressures, the interstitial volume was reduced about 30%. Changing the colloid osmotic pressure from 40 to 20 mm Hg produces relatively slight changes in the interstitial volume. As the plasma osmotic pressure is reduced to less than 15 mm Hg, however, each stepwise change in oncotic pressure leads to progressively larger changes in interstitial volume. When the plasma oncotic pressure is reduced to zero, the interstitial volume increases by 500%. The change in interstitial volume is associated with a moderate increase in tissue pressure, from a low of less than +1 mm Hg in the hypertonic range to a peak of +8 mm Hg when the plasma oncotic pressure is zero. It is particularly noteworthy that each incremental change in plasma oncotic pressure is followed by an almost identical change in oncotic pressure of tissue fluid, as shown in the third recorder channel. As the plasma oncotic pressure is reduced below 20 mm Hg, however, the changes in tissue oncotic pressure become progressively smaller. Filtration increases moderately as the plasma oncotic pressure is decreased; reabsorption is markedly reduced, however. In fact, when the plasma oncotic pressure is reduced to 10 mm Hg, reabsorption in the venous capillary is supplanted by filtration. Plasma leakage shows a gradual decrease

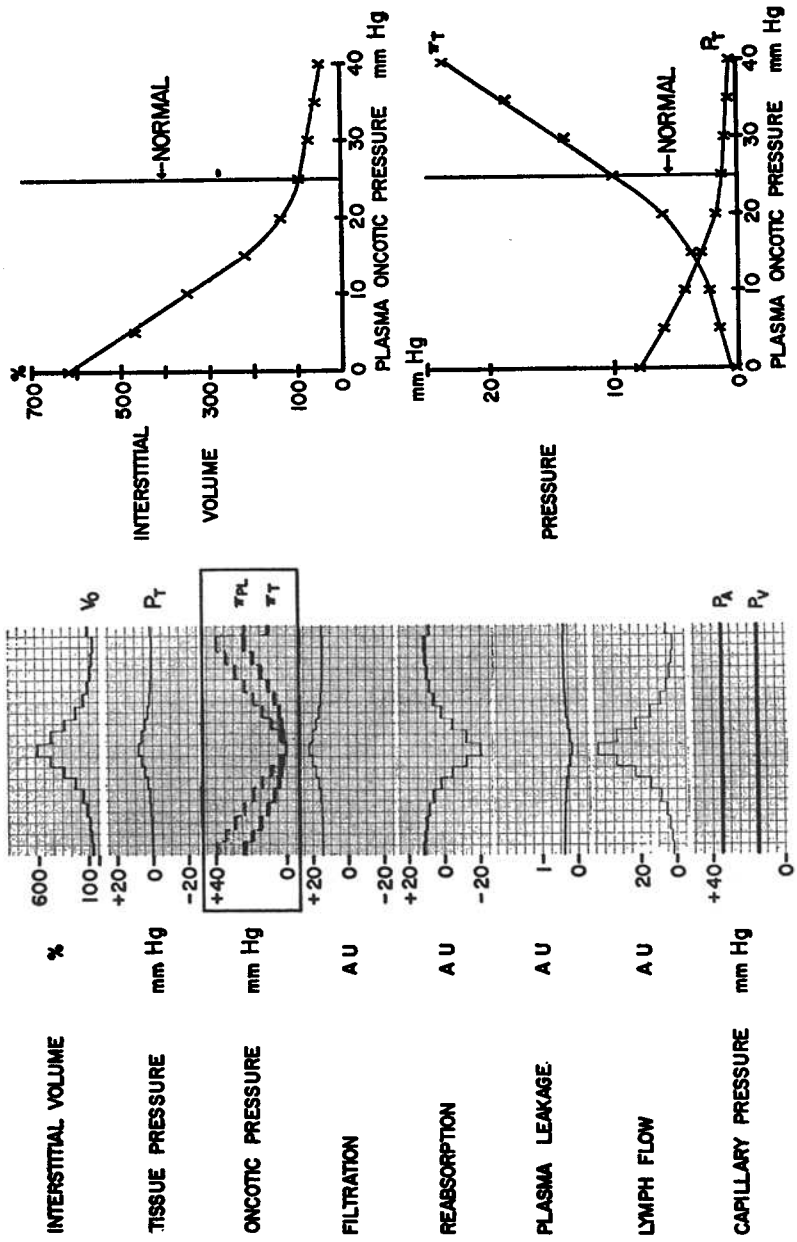

FIGURE 5. Computer simulation of response to changes in plasma oncotic pressure.

associated with progressively increasing tissue pressure. Lymph flow increases dramatically, and reaches a peak of 43 AU as compared with a control value of 5 AU.

The maintenance of a relatively constant interstitial volume can be demonstrated by plotting interstitial volume against plasma oncotic pressure (Fig. 5, top right). Over a range of plasma oncotic pressure from 40 to 20 mm Hg, the change in the interstitial volume is relatively insignificant. When the plasma oncotic pressure is reduced below 15 mm Hg, interstitial volume increases dramatically. At a plasma oncotic pressure of 15 mm Hg, the interstitial volume has approximately doubled, equivalent to 1+ edema. The compensatory changes which maintain the interstitial volume relatively constant are illustrated in the graph in Fig. 5, bottom right. This graph shows a composite plot of tissue pressure and tissue fluid oncotic pressure against plasma oncotic pressure. At hypertonic plasma oncotic pressures, the oncotic pressure of tissue fluid is high. Each decremental decrease of plasma oncotic pressure is reflected in an almost identical decrease in the tissue fluid. This may be considered a compensatory mechanism, in which a new equilibrium of filtration and reabsorption is established by dilution of tissue fluid proteins. In the range of hypotonic plasma oncotic pressures ($\pi_{PL} < 15$ mm Hg), tissue fluid protein is diluted to a point where compensation is no longer possible. Under these circumstances, reabsorption decreases progressively, leading to an increased net flux of fluid into the tissues. The limiting factor for fluid transfer at this level shifts from one of dilution of proteins to the compliance of interstitial space, reflected in pronounced increases in tissue pressure and interstitial volume. Thus two separate mechanisms limiting the net transfer of fluid across the capillary wall appear to exist. At high plasma oncotic pressures (15–40 mm Hg), the limiting factor is mainly dilution of the tissue fluid protein. At lower oncotic pressures, the distensibility of the interstitial space is the major factor.

An essentially similar picture evolves in computer runs where the venous pressure was changed (Fig. 6). The venous pressure, in this instance considered to represent pressure in the smallest venous tributaries, was changed from 0 to 35 mm Hg. Corresponding venous capillary pressures are somewhat higher, as shown in the record (channel 8), as a result of the postcapillary resistance between the venous capillary and the small vein. Each incremental change in venous pressure is initially associated with relatively small changes in the interstitial volume. However, when the venous pressure is increased beyond 20–25 mm Hg, interstitial volume increases markedly. Tissue pressure shows a moderate increase from less than +1 mm Hg to a maximum of +5 mm Hg. Each incremental change in venous pressure is also associated with an almost identical decrease in the oncotic pressure of tissue fluid. At the highest venous pressure, dilution of the tissue fluid has progressed to a point where compensa-

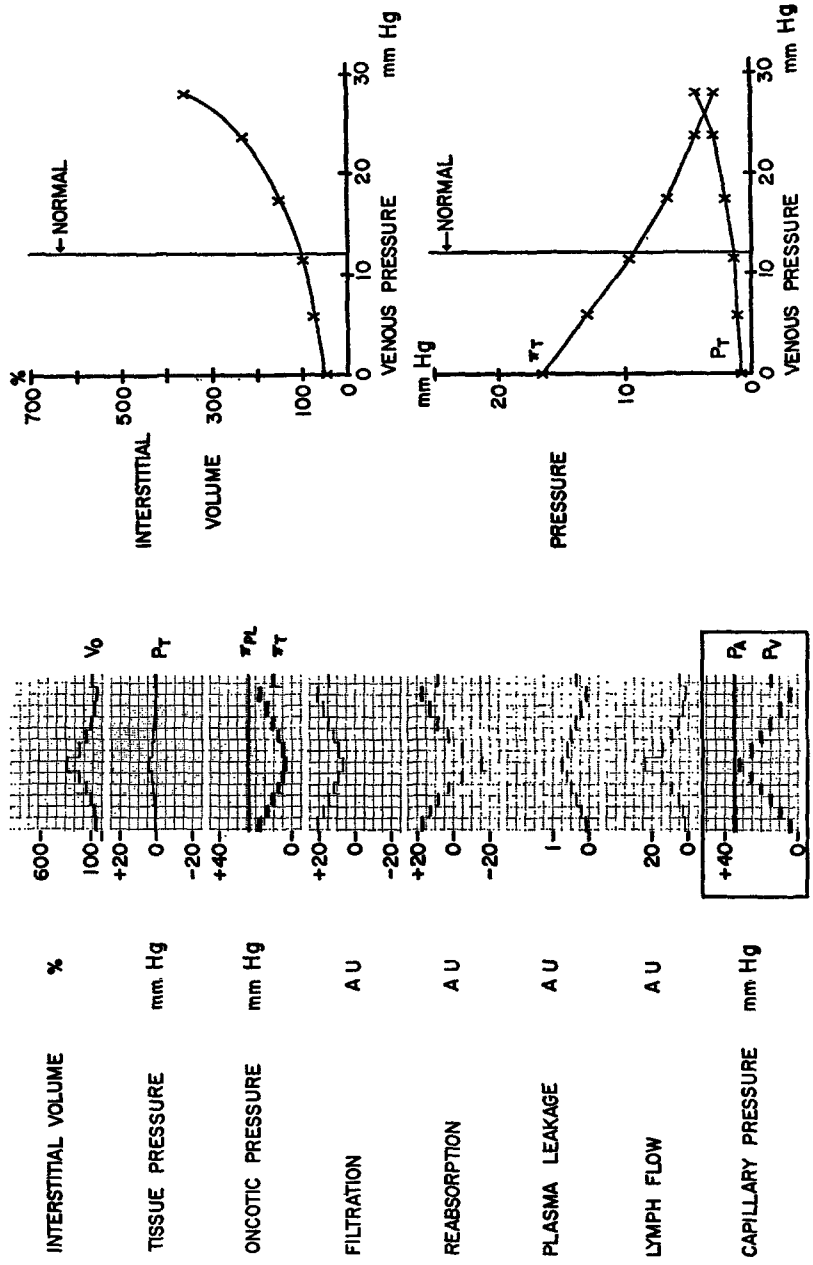

FIGURE 6. Computer simulation of response to changes in venous pressure.

tion is no longer possible. Filtration shows a progressive decrease, associated in part with the decrease in tissue fluid oncotic pressure and in part with the increase in hydrostatic tissue pressures. Reabsorption, as would be expected, is profoundly affected by changes in the venous pressure, and is converted to filtration when the venous capillary pressure reaches 25 mm Hg. Plasma leakage progressively increases with increasing venous pressures, since bulk flow through the large-pore system is determined by the venous capillary pressure. Lymph flow also shows a progressive, but nonlinear, increase. When interstitial volume is plotted against venous pressure (Fig. 6, top right), the interstitial volume does not change significantly until venous pressure has been elevated by 10–15 mm Hg. Beyond this level, the interstitial volume increases rapidly to levels equivalent to edema. Tissue fluid oncotic pressure and tissue pressure plotted against venous pressure (Fig. 6, lower right) show the same basic features as when plasma oncotic pressure was changed. At low venous pressures, the tissue fluid oncotic pressure is high. Increasing venous pressures are counteracted to a large extent by equivalent changes in tissue fluid oncotic pressure. The limit of the compensatory range is reached at venous pressures of about 25 mm Hg, at which time tissue pressure begins to rise abruptly.

Effect of Changing Arterial Capillary Pressures

That edema is not a typical feature of hypertension until heart failure intervenes is well established clinically. Changes in arterial pressure have also been shown to have considerably less effect on capillary filtration rates than corresponding changes in venous pressure (30). Similar results have been obtained in computer runs in which the arterial capillary pressure was changed in steps from 40 to 15 mm Hg (Fig. 7). Over this pressure range, only insignificant changes were observed in interstitial volume and tissue pressure. Tissue fluid oncotic pressure increased from a normal of 10 mm Hg to 13 mm Hg, which may have been due to the reduction in filtration in the face of relatively constant plasma leakage. The reduction in filtration is a direct consequence of the reduced net filtration pressure in the arterial capillary. The reduction in reabsorption, in turn, reflects the increase in tissue oncotic pressure, which at its peak approaches the venous capillary hydrostatic pressure.

Computer Analysis of Tissue Pressure

It was pointed out in the first section of this report that measurements of tissue pressure by different techniques have yielded divergent results. Pressures recorded by hypodermic needle or ultramicroscopic glass micropipettes generally yield pressures ranging from 0 to +5 mm Hg, whereas hydrostatic pressures recorded in the free fluid space in subcutaneous capsules are nor-

mally negative. These divergent findings may be reconciled by considering the interface between the interstitial space and the free fluid within the capsule as a semipermeable membrane (Fig. 8). This figure illustrates diagrammatically the relationships between the blood vessels in the tissues surrounding the capsule and the free fluid within the capsule. The hydrostatic pressure within the capsule (P_c) is determined by the net flux of fluid from the inter-

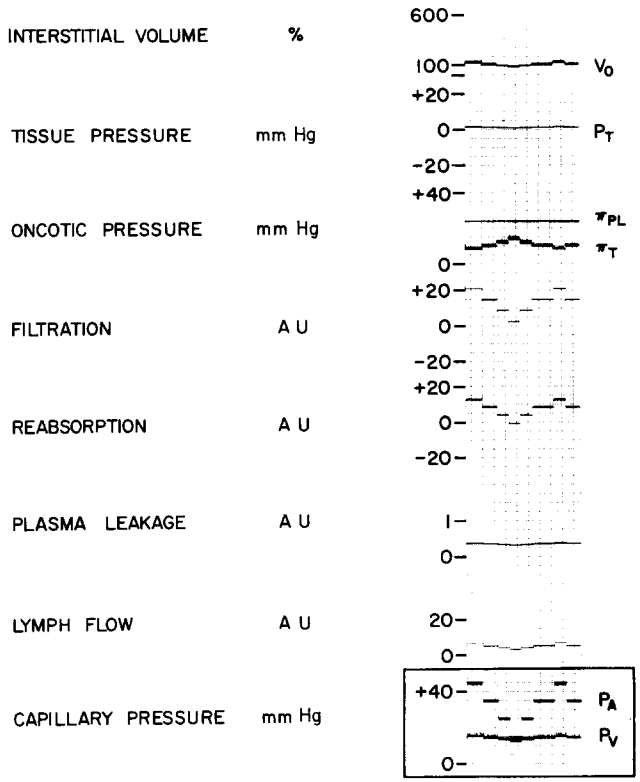

FIGURE 7. Computer simulation of response to changes in arterial capillary pressure.

stitial space of surrounding tissues into the free fluid space ($\dot{V}_{fluid}$). The free intracapsular fluid has an average protein content of 1.9%, with a corresponding colloid osmotic pressure (π_C) of 4.3 mm Hg (28). In the interstitial space surrounding the capsule exists a hydrostatic pressure (P_T) and protein with a colloid osmotic pressure (π_T). If the interface between the free fluid and the interstitial space has the properties of a semipermeable membrane, the net fluid exchange may be defined by the following equation:

$$\dot{V}_{fluid} = K_6 (P_T - P_C - \pi_T + \pi_C) \quad (14)$$

where K_6 is a virtual permeability coefficient for the interface, and P_T and π_T denote the tissue pressure and tissue fluid oncotic pressure, respectively. P_C and π_C refer to hydrostatic and oncotic pressures of the free fluid in the capsule. The change in fluid volume within the capsule (ΔV_C) would then be an integral function of the fluid flux:

$$\Delta V_C = \int_0^t \dot{V}_{\text{fluid}}\, dt \tag{15}$$

The pressure in the capsule may be assumed to be a linear function of the volume change

$$P_C = K_7 \Delta V_C \tag{16}$$

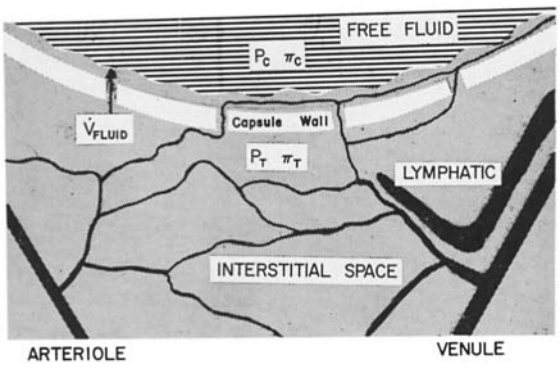

FIGURE 8. Fluid exchange between interstitial space and subcutaneous capsule. $\dot{V}_{\text{fluid}}$ = net fluid flux; P_C = hydrostatic pressure in capsule; π_C = oncotic pressure of free intracapsular fluid.

where K_7 represents the compliance of the capsule. In simulation of steady-state conditions, the steps described by equations 15 and 16 need not be performed; determining the capsule pressure for which the fluid flux is zero is sufficient. Three of the quantities describing the net driving pressure are known. P_T and π_T may be derived from the computer simulation program as described in previous sections, and π_C is 4.3 mm Hg (28). Thus the equation can be solved for P_C as the tissue pressure and tissue fluid oncotic pressure vary in response to changes in the input parameters. The results of a computer simulation run are shown in Fig. 9, in which the plasma oncotic pressure was changed and tissue fluid oncotic pressure, hydrostatic tissue pressure, intracapsular hydrostatic pressure, and interstitial volume were recorded. The changes in tissue fluid oncotic pressure, tissue pressure, and interstitial volume are identical with those described previously, and need not be elaborated here. The computed hydrostatic pressure within the capsule is generally

negative, however. When the plasma oncotic pressure is 40 mm Hg, the capsule pressure is about −19 mm Hg. As the oncotic pressure of plasma is reduced in 5 mm steps, the capsule pressure increases progressively. At the normal plasma oncotic pressure of 25 mm Hg, the intracapsular pressure is −5 mm Hg, which agrees well with Guyton's experimental measurements. A composite plot of the tissue hydrostatic, oncotic, and capsule pressures vs. plasma oncotic pressure is shown in Fig. 9, right. It is noteworthy that at normal plasma oncotic pressure the computed tissue pressure is about +1 mm

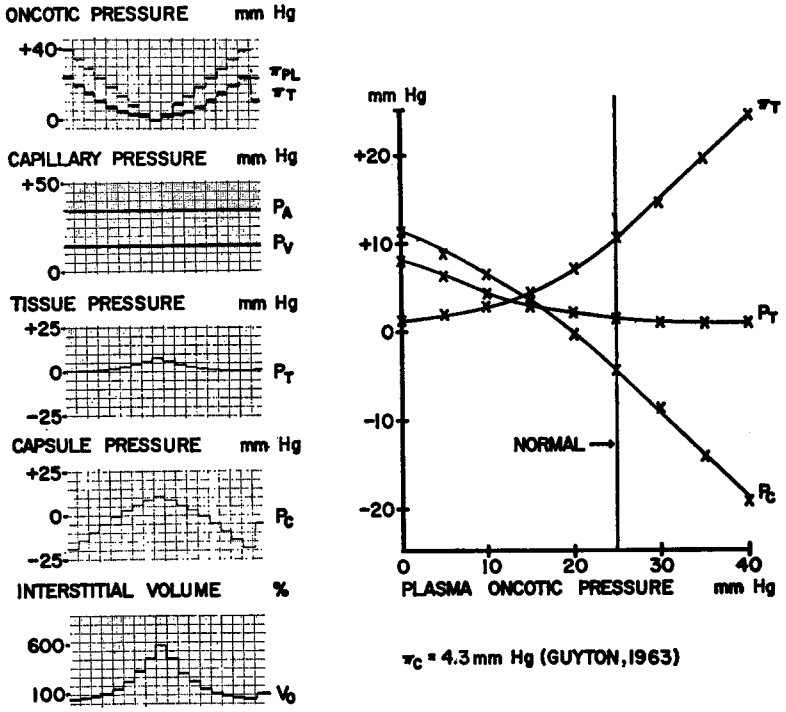

FIGURE 9. Computer simulation of equilibrium of fluid exchange between interstitial space and intracapsular fluid under conditions of changing plasma oncotic pressure.

Hg and the tissue fluid oncotic pressure about 10 mm Hg, whereas the computed intracapsular pressure is −5 mm Hg. As plasma oncotic pressures are increased from the normal level, correspondence between changes in tissue fluid oncotic pressure and capsular pressure is almost 1:1. As plasma oncotic pressures are reduced below 15 mm Hg, the capsule pressure approaches the tissue pressure. At the lowest values for plasma oncotic pressure, the capsule pressure parallels the tissue pressure with an offset corresponding to the oncotic pressure of the free fluid in the capsule (4.3 mm Hg). A basically similar response is seen when venous pressure is changed.

The data generated by the computer simulation yield a reasonable fit to

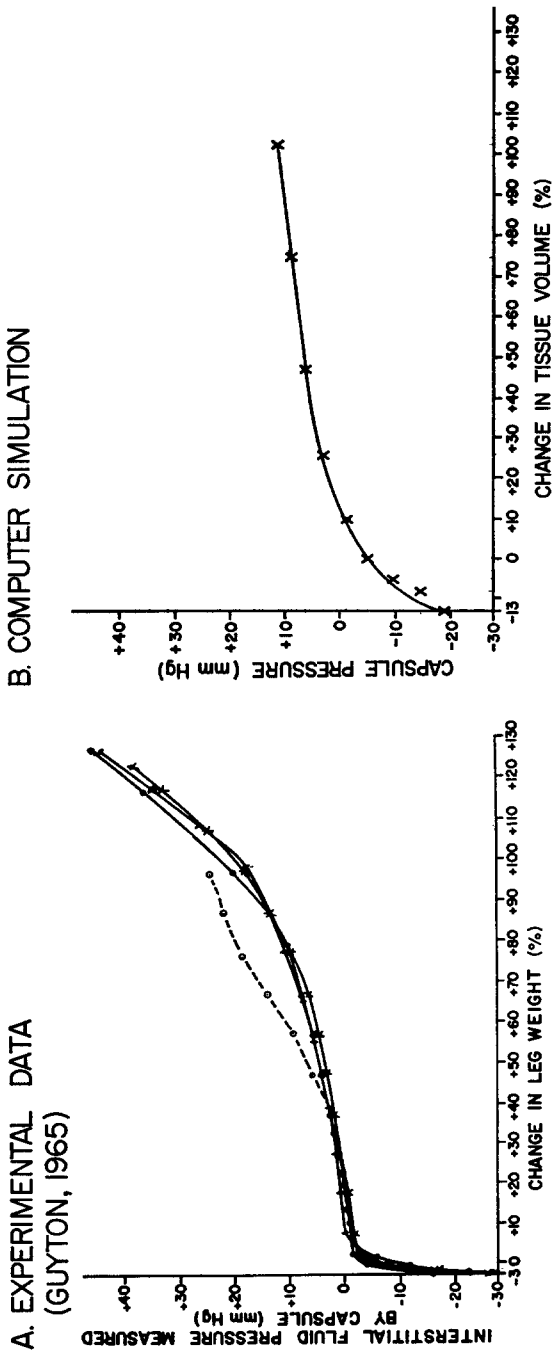

FIGURE 10. Comparison of experimental data of Guyton (31) with computed solution for intracapsular pressure. *Left-hand graph reprinted by permission from Dr. A. C. Guyton and The American Heart Association, Inc. and from Circulation Research (Interstitial fluid pressure: II. Pressure-volume curves of interstitial space, 1965, 16:452).*

Guyton's experimental data (28, 31). When computed intracapsular pressure is plotted against interstitial volume, expressed as a percentage change in total tissue volume, a fair fit is obtained over a relatively wide range (Fig. 10 *A* and *B*). The deviation in the lower range may be explained in part by the fact that the experimental data of Guyton were obtained in dehydrated preparations. Deviation in the high range may have been due to the simplifying assumption in the computer program that the interstitial volume is a linear function of tissue pressure. Deviations from linearity would be expected at large volume changes, however.

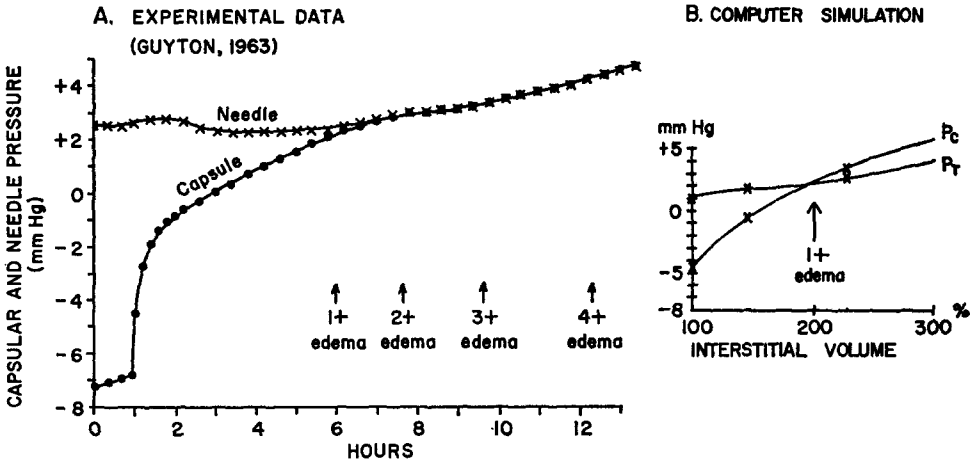

FIGURE 11. Comparison of experimental data on intracapsular and needle pressures in animal experiments with computed values of tissue and intracapsular pressures. *Left-hand graph reprinted by permission from Dr. A. C. Guyton and The American Heart Association, Inc. and from Circulation Research (Concentration of negative interstitial pressure based on pressures in implanted perforated capsules, 1963, 12:399).*

A second comparison of computer-generated data with experimental data is shown in Fig. 11. Guyton plotted intracapsular pressure and tissue pressure, recorded by needle, during development of edema (Fig. 11 *A*) (31). Computed values of tissue pressure and intracapsular pressures show a similar divergence, which initially amounts to 6.5 mm Hg. After 1 + edema has developed, the pressure recorded within the capsule and by the needle coincide in Guyton's experiments. In the computer simulation, tissue and capsule pressures cross over when the interstitial volume is increased by 100%, corresponding to 1 + edema (Fig. 11 *B*). In the animal experiments, needle and capsule pressures were identical as edema increased. In the computer simulation, however, capsule pressure slightly exceeded tissue pressure.

COMPARISON OF COMPUTER SIMULATION DATA WITH EXPERIMENTAL DATA

Normal Values

Despite its relative simplicity, the computer model has agreed surprisingly well with experimental data in the literature. Normal tissue pressure in the range of +1 mm Hg is in line with McMaster's (26) measurements and also with independent measurements in our laboratory. The relative magnitude of filtration, reabsorption, and lymph flow is somewhat higher than estimated by Landis and Pappenheimer (3). Computer analysis indicated that lymph flow normally amounts to 30% of filtration; the estimate of Landis and Pappenheimer ranged from 10 to 20%. Adjustment of some computer parameters may bring these results closer in line. The most striking divergence from previously published normal values were obtained for oncotic pressure of tissue fluid, since computer runs averaged 10 mm Hg, which corresponds to a tissue fluid protein concentration of 3.5%. Whereas the protein content of lymph derived from cardiac muscle, lungs, or gastrointestinal tract may exceed this value, lymph from skin and skeletal muscle usually has a lower protein content. This unexpectedly high value can be explained, however, on the basis of physical chemical properties of the ground substance, which occupies a substantial fraction of the interstitial space. The ground substance in the connective tissue of skin is composed mainly of hyaluronic acid and chondroitin sulfate (32). Characteristically the macromolecules of these mucopolysaccharides form an intertwining meshwork, in which the mesh diameter is sufficiently small to exclude the protein but is freely permeable to smaller molecules such as water, electrolytes, and glucose. The fluid bound in the ground substance thus is not available to the protein; i.e. the plasma protein space is not identical with the total interstitial space.

Biochemical assay of human skin shows an average content of 0.2% mucopolysaccharides (32). Since the interstitial space occupies approximately 20% of the total skin volume, the concentration of mucopolysaccharides within the interstitial space could be as high as 1%. The osmotic pressure of a 1% solution of hyaluronic acid has been estimated to be 4.5 mm Hg (33). 1 g of hyaluronic acid may bind as much as 100 ml of water in the absence of other sterically excluded macromolecules, such as plasma albumin. In the presence of albumin, however, water will be extracted from the meshwork of the ground substance, and free fluid spaces will exist within the ground substance. The water bound in the ground substance thus is in a dynamic equilibrium with the free fluid phase. The syneresis is associated with a pronounced nonlinear increase in osmotic pressure. For instance, a 2% solution of hyaluronic acid exerts an osmotic pressure of 18 mm Hg (33).

The basic concept of the interstitial space as a two-phase structure has been supported by a number of physiological and morphological studies, as summarized in a previous publication (34). Of particular interest is a study by Gersh and Catchpole (35), in which Evans blue was used as a vital dye. The Evans blue, which presumably was bound to plasma albumin, was found to be distributed in discrete areas within the ground substance. In these same

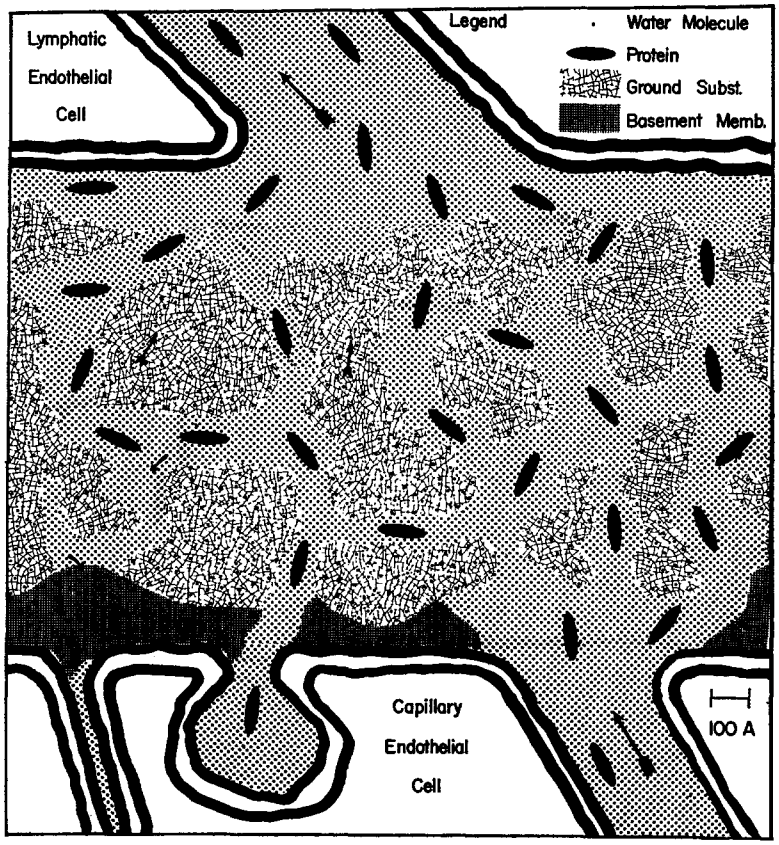

FIGURE 12. Interrelationship of ground substance, free fluid, and fluid and protein exchange in interstitial space. The large pore and the tight junction between capillary endothelial cells and protein molecules are drawn approximately to scale.

areas soluble mucopolysaccharides were found, corresponding to free fluid space. The combined effect of the osmotic pressure of the mucopolysaccharides in the ground substance and exclusion of protein may account for the unexpectedly high values for tissue fluid oncotic pressure required for an equilibrium of fluid fluxes.

The interrelationship of ground substance, free fluid, and fluid and protein exchange in the interstitial space is outlined diagrammatically in Fig. 12.

The figure represents the interstitial space between a venous capillary (bottom) and a lymphatic capillary (top). The capillary endothelium shows a 250 A large pore, through which plasma, containing water and protein, is filtered. On the left side is shown a tight junction 40 A wide, through which reabsorption occurs. Protein molecules are restricted to the free fluid phase in the interstitial space, whereas water freely permeates the meshwork of the ground substance. The arrows indicate the net drift of the solvent through the free fluid spaces as well as through the basement membrane and ground substance. Since the protein molecules are restricted to the free fluid phase, they will exert a higher osmotic pressure than if the bound fluid were available for dilution. The protein concentration in the free fluid of the interstitial space may thus be considerably higher than the concentration in either lymph or capillary filtrate, and thus exert a correspondingly higher oncotic pressure. A quantitative evaluation of the role of the ground substance is not possible at this stage, however, since information on fluid fluxes in gels is scanty.

In the studies of Pappenheimer and Soto-Rivera on the isogravimetric hind limb preparation, the average protein osmotic pressure in interstitial fluid was estimated to be 1.4 mm Hg (30). This value is considerably lower than those predicted either from estimates of interstitial protein concentration by tracer studies or by the computer analysis. Pappenheimer and Soto-Rivera's data, however, can be fitted to the computer simulation data if one considers the fact that the blood used in the majority of their experiments had colloid osmotic pressures lower than normal. In 18 out of 20 experiments, the osmotic pressure of the plasma used in the perfusion procedure ranged between 8 and 20 mm Hg, with an average of 15 mm Hg. This low value was believed to result from dilution of the plasma with tissue fluid during the bleeding of the donor animals. It is noteworthy that, when the plasma oncotic pressure is reduced to 15 mm Hg in the computer analysis, the corresponding oncotic pressure of tissue fluid is +3 mm Hg (Fig. 5). Since the tissue pressure at this plasma protein concentration is almost identical with the oncotic pressure of the tissue fluid, their effects cancel out. The isogravimetric pressure under these conditions accurately represents the oncotic pressure of plasma. That Pappenheimer's experiments were carried out under these conditions is perhaps fortuitous, since the validity of the computations of capillary filtration coefficient as well as of changes in pre- and postcapillary resistance depends critically on the assumption of negligible tissue hydrostatic and oncotic pressures. Results obtained by Pappenheimer and Soto-Rivera after periods of prolonged filtration or absorption can also be duplicated qualitatively in the computer simulation. The tissue fluid in the isolated hind limb was first concentrated by allowing net absorption to occur. At minimum limb weight the isogravimetric pressure amounted to only two-thirds of the plasma protein pressure, which was attributed to increased concentration of protein in the

tissue fluid. Capillary pressure was then increased above the isogravimetric value, leading to filtration throughout the capillary network. Filtration of 15 ml of fluid into the interstitial space diluted the tissue fluid protein sufficiently to increase the isogravimetric pressure to 92% of the plasma oncotic pressure. Further filtration did not increase the isogravimetric pressure significantly. It is noteworthy that in the computer analysis the crossover of tissue hydrostatic and oncotic pressures occurs when venous pressure is elevated to levels between 25 and 30 mm Hg. In other experiments, the isolated hind limb preparation was initially perfused with plasma having an oncotic pressure of 17.2 mm Hg for 2¾ hr. Subsequently plasma rendered hypertonic by addition of bovine albumin (π_{PL} = 39.5 mm Hg) was substituted. The first measurements of isogravimetric pressure, 10–15 min after the perfusion of hypertonic plasma was begun, show isogravimetric pressures averaging 60% of the plasma oncotic pressure. During the perfusion period of 5½ hr, the isogravimetric pressure decreased to a level of approximately 25% of the plasma protein pressure without a change in the filtration coefficient. This decrease was attributed to an abnormal permeability of the capillary membranes to crystalline bovine albumin. The alternative should be considered, however, namely that the initial perfusion of the experimental preparation with hypotonic plasma would wash out protein from the interstitial space into the normally collapsed lymphatics. A new equilibrium would be restored over a prolonged time interval, since the amount of protein moving through the large pore system is small compared to the interstitial fluid volume. In comparison, when oncotic pressure is elevated to 40 mm Hg in the computer simulation, tissue fluid oncotic pressure rises to a level of 23 mm Hg. The effective osmotic pressure ($\pi_{PL} - \pi_T$) is approximately 40% of the total plasma osmotic pressure, as compared with Pappenheimer's experimental determination of 25%. Since removal of protein by lymphatic drainage is prevented in the isogravimetric hind limb preparation, the lower values at equilibrium are not unexpected. In the computer simulation of lymphedema, the corresponding effective oncotic pressure is 15% of the plasma oncotic pressure (Fig. 4).

Pappenheimer and Soto-Rivera pointed out that diffusion coefficients of plasma protein are such that relatively large concentration gradients are possible between tissue fluid undergoing reabsorption at the venous end and freshly formed capillary filtrate at the arterial end. Even if all filtration and absorption processes were stopped, some 20 min would be required to reach 90% of equilibration of the protein concentration over a distance of 50 μ. However, significant osmotic gradients associated with unequal distribution of protein are unlikely in the interstitial space, since osmotic attraction of water into areas of high concentration would lead to rapid equilibration. In contrast with the relatively slow diffusion rates of plasma proteins, only 30 sec would be required to reach 66% equilibration of protein concentration

over a distance of 50 μ by redistribution of water (34). Thus, large gradients in osmotic pressure are unlikely within the interstitial space, except on a molecular scale such as represented in Fig. 12.

Effect of Changing Plasma Oncotic Pressure

Data obtained from the computer analysis also agree reasonably with experimental studies in which plasma oncotic pressure was altered by continuous infusion of Ringer's solution. A compensatory change in tissue fluid protein concentration, as plasma oncotic pressure is altered, has been demonstrated experimentally by Morris (36). In his experiments, hemodilution was induced by infusing Ringer's solution at a rate of 100 ml/kg/5 hr. Concentrations of protein in plasma and intestinal lymph were measured. Prior to the Ringer's infusion, the difference in colloid osmotic pressure between plasma and lymph was 8 mm Hg; at the end of the experiment, 5 hr later, this difference was 9 mm Hg. The reduction in plasma oncotic pressure was thus compensated by an almost identical reduction of tissue fluid oncotic pressure. Infusion of Ringer's solution also led to a 4-fold increase in lymph flow from the intestinal lymphatics. In the computer simulation, lymph flow increases by a factor of 3 for a similar change in plasma protein concentration. Similarly, Földi, Rusznyák, and Szabó (37) found a 4-fold increase in thoracic duct lymph flow in animals rendered hypoproteinemic by plasmapheresis.

Qualitative agreement has also been obtained by comparing data from the computer analysis with experimental studies on hypoproteinemia. It was pointed out that in the computer simulation plasma oncotic pressure has to be lowered to 10–15 mm Hg before changes in the interstitial volume occur which correspond to 1+ edema. A number of studies (4–6) indicated that edema occurs after plasmapheresis only if the protein level of the plasma drops below 3–3.5%, corresponding to a colloid osmotic pressure of 10–12 mm Hg. Similar observations have been made in clinical studies on nephrotic patients.

Hypoproteinemic conditions are associated with large increases in flow of lymph with low protein concentration. The leakage of protein through the large-pore system would be expected to be decreased in hypoproteinemia, both as a consequence of the decreased protein content of plasma, and also because of the reduced pressure gradient across the venous capillary wall. This is in contrast with edema induced by elevation of venous pressure, in which the pressure gradient across the large-pore system is actually elevated. One would thus predict that the protein content of edema fluid associated with cardiac failure should be higher than that associated with hypoproteinemic conditions. This prediction is supported by data summarized by Landis and Pappenheimer (3), which indicate that in hypoproteinemic states in most instances the protein content of edema fluid ranges between 0.1 and 0.27%,

whereas in cardiac failure edema fluids contain from 0.1 to 1% protein, with an average of 0.4%.

Effects of Changes in Venous Pressure

Many features of the changes in capillary fluid exchange which occur when venous pressure is altered are also duplicated in the computer analysis. In the previously quoted study of Morris (36), changes in intestinal lymph flow and protein concentration in response to an increase in portal venous pressure were measured. Portal pressure was elevated from the control level of 8 mm Hg to 16 mm Hg by partial occlusion of the vena cava between the liver and the diaphragm. The elevation in venous pressure produced a 2-fold increase in intestinal lymph flow, and a reduction in lymphatic protein content from 4.2 to 3.4%, corresponding to a change in colloid osmotic pressure from 12 to 7.5 mm Hg. For corresponding venous pressure values in the computer analysis, lymph flow increases by a factor of 2.2, and the tissue fluid oncotic pressure decreases by 6 mm Hg.

In similar experiments, carried out by White et al. (38), protein concentration and lymph flow were measured in the forepaws of unanesthetized dogs. Spontaneous activity during the experimental period produced some variability in the data, and only average values are presented here. When the venous pressure in the forepaw was elevated from 14 to 26 mm Hg by inflation of a cuff, lymph flow increased by a factor of 2.1. A further increase of venous pressure to 34 mm Hg increased the lymph flow to a value 4.1 times the control flow. Data obtained from the computer analysis show a 3-fold and a 4.8-fold increase in lymph flow for corresponding changes in venous pressure. The absolute magnitudes of the protein concentration differ markedly in the experiment and in the computer simulation. The percentage changes are quite similar, however. In the experiments of White et al., elevation of the venous pressure to 26 mm Hg reduced lymph protein concentration to 42% of the control value. Further elevation of the venous pressure to 34 mm Hg reduced the protein concentration to 27% of the control value. In comparison, the computer analysis yielded a reduction of the oncotic pressure of tissue fluid to 50% and 33% of the control value for the corresponding changes in venous pressure.

By drastic elevation of venous pressure, excessive filtration into the interstitial space can be induced, thus elevating the tissue pressure as reported by McMaster (39). In his experiments a cuff was inflated around the forearm or thigh of human subjects, and tissue pressure was recorded by inserting fine hypodermic needles into the connective tissue of the skin of the arm or ankle. After the cuff had been inflated to a pressure of 80–87 mm Hg, the interstitial pressure reached an equilibrium ranging from 13 to 17 mm Hg

after 20–30 min. Computer simulation of this experiment yielded an equilibrium value for tissue pressure of 25 mm Hg.

Pressure in Subcutaneous Capsules

The basic question whether pressures recorded in subcutaneous capsules represent hydrostatic pressures in tissues must be regarded as unresolved. The fit of the computer data to Guyton's experimental data indicates at least that alternative possibilities may exist. The consistency with which the negative pressures in the subcutaneous capsules is reproduced in different laboratories serves as a strong indication that the observations are not artifactual The validity of the assumed identity of tissue pressures and capsule pressures, however, rests on the assumption of free communication between fluid in the capsule and the free fluid in the interstitial space. Guyton's observation that Evans blue injected into the capsule appears in the tissue surrounding the capsule seems to support this assumption. These experiments were conducted, however, with hydrostatic pressures of +10 mm Hg within the capsule, which may have been sufficient to violate the integrity of the surrounding tissues. With capsules of the dimensions used by Guyton, a pressure of 10 mm Hg would give rise to a total wall tension of 0.5–1 g/cm. In comparison, the total tension at the tip of the 30 gauge needle would only amount to 0.01 g/cm for comparative pressures.

When native capsule fluid is replaced by hypertonic protein solutions, the composition of the capsule fluid retains its hypertonicity for prolonged periods. Thus, in one instance when the capsule fluid was replaced by a 5% protein solution, concentration 1 month later was still 4.1%. This extremely slow disappearance of protein from the capsules is consistent with the concept that the interface between tissue and capsule is relatively impermeable to protein. The protein composition of the intracapsular fluid may represent an average of the composition of the free fluid phase in the interstitial space, but the time course of equilibration may be prolonged to an extent where it has not yet been measured. It is noteworthy that the computed normal protein concentration of tissue fluid, amounting to 3.5%, falls between the values of 4.1%, observed 1 month after injection of hypertonic protein solution, and 1.96%, obtained about 4 wk after implantation of the capsule. However, Guyton's observation that changes in the protein concentration in the capsule do not alter the recorded capsule pressure is difficult to explain. The volume of fluid contained within the capsule is large compared to the free fluid volume within the interstitial space, and thus the tissue immediately surrounding the capsule may be in a state of relative dehydration, which would account for the maintained negative pressures.

The fact that the intracapsular pressures reflect changes in hydrostatic tissue pressures cannot be disputed. The converse, however, is not necessarily

true, namely that the capsule pressures are independent of changes in tissue fluid oncotic pressure. The latter possibility might be excluded by experiments supplementing Guyton's studies, in which local injection of saline produced edema, resulting in a rise in capsule pressure into the positive range. Injection of an identical volume of native blood plasma should give identical results, if free communication exists between the tissue fluids and the capsule. If, on the other hand, the interface between tissues and the free fluid in the capsule has the properties of a semipermeable membrane, the elevated tissue fluid oncotic pressure should drive the intracapsular pressure to more negative levels. This experiment has not been performed.

SUMMARY AND CONCLUSIONS

Fluid balance at the capillary level has been simulated with an analogue computer program, based on experimental data on regional differences in capillary permeability, surface area, and hydrostatic pressures. The program takes into account four fluid fluxes into and out of the interstitial space: filtration, reabsorption, plasma leakage through the large-pore system in capillaries, and removal of tissue fluid by lymphatics. The program also takes into account changes in tissue fluid protein concentration, as determined by differences in protein fluxes into the interstitial space through the large-pore system in capillaries, and protein removal by the lymphatics. Solutions are obtained for tissue hydrostatic pressure, tissue fluid osmotic pressure, interstitial space volume, and lymph flow. Simulation of these processes yields qualitative agreement with clinical data. Changes in input parameters, e.g. plasma oncotic pressure, venous pressure, or arterial capillary pressure, yield results which agree well with physiological data obtained in many experimental studies. The computer analysis has shed light on the mechanism whereby edema formation is prevented when plasma oncotic pressures are reduced by 10–15 mm Hg, or, alternatively, venous pressures are elevated by a similar amount. Dilution of the interstitial plasma protein pool with a consequent reduction of its oncotic pressures appears to be the major factor, as originally surmised by Starling. When the plasma oncotic pressure is reduced below 15 mm Hg, or venous pressure elevated by more than 10 mm Hg, excessive dilution of tissue fluid protein occurs. The increased filtration leads to edema, the extent of which is limited only by lymphatic removal of tissue fluid and the compliance of the interstitial space. The computer analysis in all instances gives positive values for tissue pressure, in agreement with experimental data obtained by needle puncture. The negative tissue pressures observed in subcutaneous capsules can be reproduced in the simulation program, however, if the interface between the capsule and the surrounding interstitial space is assumed to have the properties of a semipermeable membrane.

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Discussion

Dr. Giles Filley: Dr. Wiederhielm has shown how it is possible to improve on a very simple model by considering important anatomical configurations which determine how these vessels are arranged in space. I think it is also important to remember that eventually biologists will have to deal with another oversimplification, namely, that involved in considering protein as a simple osmotically active substance in a simple fluid called water.

I call your attention to Dr. Scholander's interesting considerations in recent issues

of *Science*, which are, like the tissue capsule problem, very controversial. He says that certain hydrostatic forces do not result simply from osmotic forces, but from interactions between solute and solvent in the near vicinity of solute molecules. In other words, the structure of water is affected by the presence of solute in a way not predicted by traditional concentration and chemical potential calculations. Anatomical sophistication in improving on the Landis model is one step forward. Physicochemical sophistication may be the next step.

Dr. Wiederhielm: I would like to thank you for your comments. We have certainly recognized the importance of these factors, particularly the role of mucopolysaccharides in the ground substance and their interaction with albumin in the interstitial space. Unfortunately, we have no experimental means available at the present time to quantitate this problem. We have, however, planned model studies, which we hope will elucidate this kind of interaction and its effect on osmotic pressure of tissue fluid, perhaps in terms of its chemical potential. I think one of the basic problems may be that the albumin space and the fluid space may be entirely different because of steric exclusion of plasma proteins in the ground substance.

Dr. Chester Hyman: I was much impressed by Dr. Wiederhielm's excellent analysis of the situation. Dr. Wiederhielm, like many of us who have looked at the microcirculation, must have been impressed by the dynamic shift and changes in the area of capillary beds. In fact, the paper of McMaster, cited by Dr. Wiederhielm, stressed the instability and rapid fluctuation in tissue pressure which can be ascribed to changes in the dynamics of the microvascular bed; therefore, absorption and filtration in specific and rigidly defined areas may not be as simple as was here set forth. Dr. Wiederhielm, I am sure, is fully aware of this, and I can understand the necessity of oversimplifying in order not to confuse the simple computer; although I suspect Starling could have managed the problem.

The other point I wondered about was Dr. Wiederhielm's model of inflammation, in which he made the capillaries 40 % more permeable to protein than they had been previously; yet the tracing shows no change in the oncotic pressure of the plasma protein. I wonder if the computer was confused or if there was an implicit assumption that making the capillaries leaky does not change the oncotic pressure of protein.

Dr. Wiederhielm: First of all, I would like to refer to your question about the variability of surface area due to vasomotion. The simulation program presented today is of course a first-generation computer analysis, designed specifically to analyze steady-state condition. The second generation of the program is already under way, and we hope, by incorporating proper time scaling, to be able to follow the time course of events with some degree of precision and match it against the physiological data. The variability in the capillary pressures could not be included in the current computer analysis because of the lack of time scaling. We have, however, recorded capillary pressures for some time, as summarized in the paper given at the 1967 Federation Meetings. These studies have been the direct stimulus for the current computer simulation studies. Later, when our program includes proper time scaling, we plan to play back data tape recordings of the arterial and venous capillary pressures, obtained by measurements in living mammals, as input variables in the computer program and then see what comes out.

Dr. Hyman's second question refers to the constancy of the oncotic pressure of plasma protein when there was a 20-fold increase in permeability to protein. In the computer program the oncotic pressure of the plasma protein was considered an input variable, and held constant. It would be possible to include the circulation as a separate compartment, which then should reflect the effects of added plasma leakage.

Dr. Zierler: I hope that before the session is over we may argue as to whether or not it is appropriate to make a model in which there are pores, meaning morphological holes fixed in capillaries, or indeed in any biological interface. I would like to see a calculation of the effect of such large pores on pressure drops across the capillary, on changes in electrical conductivity, and so on.

Dr. Wiederhielm: When using the term "pore" we of course are not limiting our thinking to a cylindrical structure going through the endothelial cells, or even a Pappenhiemer slit. Perhaps we should rather consider the large pores as defects in the junction of several endothelial cells. In other words, capillaries are something less than a perfect, leakproof system, as evidenced by the fact that some substances with large molecular radii do after all enter the interstitial space from the circulation, and then appear in the lymph. Our information at this stage of the game is more functional than structural, I am afraid.