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Interstitial Fluid Pressure

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PRIOR TO 1960, a century of studies on tissue pressure had led to the almost universal belief that normal interstitial fluid pressure is positive with respect to atmospheric pressure (for reviews see refs 80, 81). At that time a report from our laboratory (51) suggested that normal interstitial fluid pressure is negative instead of positive in most parts of the body. Today, a decade later, much additional evidence supports this concept of negative interstitial fluid pressure, but some investigators still feel that the measurements of negative interstitial fluid pressures might have been caused by peculiarities of the measuring techniques rather than

by true existence of negative pressure. The purpose of this paper, therefore, is to review this controversy and to present the available evidence. Unfortunately, since a major share of the evidence in favor of the concept of negative interstitial fluid pressure has come from our laboratory, this analysis is likely to be biased, but an attempt has been made to prevent as much of this bias as possible or at least to substantiate the bases for it. Other discussions of interstitial fluid pressure that present primarily an opposite viewpoint, that interstitial fluid pressure is normally positive in essentially all tissues of the body, have been presented by Wiederhielm (142), McDonald (87), and Rodbard and Kuramoto (108, 110). A more recent review by Gauer et al. (35) in general concurs with our concept of negative interstitial fluid pressure.

THREE TYPES OF TISSUE PRESSURE

It is our belief that most of the controversy regarding negative versus positive interstitial fluid pressure is caused by confusion about the types of pressure that can exist in tissues. Prior to the past few years essentially all papers dealing with tissue pressure have made the assumption that there is a unitary type of tissue pressure. Yet, consideration of several simple physical principles leads one to the concept that at least three different types of pressure are normally operative in tissues and that each of these has different types of significance (46). Furthermore, methods for measuring the different types of pressure are theoretically different.

To explain the types of pressure, let us consider a surface existing in a tissue, such as the outer surface of a blood vessel. In what ways can force be exerted against this surface by the surrounding tissues? First, wherever tissue fluids are in contact with the surface, force is applied to the surface by kinetic impact of the fluid molecules. Second, where solid structures are in contact with the surface, force is applied by the contact points between the solid structures and the surface. Therefore, the total force exerted on any unit area is the sum of the forces exerted by both the fluid and the solid structures. To translate these forces into pressures one simply divides by area, because pressure equals force per unit area. Thus, where fluid contacts the surface of a blood vessel, there exists an *interstitial fluid pressure*. On the other hand, where the solid structures contact the surface, there exists *solid tissue pressure*. And, finally, the *total tissue pressure* exerted against the vessel surface is equal to the algebraic sum of interstitial fluid pressure acting on its part of the surface and solid tissue pressure acting on the remainder. This summation is expressed by the following equation:

$$\text{Total tissue pressure} = (\text{interstitial fluid pressure} \times \text{proportion of total surface against which fluid pressure is operating}) + (\text{solid tissue pressure} \times \text{proportion of total surface against which solid tissue pressure is operating}) \quad (1)$$

Solid tissue pressure is transmitted not only by direct contact between cells, but also through the intermediation of fibers and other solid elements in the interstitial spaces. That is, the collagen, elastin, and other types of fibers are all solids

with elastic moduli. Therefore, force can be transmitted from one tissue cell to another through these fibers. Yet, even so, the points of contact between the fibers and the cells usually cover only a minute portion of the total surface area. It is this observation that has led many investigators to believe that only the fluid-transmitted pressure is significant. If this were true, the interstitial fluid pressure would be equal to the total tissue pressure. On the other hand, if the contact pressures of the fibers (or of the contact points of cell upon cell) should be high in comparison with the interstitial fluid pressure, a major share of the total pressure could be solid tissue pressure despite the minuteness of the areas of contact. The fact that solid structures in tissues deform the shapes of cells indicates that this is the case and therefore indicates that the total tissue pressure and the interstitial fluid pressure are not equal to each other. That tremendous forces can be transmitted through the small interstitial spaces by compression of fibers between adjacent cells is supported by two important facts. First, the strength of collagen fibers, the main type of fiber found in tissue spaces, approaches that of steel in relation to its size (20). Second, because of the extremely short distances within the interstitial spaces, the bending moments exerted on the fibers by even large compression forces are almost infinitesimally small.

Still another type of solid structure exists in the tissue spaces. This is interstitial gel, composed primarily of mucopolysaccharides (94) possibly or probably cross-linked with collagen (95) and containing interstitial fluid entrapped within the interstices of the gel meshwork. A gel has a modulus of elasticity (28, 32, 72), which means that it has the property of a solid and therefore can transmit solid tissue pressure from cell to cell. However, the basic physics of forces and pressures related to gels is complex and requires further elaboration later in this paper. Suffice it to say here that the modulus of elasticity of the gel allows it, as well as the actual structural solids of the tissue spaces, to transmit solid tissue pressure (58).

Figure 1 gives schematically a summary of the methods by which pressures can be transmitted from one tissue surface to another. Figure 1*A* shows that pressure can be transmitted from surface 1 to surface 2, respectively, by fluid, by solid fibers such as collagen and elastin fibers, and by gel. Transmission through the

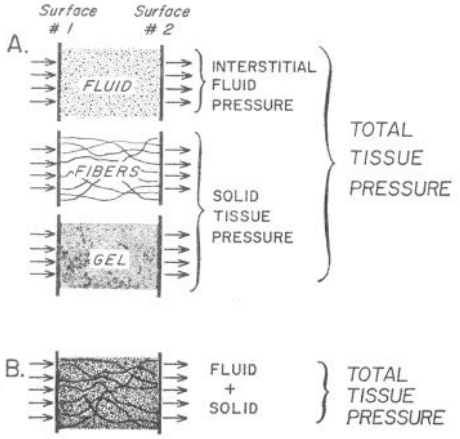

FIG. 1. *A:* transmission of pressure separately through fluid, fibers, and gel. *B:* transmission of pressure through all media simultaneously.

fluid is denoted as "interstitial fluid pressure," while transmission through the solid elements is denoted as "solid tissue pressure." The sum of these two types of pressures is then equal to "total tissue pressure." In Figure 1*B* all three methods for pressure transmission are shown acting together, illustrating that each element of pressure transmission is a type of partial pressure and that the total tissue pressure is the sum of all the partial pressures.

Relationship Between Surface-Averaged Interstitial Fluid Pressure, Surface-Averaged Solid Tissue Pressure, and Total Tissue Pressure

In the above analysis of pressures acting on a tissue surface, it was pointed out that fluid pressure acts only where the fluid is in contact with the surface. Likewise, solid tissue pressure acts only where solid elements are in contact with the surface. Yet, because it is not possible to measure pressures exerted at individual solid contact points, we must introduce the terms *surface-averaged interstitial fluid pressure* and *surface-averaged solid tissue pressure.* If interstitial fluid pressure is −10 mm Hg but is exerted on only 90% of the total surface area, the surface-averaged interstitial fluid pressure is −9 mm Hg. If the solid tissue pressure at each contact point is +100 mm Hg but is exerted on only 10% of the surface area, the surface-averaged solid tissue pressure is +10 mm Hg. Once the interstitial fluid pressure and the solid tissue pressure have been averaged in this manner, the following simple relationship between total tissue pressure and the other two pressures holds:

$$\text{Total tissue pressure} = \text{surface-averaged interstitial fluid pressure} + \text{surface-averaged solid tissue pressure} \quad (2)$$

In this example, the total tissue pressure is −9 + 10 or a total of +1 mm Hg.

If the total area of the solid contacts is very small, for instance less than 1% of the total area on the surface of a capillary, the fluid pressure will be exerted over essentially the entire surface. In this case the surface-averaged interstitial fluid pressure will, for practical purposes, be equal to the interstitial fluid pressure, and *equation 2* can be simplified for this special condition to:

$$\text{Total tissue pressure} = \text{interstitial fluid pressure} + \text{surface-averaged solid tissue pressure} \quad (3)$$

Interrelationships Among Different Types of Tissue Pressure when Interstitial Fluid Pressure is Negative

Several investigators have suggested that the interstitial fluid pressure could not possibly be negative because the body is exposed to atmospheric pressure that is transmitted through the body's soft tissues and because turgor of the skin and of other tissues creates still additional positive compressive forces on the tissue spaces (87). However, since these forces are transmitted by a combination of both fluid and solid media, the resultant tissue pressure is the total tissue pressure and is not necessarily equal to either interstitial fluid pressure or solid tissue pressure.

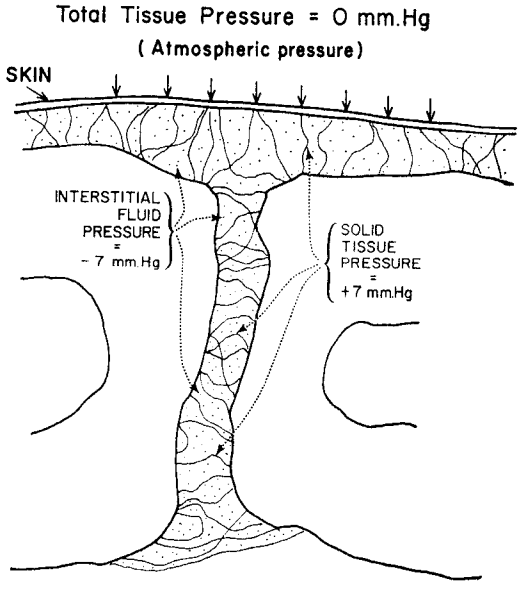

FIG. 2. Effect of negative interstitial fluid pressure on other types of tissue pressure. (Interstitial fluid pressure and solid tissue pressure are represented as surface-averaged pressures.)

Yet, what effect would the presence of negative interstitial fluid pressure have on the other types of tissue pressures? To answer this, let us refer to Figure 2, which shows an interstitial fluid pressure of −7 mm Hg in the spaces between the two cells and between the skin and the cells. This negative pressure is pulling the inner surface of the skin toward the cell surfaces, and it is also pulling the adjacent cell surfaces toward each other. In turn, the solid fibers in the spaces are holding the surfaces apart. The greater the negative pressure exerted on the fluid, the greater becomes the solid tissue pressure exerted by the solid fibers. This is a simple application of the physical principle 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.

One might also ask how increasing negativity in the fluid spaces of Figure 2 would affect total tissue pressure. In this example, the total tissue pressure is caused by pressure of the atmosphere on the outer surface of the skin. If we assume that the skin is flaccid enough to transmit this pressure faithfully, the total tissue pressure on the under surface of the skin is equal to zero with respect to atmospheric pressure. This same amount of pressure is then transmitted through the fluid and solid structures to the surfaces of the cells because the fluid and solid pressures are equal to each other but of opposite sign, one negative and one positive. Furthermore, if the fluid pressure should be made still more negative, suction of the surfaces toward each other would increase the solid tissue pressure by exactly the same amount, but the total tissue pressure would still remain zero.

The problem is not this simple when turgor in the skin or in other tissue structures causes additional forces in the tissues, but the above example does illustrate that total tissue pressure does not necessarily change when interstitial fluid pressure changes.

Physical Effects of Edema Fluid in Interstitial Spaces

Edema has several effects on the three different types of tissue pressure. If the fluid excess is great enough to separate the solid elements of the tissue completely from each other, no solid compressional forces will remain between the solid elements of the tissues. In this condition the solid tissue pressure becomes zero and, referring again to *equations 1–3*, one finds that the interstitial fluid pressure then becomes equal to the total tissue pressure. Therefore, theoretically, a method for measuring total tissue pressure would be to inject a small amount of fluid into the tissues, barely enough to relieve the compressional forces between the solid elements (but not enough to cause tension in the attachments between the solid tissue elements), and then to measure the pressure in the injected fluid. We shall see that this method has often been used to measure tissue pressure, but usually without the realization that it is probably total tissue pressure that is being measured.

PHYSIOLOGICAL SIGNIFICANCE OF THE DIFFERENT TYPES OF PRESSURE

Total tissue pressure is defined as the total pressure exerted on a surface within the tissues, a typical surface being the outside of a blood vessel. Obviously, interstitial fluid pressure and solid tissue pressure both exert their influences on such a surface, the algebraic sum of which equals the total tissue pressure. Therefore, it is the total tissue pressure that tends to compress blood vessels or other intratissue structures. In most soft tissues that are freely exposed to atmospheric pressure this total tissue pressure remains very slightly higher than atmospheric pressure (+1 to +3 mm Hg). However, the total tissue pressure can be increased markedly by such a maneuver as placing an inflated blood pressure cuff around a soft tissue, in which case many hundred millimeters Hg positive pressure can be transmitted to the surfaces of blood vessels by a combination of interstitial fluid and solid tissue pressures. The total tissue pressure at the surface of the blood vessel is then very nearly equal to the pressure exerted by the pressure cuff.

Interstitial fluid pressure has significance principally for causing fluid movement through pores and tissue spaces. Solid tissue pressure cannot have similar effects because it, by definition, is the pressure exerted by solid contact points, and these are entirely independent of the fluid molecules. For instance, though the total pressure compressing the outside of a blood capillary might be +1 mm Hg, it is theoretically possible for the fluid pressure in the space between the capillary and the surrounding tissues to be very negative, in which case the negative pressure outside the capillary would cause increased bulk movement and increased diffusion of fluid and solute molecules outward through the capillary pores. A special example of this occurs when solutions having high colloid osmotic pressures are perfused through tissue capillaries—for instance, dextran solutions with colloid osmotic pressures as high as 150 mm Hg. Such solutions cause osmosis of fluid out of the interstitial fluid spaces into the blood and thereby create negative pressures in the interstitial spaces as great as −30 to −100 mm Hg, an effect demonstrated by

experiments that are explained in more detail later (46, 47). Thus, it is the interstitial fluid pressure, not total tissue pressure nor solid tissue pressure, that determines the rate of fluid movement through the capillary membrane.

Solid tissue pressures cause deforming forces between solid tissue structures because this type of pressure is never evenly distributed. For instance, if a clump of cells is being compressed by a surface-averaged solid tissue pressure of 10 mm Hg, the deforming effect of this on the cells is 10 times as great as that of a surface-averaged solid tissue pressure of 1 mm Hg. High solid tissue pressures are, for obvious reasons, associated with minimum-sized tissue spaces—that is, with compacted tissues—while low solid tissue pressures causes the spaces to enlarge because of elastic recoil of the cells, interstitial fibers, and other solid structures around the spaces.

Thus, each one of the different types of tissue pressure plays a very significant physiological role. And, most important, it seems to be clear that a single unitary type of pressure rarely, if ever, exists in any tissue. Therefore, any discussion of tissue pressure that fails to take cognizance of the differences among the different types of tissue pressure is likely to be in error.

INTERSTITIAL GEL AND PRESSURES RELATED TO GELS

The discussion thus far has assumed mainly that the interstitial spaces are filled with mobile fluid capable of transmitting pressure in all vectorial directions. However, histologists (19, 21, 36), biochemists (94, 118), and physiologists (32, 138) have all pointed out that almost all normal tissue spaces are filled with a gel or a gel-like substance. The most important chemical constituent of the gel reticulum in most tissues is hyaluronic acid, which is present in tissue spaces in the form of long coiled filaments and in concentrations usually between 0.3 and 1.2% (20, 98). Since the coiled filaments are oriented in all directions, the mass of filaments in the interstitial spaces has been likened by Fessler (26) and Ogston and Sherman (99) to a "brush pile" that, with its entrapped fluid, gives a gel-like consistency. Because there are no cross-linkages between pure hyaluronic acid filaments, some investigators prefer not to call the gel-like material a true gel. However, collagen, which is present in the tissue spaces in addition to the hyaluronic acid, does form cross-linkages with the different mucopolysaccharides (hyaluronic acid, chondroitin sulfate, etc.) and the combination thereby becomes a true gel (85, 95, 132). Yet, whether or not a true gel exists in tissue spaces is immaterial to the present discussion, because the pertinent physical characteristics are those of a gel nevertheless. Therefore, this material is called a gel here with full knowledge of possible lack of, or sparsity of, cross-linkages.

The pertinent properties of the tissue gel in relation to the present discussion are the following: *1*) the gel tends to swell when it is surrounded by free fluid, *2*) the gel impedes bulk flow of interstitial fluid through the spaces, and *3*) the gel does not impede significantly the diffusion of small molecules and ions through its substance.

Swelling of Tissue Gel and Pressures Exerted by Gel

The swelling property of tissue gel is caused by its ability to imbibe water, and this ability in turn results at least partly from the presence of strong negative electrical charges on the hyaluronic acid filaments (60, 63, 72). These charges attract positive ions, such as sodium ions, into the gel, creating a higher total density of ions in the gel than in the surrounding nongelled fluid. These ions, in turn, cause osmotic pressure that pulls fluid from the free fluid into the gel. This is a Donnan equilibrium effect, the dynamics of which have been discussed elsewhere and substantiated in detail (28, 129). Ogston (98) has found the colloid osmotic pressures of hyaluronic acid solutions with concentrations similar to those found in tissue spaces to be approximately the following: 0.5% hyaluronic acid, 1 mm Hg; 1% hyaluronic acid, 4.5 mm Hg; 2% hyaluronic acid, 18 mm Hg. The presence of protein, collagen, or other substances in the gel can add significantly to these colloid osmotic pressures.

Other factors that can cause gels to swell include: *1*) expansion of the gel reticulum because of mutual repulsion of electrostatic changes on the adjacent filaments of the reticulum and *2*) hydration of some of the substances in the gel (28, 72).

The elastic nature of the gel reticulum gives the gel an elastic modulus (28), which is a characteristic of a solid. Therefore, the gel can transmit solid tissue pressure from one solid element of a tissue to another.

From the above considerations, one can immediately understand that several different types of pressure besides the three tissue pressures already discussed are associated with the gel itself (28, 49, 63, 72). These include: *1*) pressure of fluid inside the gel, *2*) recoil pressure of the gel reticulum (this can be either positive or negative depending on whether the gel is compressed or stretched), and *3*) total pressure exerted on the surface of the gel. (In addition to these three pressures the gel also exhibits a colloid osmotic pressure caused partly by the Donnan effect discussed above.)

Thus, essentially the same types of forces and pressure occur in tissue gel as occur in tissue spaces as a whole. One of these pressures is a fluid pressure, another is a "solid" pressure caused by the elastic modulus of the gel reticulum, and a third is the total pressure. Obviously, it is the third pressure, the total pressure, that is important for transmitting pressures in tissue spaces, but the other two are important because of the effect that they have on the total pressure.

Wiederhielm (143) recently developed a method that he believes measures the colloid osmotic pressure of tissue gel; the average value he found was 11 mm Hg. This compared with the average value of about 4 mm Hg usually found in the free fluid of the tissue spaces. Therefore, the net osmotic pressure exerted at the surface of the gel would be $11 - 4 = 7$ mm Hg. If the pressure in the free fluid surrounding the gel is -6 mm Hg, then the fluid pressure in the gel calculates to be approximately $+1$ mm Hg, but this has never yet been measured.

Impeded Movement of Fluid and Solutes Through Gel

The reticulum of the gel (the "brush pile") impedes bulk movement of fluid and solutes through the gel (24, 61, 62, 99). Preston et al. (103) have calculated that bulk fluid movement through a 0.1-mm-diameter capillary tube containing a 1% hyaluronic acid solution is impeded by a factor of approximately 10,000,000-fold. This means that, for a given pressure gradient, *flow* of tissue fluid through tissue gel would be extremely slight in comparison with flow in tissue spaces where gel does not exist. Studies by McMasters and Parsons (91) more than 30 years ago and more recently by Wiederhilem (138) have demonstrated that only minute spicules of flowing fluid exist in tissue spaces in contrast to large areas of nonflowing fluid, presumably impeded by the presence of gel. Also, Guyton et al. (57) studied the rate of fluid flow through tissue spaces between two perforated capsules implanted 2.5 cm apart and observed that flow was nearly zero in normal tissues despite pressure differences of several millimeters Hg between the two capsules. Yet, when the tissue was made edematous so that there were large amounts of free, nongelled fluid in the tissue spaces, the rate of fluid flow increased 10,000-fold.

METHODS FOR MEASURING TISSUE PRESSURE

Three major types of methods have been used for measuring tissue pressure: *1*) an intratissue balloon method; *2*) a needle or capillary pipette method; and *3*) fluid equilibration methods, of which the most widely used thus far has been the implanted perforated capsule procedure. The first two give almost exactly the same values, positive tissue pressures of +1 to +3 mm Hg (when referred to the hydrostatic pressure level of the tissue itself) for normal soft tissues (13, 77, 89, 90, 142) and as high as +10 to +15 mm Hg in tissues limited by tight membranes such as in the kidneys (40, 131, 133). The fluid equilibration techniques give an entirely different set of values, with measurements ranging from −2 to −8 mm Hg in normal, nonedematous soft tissues of mammals (5, 46, 66, 79, 116, 126) and about +6 mm Hg in the kidney (Ott and Navar, unpublished observations). Because of these differences it is important to determine what each of these tissue pressure-measuring techniques actually measures. The balloon technique is considered first because it is very clear what type of pressure it measures.

Intratissue Balloon Techniques for Measuring Tissue Pressure

Several different types of minute balloons have been used for measuring tissue pressure. One method has been to construct a balloon on a needle and to thread this through the tissue (76). A second has been to tie off a small vein in a tissue and to utilize this as a balloon (77). A third has been to thread an excised blood vessel through the tissue and to use this as a balloon (43, 69). In each of these methods, the pressure barely required to begin distending the balloon while it is still flaccid is considered to be the tissue pressure.

But what type of tissue pressure does the balloon method measure? The balloon obviously is in contact with both the solid elements and the fluid in tissues. Therefore, the balloon method theoretically measures *total tissue pressure* but gives no measure of either solid tissue pressure or interstitial fluid pressure.

Tissue Pressures Measured by Needle or Capillary Pipette Techniques

Perhaps the most widely used procedure for measuring tissue pressure has been the needle technique (12, 13, 38, 40, 65, 86, 90, 114, 122, 128, 136, 144). One would think, on first thought, that pressure in the tissue fluids could be measured by a needle inserted into the tissues.

However, the intercellular spaces ordinarily are less than 1 μ in width (25, 31) and the free fluid spaces in the amorphous tissue gel are closer to 0.05 μ in width (19, 36), while even the smallest hypodermic needles usually used for tissue pressure measurements are several hundred microns in diameter. Therefore, the presence of a needle in the tissues can so distort the spaces that the pressures measured should theoretically be invalid. Furthermore, when one inserts a needle into a tissue, the tip of the needle usually becomes occluded with tissue or gel, which makes it impossible to measure any significant value at all. To overcome this difficulty, essentially all investigators have injected a minute quantity of fluid at the tip of the needle, usually about 1 μl, after which a very stable pressure can be measured for 30 sec to 1 min or more. Beyond that time, when the minute quantity of fluid has been absorbed into the surrounding tissues the pressure measurement becomes erratic once again, with the tip of the needle becoming occluded again or occasionally with a ball-valve effect occurring at the tip of the needle so that each movement of the tissue sucks fluid out of the needle into the tissue and the pressure in the needle falls artifactually to extremely low values, sometimes to as low as -30 to -50 mm Hg (Guyton, unpublished observations).

The basic theory of the needle technique has been the following: since fluid is injected at the tip of the needle, this fluid supposedly makes contact with the fluid of the tissues and, therefore, supposedly provides a fluid connecting link for measuring fluid pressure in the tissues. However, the time factor has been ignored, namely, the fact that a stable pressure can be measured only so long as the bolus of fluid remains at the tip of the needle but not after the injected fluid has been absorbed. From the earlier discussion relative to the presence of excess fluid in tissue spaces and its effect on interstitial fluid pressure, solid tissue pressure, and total tissue pressure, it will be recalled that fluid placed into a tissue space in enough quantity to separate the solid elements from each other causes the solid tissue pressure to be reduced to zero, while the local interstitial fluid pressure rises to equal total tissue pressure. Therefore, during that period of time that the tissue elements are separated from each other—that is, so long as the minute bolus of fluid remains at the tip of the needle—the pressure that the needle measures should theoretically be equal to total tissue pressure and not to normal interstitial fluid pressure. Many investigators who have used the needle technique for measuring tissue pressure have recognized this limitation. Indeed, McMaster (89), one of the

most important users of the needle technique, actually refused to call the pressure measured in this manner "tissue pressure" but instead called it "tissue resistance," indicating by this term that the pressure measured was the pressure at which the tissues yielded to allow free flow of fluid away from the tip of the needle.

To obviate some of the difficulties of the needle technique for measuring tissue pressure, Wiederhielm (141, 142) has used a capillary micropipette (tip diameter of about 0.5 μ) to measure pressures in the tissues of bats' wings. In using this technique it has not been necessary to inject fluid preliminary to making the pressure measurement, but other problems have been encountered. First, even this size pipette is much larger than the usual free-fluid spaces interspersed among the tissue space gel. Therefore, it has been necessary to find unnaturally large interstitial spaces where free fluid exists. Wiederhielm has found such spaces by observing Brownian motion of particles within the tissue spaces of the bat's wing. Where Brownian motion occurs, the fluid is free and mobile. On the other hand, where there is no Brownian motion, the fluid is in a fixed state, presumably in a gel. Wiederhielm has found large chambers of fluid having dimensions of 5 μ or more in the bat's wing, and it is in these chambers that fluid pressures have been measured. These pressures have ranged from slightly negative to slightly positive with an average value of +1.25 mm Hg (142). (Unfortunately, it has not been possible to make similar measurements of tissue pressure in other tissues because similar free-fluid pockets have not been found elsewhere.) Referring again to the earlier discussion of the relationship of excess fluid in tissues to its effect on the different types of tissue pressure, one can see that where excess fluid can be found, such as in these tissue fluid spaces, the solid tissue elements have already been separated from each other, and solid tissue pressure has fallen to zero. Under these conditions the interstitial fluid pressure should be equal to the total tissue pressure. To interpret this another way, it is the authors' belief that these large fluid chambers represent points of local tissue edema in which one would expect to measure positive pressures. On the other hand, Clark and Clark (21), in microscopic studies of subcutaneous tissue in skin chambers of the rat, were unable to find any pockets of Brownian motion, indicating that such pockets are not a normal characteristic of tissue spaces. Therefore, one possible interpretation of these micropipette measurements is that they represent measurements of pressure in naturally edematous spaces that have not yet been found in other tissues.

Another special use of the needle technique for study of tissue fluids was that reported by McMaster (88) in which he inserted a 30-gauge hypodermic needle very carefully into the subcutaneous tissue of a mouse under microscopic observation so that no blood capillaries were ruptured. Then he observed whether or not fluid flowed out from the needle or into the needle. Approximately once every 2 min a minute quantity of fluid was sucked into the tissue from the needle, this sucking effect occurring intermittently. Unfortunately, McMaster did not measure pressure from the needle, but the results indicated that the equilibration pressure would have been less than atmospheric pressure had he made such a measurement. McMaster further observed that, if the tissue were made edematous, fluid moved in the opposite direction, out of the tissue into the needle, which indicated positive pressure in the tissue fluid during the edematous state.

In summary, despite the early assumption that needle techniques can be used to measure interstitial fluid pressure, these techniques seem to be capable of valid pressure measurements only in the presence of excess free fluid as found naturally in occasional tissue spaces, as found in edema, or as found in fluid spaces artificially created at the tip of a needle by injection of fluid. Under these conditions the interstitial fluid pressure would be expected to be positive and to be equal to total tissue pressure but not equal to the interstitial fluid pressure in normal tissues where large spaces of free fluid do not exist.

Fluid Equilibration Techniques for Measuring Tissue Pressure

Between 1960 and 1967 four new methods for measuring tissue pressure appeared (46, 48, 51, 116), all based on the principle of allowing a considerable period of time for fluids of the tissues to come to equilibrium with fluids in the measuring device. The first of these techniques was the implanted perforated capsule (5, 15, 29, 30, 42, 46, 66, 107, 126). Though almost any shape or size of capsule will give the same result, the usual embodiment of this technique has been to implant a small hollow plastic sphere approximately 1.5 cm in diameter and having in its shell 100–200 holes 1 mm in size. After such a capsule has been implanted for several weeks (if it had been implanted without gross hemorrhage), its central cavity is found to be filled with fluid, and pressure in the capsule can be measured from a needle inserted through one of the capsule holes into the fluid cavity. Or the pressure can be measured from a catheter the tip of which had been fixed in the middle of the cavity at the time of implantation. This implanted capsule method gives a pressure value averaging -6 to -7 mm Hg in normal subcutaneous tissues.

The second method has been to implant a simple flat piece of plastic material into the tissue and to allow healing for several weeks; then a needle is inserted into the tissue with its beveled surface against the plastic (48). Fluid in the needle makes contact with a thin film of fluid along the flat surface of the plastic, and negative pressures similar, on the average, to those measured by the perforated capsule are recorded. However, this method has not been used for many studies because these pressures are very variable, ranging from -13 to $+1$ and also changing with movement of the tissues. The theory was to provide a large tissue space (along the surface of the plastic) from which the fluid pressure could be measured. The importance of this method has been to demonstrate that tissue fluid does not have to be inside the cavity of a sphere to give negative pressure measurements.

The third method has been to lift the outer surface of an area of skin by applying an adhesive to the skin and pulling upward (48). The skin remains lifted for 24 hr until free fluid is pulled into the space beneath the skin. Then a needle is inserted into the space while the skin is kept lifted; the presence of free fluid in the tissue space allows easy measurement of the pressure, and the needle tip does not become occluded. This method, too, measures negative values that range from -2 to -10 mm Hg in normal subcutaneous tissues. Obviously, these initial pressures could have been caused by the initial distortion of the tissue. However, if extra

fluid is injected into the tissue, the pressure can be made positive. Then, upon returning 24 hr later and measuring the pressure in the same space once more, it is again in the negative range that it had been initially. Since the fluid in this space is in contact with the tissue spaces of the surrounding tissues and since fluid is reabsorbed from the space after injection, it is deduced that a specific absorptive mechanism is available in the tissues to cause the negative pressures so recorded. The value of this technique, also, has been to show that fluid does not have to be in the cavity of an implanted capsule to give negative fluid pressure. All that is required is to prevent atmospheric pressure from acting through the tissues to collapse the tissue spaces, in which case large amounts of free fluid can be caused to collect in the tissue spaces even in the presence of negative fluid pressures; then fluid contact can be made by an inserted needle to measure the interstitial fluid pressure, an effect that cannot be achieved in normal tissues because of lack of sufficient free fluid.

The fourth fluid equilibration method is to insert into the tissue a minute Teflon tube having approximately eight cotton fibers protruding from its tip, a technique pioneered by Scholander and his colleagues (116, 127) and more recently used by others (79; H. Engel, personal communication). These cotton fibers are actually minute tubules with many side pores, and their large surface areas within the tissue provide a large area for contact between the normal tissue fluids and the fluid inside the Teflon tube. After insertion of such a wick into a tissue, the pressure measured from the protruding Teflon tube comes to equilibrium in approximately 30 min. This pressure, too, is a negative value with respect to atmospheric pressure. The pressures measured by this technique generally are slightly less negative than those measured by the perforated capsule technique, averaging about -2 mm Hg in the subcutaneous tissue of man (H. Engel, personal communication), and -5 mm Hg in the rat, mouse, or guinea pig (127), in contrast to -6 to -7 mm Hg for capsule pressures in the dog (46). Some investigators have suggested informally that the negative pressures measured by the Scholander technique might be caused by a colloid osmotic effect at the surface of the cotton fibers. However, this has been disproved by observations of Scholander and his colleagues (116) and of Ladegaard-Pedersen (79) that placing the measuring device alternately in saline solution, plasma, or even 22% albumin does not cause any effect on pressure measured by the device.

The universal features of all four of the above techniques are: *1*) large areas of tissue spaces are exposed to the measuring device and *2*) sufficient time is allowed for the pressure in the measuring device to come to equilibrium with the pressure in the tissue spaces. For these reasons, it is our belief that the pressure recorded by these techniques is the true interstitial fluid pressure in contrast to the measurement of total tissue pressure by both the tissue balloon technique and the needle technique. This could explain why both the balloon and the needle techniques measure positive pressure, presumably equal to the total tissue pressure, while the fluid equilibration methods universally measure negative pressures in normal tissues but positive pressures in the state of edema (46, 47), discussed in more detail later.

Because most of the studies on interstitial fluid dynamics have been based

on continuous measurement of interstitial fluid pressure using the perforated-capsule technique, the theoretical basis of this technique, its history, and its validation are discussed next.

HISTORY AND DEVELOPMENT OF CAPSULE METHOD FOR MEASURING INTERSTITIAL FLUID PRESSURE

Development of the capsule method for measuring interstitial fluid pressure was not a chance discovery but, instead, was a deliberate attempt to find some method for measuring interstitial fluid pressure that would avoid the pitfalls of the needle and balloon techniques. This work was begun on the basis of two theoretical considerations. First, an analysis of tissue mechanics and of pressure transmission through tissues similar to that presented at the outset of this paper had indicated that pressures can be transmitted by both fluid and solid elements, which would give rise to the three different types of tissue pressure already discussed. Therefore, there was reason to believe that interstitial fluid pressure should be different from total tissue pressure as measured by the balloon technique. In view of the failure of the needle technique to measure a pressure different from the balloon pressure, it seemed that there must be some better method for establishing pressure equilibrium between the tissue fluids and the measuring device than by use of an inserted needle. The basic theory of the capsule technique was: *1*) to provide a large surface area of exposure to the tissue fluids, *2*) to provide adequate time for equilibration to take place, and *3*) to provide a tissue space that could not be collapsed by transmission of atmospheric pressure through the tissues in case the measured pressure should prove to be subatmospheric. The surface area provided by the capsule technique is roughly 100 times the surface area provided by the small bolus of fluid at the tip of a needle, and the time allowed for equilibration to take place is in the order of days rather than seconds. Multiplying these two factors together the perforated-capsule technique theoretically should have about 100,000 times the capability of approaching the true interstitial fluid pressure as would the space at the tip of an inserted needle.

The second theoretical consideration that led to the deliberate search for new methods for measuring interstitial fluid pressure was that many clinical and basic physiological observations made prior to 1960 had already indicated the existence of negative interstitial fluid pressure. Also, a number of observations had led to the deduction that in the edema state the fluid pressure is positive. Some of these many observations follow.

1) Krogh, Landis, and Turner in 1932 (78) pointed out that there is a "margin of safety" against edema. That is, the venous pressure of the limb must be raised to +15 to +20 cm H_2O before large amounts of edema fluid suddenly begin to appear in the limb. If the tissue pressure were already positive and if edema resulted simply from further elevation of this positive pressure, there should be progressive increase in fluid in the tissue spaces at a steady rate as the venous pressure is raised rather than the observed discontinuity at a critical venous pressure of +15 to +20 cm H_2O. Though those observations could be explained in other ways, they are also

consistent with the concept that the tissues are normally in a compacted state but expand rapidly when the interstitial pressure changes from suction in the tissue spaces to a positive expansive force.

2) Large numbers of clinicians had observed that low protein in the circulating blood usually will not cause generalized edema until the plasma colloid osmotic pressure falls below about 10 mm Hg (80). Furthermore, a very slight additional decrease in plasma protein level is likely to be associated with rapidly developing generalized edema while a very slight improvement in the plasma protein level is likely to be associated with rapid disappearance of the edema. This effect has been emphasized especially by the discovery of persons with analbuminemia who are not edematous (8, 67). From electrophoretic patterns of the plasma proteins in two of these persons, their plasma colloid osmotic pressures calculated to be respectively 6 mm Hg and 7 mm Hg. Yet neither had edema despite the fact that their colloid osmotic pressures were respectively 22 mm Hg and 21 mm Hg below the average normal value for the human being. On the other hand, each was highly susceptible to the development of edema after trauma, being particularly susceptible to accumulation of fluid in a joint when it was bruised. Here again, there was a tremendous safety factor against edema, and, though these two persons with low albumins in their blood did not normally have edema fluid in their tissues, they nevertheless were on the critical verge of developing edema with the slightest provocation.

3) Southey tubes inserted into edematous tissues allow fluid to drain to the exterior, indicating the presence of positive pressure in the tissue fluid (124). Conversely, similar tubes placed in normal tissue will not drain fluid.

4) A denuded skin area that is kept moist does not continue to drain fluid beyond the first few hours unless it is continually abraided by a rubbing bandage, though such would be expected if the pressure in the tissue spaces were positive. Furthermore, during subsequent days when granulation tissue develops, even though this consists of newly growing cells on the very surface of the granulation and even though the cells are kept moist to prevent encrustment, there is still no flow of fluid to the exterior.

5) As a blister resorbs, the outer surface of the blister at first becomes entirely flaccid. Since the pressures on the two sides of a flaccid membrane are exactly equal, the pressure within the flaccid blister is exactly equal to atmospheric pressure, or is 0 mm Hg. In subsequent days the fluid from the flaccid blister is completely resorbed, and the outer surface of the blister is pulled against the inner surface. The outer skin of the blister then crinkles, which in itself requires a negative pressure in the space between the two surfaces of the blister. Thus, the absorption of fluid from a blister to the point that skin crinkling occurs is circumstantial evidence for negative interstitial fluid pressure.

6) An effect similar to the blister sequence occurs when fluid is injected by the process known as clysis into the subcutaneous tissue. Fluid can be injected to an extent that the skin protrudes outward in the shape and size of an orange. Gradually the fluid is resorbed. During the resorptive phase, the skin becomes totally flaccid, indicating once again that the pressure in the fluid beneath the skin is at

exactly atmospheric pressure, or at zero pressure. Yet the fluid continues to be resorbed until the skin becomes again tightly approximated to the surface of the body. This observation also demonstrates still another phenomenon essential to understanding tissue fluid dynamics—that normal subcutaneous tissues can be stretched to a tremendous extent and still have flaccidity, indicating zero pressure within their spaces, as occurs during the resorptive phase of edema. This same observation has been repeated in dogs in a much more quantitative manner (Guyton, unpublished observations): 400 ml of saline were injected in the axillary spaces of dogs; the pressure measured by an inserted needle during the resorptive phase of this fluid 20 min after injection averaged +3 mm Hg; 1 hr later it was 0 mm Hg even though less than half of the fluid had been absorbed. A similar effect was shown by Burch (11) 20 years ago when he injected sufficient fluid in the lower eyelid of the human being to increase the interstitial fluid volume of that area approximately 10,000% and still found the pressure of the fluid to be only +2 to +3 mm Hg immediately after injection. Thus, the concept that the tissue spaces are enclosed by strong elastic boundaries, a concept that had been necessary for previous theories of tissue fluid dynamics, does not fit with actual measurements of pressures in some edematous tissues.

7) A skin graft is occasionally placed in a concavity of the body, such as in the eye socket of a person whose orbit has been almost completely cleared of tissue because of cancer. Furthermore, such a graft frequently develops fluid beneath the skin during the healing process. Yet, if this fluid does not become infected and if the skin itself does not slough for lack of nutrition, the skin will eventually be pulled back into the socket (Guyton, unpublished observation). Since there is a natural tendency for skin to contract and to pull away from the socket, there is only one means by which the skin could be made to move back into the socket, and this would be for negative pressure to occur in the fluid in the cavity. This indicates that fluid can be absorbed from a cavity of the body despite the fact that the fluid exists in a state of negative pressure.

8) With the exception of the eye and cerebrospinal fluid system, both of which have special dynamics, pressures in essentially all the special tissue spaces of the body have all been measured and found to be in the negative range. These include negative pressure in the intrapleural space (2), in joint spaces (7, 105), in the epidural space (64), and in the pericardial space (E. H. Wood, personal communication). Thus far no measurements have been made of fluid pressure in the peritoneal space, but there is reason to believe that this also is negative because many persons are born with direct communications between their abdominal cavity and pleural cavity, and fluid does not (at least grossly) flow from the abdominal cavity into the pleural cavity where negative pressure is known to exist.

9) In recent years identically the same dynamics as those presented in the first part of this article (the concepts of fluid pressure, solid tissue pressure, and total pressure) have been developed for the pleural cavity by Agostoni et al. (2) and others (102). That is, their measurements show the intrapleural fluid pressure to be about −10 mm Hg. There are also a recoil pressure of the lungs tending to pull the lungs away from the chest wall with a pressure of approximately −5 mm Hg and a

contact pressure of about +5 mm Hg between the parietal pleura and the visceral pleura. Thus the balance of forces is −10 mm Hg + 5 mm Hg = −5 mm Hg. In other words, the principles developed earlier in this article for the peripheral tissues spaces have been shown to apply completely to the intrapleural space.

Thus, even before the advent of the capsule method for measuring interstitial fluid pressure, the above observations already pointed toward negative fluid pressures in normal tissue spaces. Furthermore, the large safety factor against edema indicated that the solid and semisolid elements of the tissues are normally compacted on themselves, which would mean a significant level of solid tissue pressure. Therefore, according to the formulations presented earlier in *equations 1–3*, interstitial fluid pressure should theoretically be some value considerably less than total tissue pressure, probably some value less than atmospheric pressure.

It was on the basis of this type of reasoning that a method for measuring interstitial fluid pressure, and especially a method that could demonstrate a negative interstitial fluid pressure, if such existed, was sought. It was also recognized that the tendency for compacted tissues to occlude needle tips probably made it impossible for the needle method to measure a negative pressure even if it did exist.

CHARACTERISTICS OF PERFORATED-CAPSULE METHOD FOR MEASURING INTERSTITIAL FLUID PRESSURE

The basic theory of the perforated capsule technique is to create a tissue fluid cavity that is large enough to measure pressure from it and yet at the same time has a rigid enough outer structure to prevent atmospheric pressure from collapsing the cavity (46). An implanted plastic capsule, even though perforated by tremendous numbers of small holes, provides a rigid skeleton that becomes imbedded in the tissue, and this rigid skeleton prevents atmospheric pressure from being transmitted to the inside of the capsule. Nevertheless, subcutaneous tissue does grow through the perforations and lines the inside of the capsule. The thickness of this lining continues to increase with age of the implantation. When using very small capsules (½ cm diam) the tissue grows to the center of the capsule in approximately 3 weeks. When using very large capsules (about 3 cm diam) the time period required for the capsule to fill completely with tissue is about 6 months. And for capsules of the size most usually used, about 1.5 cm, tissue grows to the center in approximately 4 months.

The tissue that grows through the perforations is at first loose areolar fibrous tissue, but its density increases with time so that 7–8 weeks after implantation the lining is dense fibrous tissue.

When a capsule is first implanted, the surrounding tissues are severely traumatized and there is usually extravasation of blood. Furthermore, air is often either purposely or inadvertently left inside the implanted capsule. The air is absorbed within 7–9 days while the capsule fills with fluid. The clotted blood becomes liquified, and the hemoglobin content of the fluid gradually disappears over a period of 2–3 weeks. During this entire first 2 weeks, severe inflammatory edema exists around the implanted capsule, and the pressures measured from the capsule

are invariably positive. However, as the inflammatory edema recedes, the pressure in the capsule becomes less than atmospheric pressure, that is, becomes negative. At about 4 weeks after implantation, the pressure reaches its lowest value and remains stabilized at that value until the capsule fills with tissue.

Two different methods have been used for measuring pressure in the capsules. One of these is to insert a small needle, usually 22 gauge or smaller, through the skin and then through one of the perforations into the cavity of the capsule. The pressure is then measured from the needle using a low-displacement electronic transducer. Another procedure is to connect a catheter to the perforated capsule prior to implantation, with the tip of the catheter positioned in the very center of the capsule cavity. The free end of the catheter is either brought to the exterior of the animal or is closed off completely and left underneath the skin until such time that it is desirable to lift the catheter and make measurements.

The nature of the material from which the capsule is made makes little difference. Approximately the same pressure levels have been measured from capsules made of plexiglass, celluloid, vinyl, nylon, and stainless steel.

Pressure Values Measured from Implanted Capsules

Pressures (mm Hg) measured from capsules implanted in dogs have been approximately the following for different tissues (46):

Mean of all subcutaneous tissues	−6.4
Axillary subcutaneous tissue	−7.1
Scrotal subcutaneous tissue	−6.6
Subcutaneous tissue of lower leg of the dog	−6.3
Subcutaneous tissue of abdominal wall	−5.5
Retroperitoneal tissue	−6.0
Muscle	−2.6
Lung (93)	−5.0
Kidney (Ott and Navar, unpublished observations)	−1 to +8.0

Implantations in lung and kidney have not been satisfactory enough for one to feel confident of the pressures measured. For instance, in kidneys the capsules must be very small to prevent serious distortion of the tissue, and fibrous tissue usually fills the capsule before the period of inflammation surrounding the capsule is gone Therefore, the validity of pressures measured from such capsules is questionable. In lungs it is difficult to eliminate all infection and inflammation from around the capsules so that the measured pressures are probably less negative than the true pressures.

Several implantations have been made in the abdominal wall of man. Floyer (personal communication) implanted a nylon capsule in himself and found the pressure to average −4 mm Hg, and Holt (15) implanted capsules in man and also found negative pressures, though the values were not given. Subcutaneous implantations by Floyer (30) in rats have given values averaging −5.0 mm Hg.

Scholander et al. (115–117, 127) have pointed out that negative interstitial fluid pressure is a phenomenon of essentially all multicellular biologic life. Using his

wick method for measuring interstitial fluid pressure, he has demonstrated negative pressures in many different species of animals lower than mammals. And, even more important, he has also shown that sap pressure in plants is extremely negative, indeed, many thousands of mm Hg negative with respect to atmospheric pressure, values three orders of magnitude greater than the negative pressure values found in animals.

Pressures Measured in Edema

Pressures have been measured from implanted perforated capsules in different types of edema, including more than 500 separate measurements in typical extracellular edema, noninfectious inflammatory edema, and edema associated with infection. In all these conditions without exception, the pressure has been equal to or more usually greater than atmospheric pressure, that is, positive pressure (46, 47). In usual subcutaneous pitting edema, the pressures have ranged from 0 mm Hg to +6 mm Hg except when the edema occurs after acute injection of fluid into tissues, in which case the pressure can be as high +15 to +20 mm Hg. These high pressures decrease very rapidly even though the same degree of edema is maintained by continual injection of fluid; for instance, the pressure falls from +15 mm Hg to approximately +5 mm Hg within 1–2 hr (47). This effect illustrates that there is a high degree of stress-relaxation of the expanded tissue spaces.

Persistent pressures as high as +12 to +15 mm Hg have been measured in nonpitting edema, especially edema of the indurated infectious types (46).

Validation of Perforated-Capsule Method for Measuring Interstitial Fluid Pressure

If the capsule method is to measure interstitial fluid pressure instead of some artifactual pressure, the fluid pressure in the cavity of the capsule must come to equilibrium with the fluid pressure in the interstitial spaces surrounding the capsule Therefore, various procedures have been performed to determine whether or not equilibration does occur between the fluids in the capsule and the surrounding fluids, some of which follow.

1) Sufficient fluid is present in edematous tissues to make direct fluid contact with a needle inserted in the tissue, in which case true fluid pressure in the tissue spaces can be measured by the needle (46). In more than 100 measurements in which pressures were measured simultaneously from an implanted capsule and from a needle inserted into edematous tissue within 2 cm of the capsule in frankly edematous tissues, the pressures never varied more than 1 mm Hg and the average difference was less than 0.3 mm Hg. Furthermore, dynamic pressure changes recorded simultaneously by these two methods gave almost identical results; for example, a typical experiment was one in which both methods were recording +4 mm Hg pressure in the edematous tissue and sudden compression of the edematous tissue on the outside of the tissue caused both measurements to rise to a new pressure level of +15 mm Hg. Upon removal of the external pressure, both measure-

ments returned exactly to +4 mm Hg. The only difference between the two dynamic responses was that total response from the capsule occurred within 2 sec and was four or more times as rapid as the response from the needle. Thus, the capsule is capable of measuring pressure changes faithfully and almost instantaneously in edematous tissue, indicating wide-open communications between the tissue spaces and the capsule cavity. If an impermeable membrane should exist at the surface of the capsule cavity, these results should be impossible.

2) It has also been important to establish that no pressure difference develops between the cavity in the capsule and the surrounding tissue spaces because of osmotic factors. Osmotic pressure could develop only in case a semipermeable membrane should develop around the intracapsular cavity, that is, a membrane that would allow small molecules to pass more easily between the capsule cavity and the surrounding tissue spaces than could larger molecules such as protein. Therefore, several different types of studies were performed to determine how freely proteins can pass between the cavity of the capsule and the surrounding tissue spaces. The first study was to inject the vital dye T-1824 into capsules and to study movement of this dye from the capsules into the surrounding tissues (46). Since the dye attaches almost entirely to protein, it was assumed that wherever the dye went so did protein. A positive pressure of +10 mm Hg was created inside the capsule by connecting an inserted needle to a pressure source. The capsules were removed from the animals an hour later, and the tissues surrounding the capsules were studied. At each point where a hole perforated a capsule, a stream of dye had penetrated into the surrounding tissues, presumably indicating free mobility of dye and protein into the surrounding tissues. However, it is theoretically possible that the dye that moved through the holes was the dye that was not bound with proteins.

Similar studies have been performed more recently using radioactive albumin. One such procedure has been to study the relative appearance times and courses of concentration changes of albumin both in a capsule and in lymph from the same tissue after injection of radioactive albumin intravenously (37). Results from these studies in six experiments showed that the radioactive albumin concentrations in the capsules and in the lymph 100 hr after injection were identical. This illustrated an almost exact correspondence between the concentrations of albumin in the capsule fluid and in lymph. Measurements of A/G ratio were 1.78 and 1.42 for lymph and capsule, respectively, and these values were not different statistically (Gibson and Taylor, unpublished observations). Studies of protein concentrations in capsules have averaged (in studies from different laboratories) 2.2 g/100 ml (126), 1.96 g/100 ml (46), and one-third the concentration of plasma (30), which are values comparable to protein concentrations in lymph draining from peripheral tissues (112). Gibson and Taylor (unpublished observations) have recently compared lymph and capsule protein concentrations in the same tissue and found average values of 2.3 and 2.0 g/100 ml, respectively, for a total of 28 and 54 determinations.

Finally, the permeability of the pericavity tissues to protein was studied in eight capsules by the following direct method (41). Capsules were removed from

the body and sliced in half. The capsular membrane was carefully isolated and placed in a diffusion chamber to measure transport of albumin from the inside of the membrane to the outside or vice versa. These studies showed that protein diffused through the membrane at a rate approximately one-third that which one would expect through pure water. In other words, the conductivity of the membrane for protein was as if one-third of its mass were water in freely diffusible tissue spaces. Since this is near the ratio of interstitial fluid volume to total volume of normal tissues, it was concluded that the communications between the cavity in the capsule and the surrounding tissue spaces are too large to cause any significant osmotic effects.

Despite the above procedures to study protein movement between the capsule cavity and surrounding tissue spaces, Stromberg and Wiederhielm (126) have suggested, on the basis of indirect evidence, that a membrane barrier might nevertheless exist. The two principal bases of their suggestion are: *1*) injection of hyaluronidase or collagenase into a capsule causes the pressure to rise from the normal negative value to a positive value over a period of several hours; *2*) injection of a high concentration of serum albumin into the capsule also causes the pressure to rise from a negative value to a positive value, but again an hour or more is required for the effect to begin and several hours are required to reach equilibrium. Both these effects can be explained on the basis of well-known characteristics of the capsule technique without implicating a membrane barrier lining the capsule cavity. First, any type of inflammation in the tissue surrounding a capsule will ordinarily cause fluid to transude from the surrounding capillaries and thereby cause the pressure in the capsule to become positive. For instance, injection of Zephiran (benzalkonium), a detergent, into a capsule will cause the same effect as injection of collagenase or hyaluronidase (Guyton, unpublished observations). Also, hyaluronidase could liquefy the hyaluronic acid gel in the surrounding spaces, releasing fluid from the gel into the free state, and thereby elevate the pressure of the free fluid even without increasing the total tissue fluid volume. Furthermore, Chambers and Zweifach (18) demonstrated in frog mesentery that hyaluronidase actually causes fragmentation of capillary membranes, which supports the idea that the hyaluronidase in Stromberg and Wiederhielm's experiments perhaps caused a local inflammatory state.

The fact that the pressure rises in the capsule when concentrated protein solutions are injected, but begins to rise only after an hour or more, actually seems to support the validity of the capsule technique for measuring interstitial fluid pressure rather than detracting from it for the following reasons. First, when concentrated dextran solution is injected into the circulation of a tissue in which a capsule is implanted, fluid is pulled osmotically into the capillaries from the tissue, and the pressure in the capsule falls to approach a new equilibrium value. This effect occurs with a half-time of 1–2 min (46). Second, if a small amount of fluid is injected into or removed from a capsule, the intracapsular pressure is at first acutely altered, but the half-time for reapproach of a new equilibrium pressure is again approximately 1–2 min. Yet, when protein is injected into the capsule, the half-time for increase in intracapsular pressure is several hours rather than the 1–2

min that occurs when the hydrostatic or osmotic pressure is changed in other ways. Therefore, the only interpretation of Stromberg and Wiederhielm's data that seems logical is that the long half-times for equilibrium (two orders of magnitude too high) are caused by necessity for the injected protein to diffuse out of the cavity of the capsule before it exerts its effect. For instance, when it diffuses into the surrounding tissues it comes in contact with tissue capillary membranes and also with the surface of the surrounding tissue gel. At either of these surfaces the elevated colloid osmotic pressure in the free fluid can theoretically cause osmosis of fluid into the free fluid and thereby elevate the free fluid pressure as well as the measured pressure in the capsule.

From a thermodynamic analysis of osmotic pressure differences across membranes, it can be shown that a leaky membrane that eventually allows equilibration of osmotically active substances on the two sides of the membrane—for instance, protein leakage through the capsule membrane, as discussed above—cannot cause a continued osmotic pressure difference between the two sides of the membrane (73). This fact again negates the importance of osmotic pressure as a factor in the capsule method for measuring interstitial fluid pressure. Yet, in a published computer model of interstitial fluid dynamics and function of the capsule (140), the tissue lining the capsule cavity was considered to be totally impermeable to protein despite the data disputing this fact. This caused the model to predict a much lower pressure in the capsule than in the free fluid of the tissue spaces, a result that would not have occurred had the information put into the computer been in accord with experimental observations.

Physiological Validation of Perforated-Capsule Technique for Measuring Interstitial Fluid Pressure

Aside from the above procedures to show that pressure in an implanted perforated capsule is in equilibrium with the free-fluid pressure of the surrounding tissue spaces, different physiological experiments have shown that pressures measured by this technique agree with those predicted from the Starling concept of pressure equilibrium at the capillary membrane (56).

Effect of changes in venous and arterial pressure on measured interstitial fluid pressure. Increasing the venous pressure, which presumably increases the tissue capillary pressure as well, causes the interstitial fluid pressure measured by the capsule method to begin rising immediately (56). Conversely, decreasing the venous pressure back to normal allows the pressure in the capsule to begin returning toward normal.

Decreasing the arterial pressure causes a biphasic response (56). First, the interstitial fluid pressure immediately begins to fall, but approximately 1 min later it stops falling and usually partially returns toward the control value. This secondary effect was believed to be caused by the ability of the tissue to autoregulate its blood flow when arterial pressure is decreased (56). That is, when autoregulation occurs, the resulting arterial dilatation presumably returns the capillary pressure

almost back to normal despite the decrease in arterial pressure, and this is believed to be the cause of the return of the interstitial pressure toward normal as well.

Study of fluid flow through capillary membranes at different intracapsular or different venous pressures. The pressure inside a capsule can be increased by infusion of fluid into the capsule cavity or decreased by withdrawal of fluid. Under appropriate conditions, the rate at which fluid must be infused or withdrawn from the capsule to hold its pressure at either an abnormally high or an abnormally low level is a measure of the rate at which the abnormal pressure can cause fluid to flow through the adjacent capillary membranes (56). Also, the capillary pressure can be raised by increasing venous pressure, and here again the rate of fluid flow through the capillary membrane can be measured by the rate of capsule fluid withdrawal required to keep the capsule pressure constant. In experiments of this type, the rate of fluid exchange across the capillary membrane has been found to be almost exactly the same whether the pressure is changed inside the capsule or changed in the capillaries, except that these changes in pressures cause exactly opposite directions of fluid movement. In four experiments the average rate of fluid flow that occurred in response to intracapsular pressure changes was 0.058 μl/min per mm Hg per gram of tissue inside the capsule. When the pressure was changed in the capillaries instead of in the capsules, the rate was 0.049 μl/min per mm Hg rise in venous pressure per gram of tissue inside the capsule. Thus, Starling's concept of the balance of forces on the two sides of the capillary membrane was satisfied.

Effect of changes in plasma colloid osmotic pressure on interstitial fluid pressure. According to the Starling concept of equilibrium at the capillary membrane, a change in colloid osmotic pressure in the plasma should cause a subsequent change in the interstitial fluid pressure. Injection of concentrated dextran solution intravenously into 12 dogs caused the interstitial fluid pressure as measured by the capsule to begin decreasing immediately and to approach a new equilibrium with a half-time averaging 1–2 min (46, 47). Furthermore, the amount of decrease was proportional to the quantity and concentration of dextran solution injected, and successive injections caused the interstitial fluid pressure to become progressively more negative in a stepwise fashion. Also, perfusion of the hindlimb of 4 dogs for 10 min with 10% dextran solution having a colloid osmotic pressure of 80 mm Hg caused the interstitial fluid pressure of the limb to fall from an average control value of -7 mm Hg to an average of -27 mm Hg. Thus, again, the implanted perforated capsule technique gave interstitial fluid pressure values and proper directional changes in pressure that were consistent with theoretical predictions.

Effect of hemorrhage and shock on interstitial fluid pressure. Hopkinson et al. (66) used the capsule technique to study the effect of hemorrhage on changes in interstitial fluid pressure. Their control values averaged -2.75 mm Hg. After hemorrhage of 35–45 ml/kg, the interstitial fluid pressure fell an additional 3 mm Hg in 1–2 hr. Then they reinfused the blood that had been removed, and the pressure returned to its original control value. On the other hand, when they maintained the animals in a state of shock for about an hour and then returned the withdrawn blood, the pressure did not return to the original control value until they infused additional electrolyte solution.

In endotoxin shock, a condition in which fluid leaks from the capillaries into the interstitial spaces, Anas et al. (5) found that the capsule pressure rose from an average control value of −5 mm Hg to an average value of −2 mm Hg while the plasma volume decreased. These results illustrate still other instances in which the capsule technique measures changes in interstitial fluid pressures that were predicted from knowledge of the physiological conditions.

Failure of needle technique to give pressure changes consistent with Starling concept of pressure equilibria. In the above procedures in which the capillary pressure was altered or in which the colloid osmotic pressure was elevated (46), tissue pressure was measured by the needle technique at the same time that it was measured by the capsule technique. The pressures measured by the needle method did not vary more than 1–2 mm Hg despite marked changes in tissue fluid conditions. Also, the very slight changes that did occur were often in the wrong direction to fit with the predictions of the Starling equilibrium concept. The lack of significant change in pressure measured by the needle technique fits well with the idea that the needle technique measures total tissue pressure rather than interstitial fluid pressure for the following reason: as explained earlier, in normal tissues in which the solid elements of the tissue are compacted upon each other, each time the interstitial fluid pressure changes the solid tissue pressure theoretically should change approximately an equal but directionally opposite amount, thus keeping the total tissue pressure essentially constant.

PRESSURE-VOLUME CURVE OF INTERSTITIAL FLUID SPACES

Probably the most important facts that have emerged from capsule measurements of the interstitial fluid pressure have not been whether or not the true interstitial fluid pressure is negative or positive but instead are the characteristics of the pressure-volume curve of the tissue spaces. To determine this curve, the interstitial fluid volume was decreased or increased while capsule pressure measurements were made (47). The method used to *decrease* volume was to perfuse the blood vessels of the tissue with concentrated dextran solution, thereby withdrawing fluid by osmosis from the tissue spaces. Methods used to *increase* fluid volume were *1*) to perfuse the tissue vessels with saline solution under pressure; *2*) to elevate venous pressure, thereby causing transudation of fluid into the tissue spaces; or *3*) to inject large quantities of balanced electrolyte solution intravenously into an animal. The general characteristics of the pressure-volume curves recorded by these different techniques have been the same. However, the most accurate measurements of pressure-volume curves were derived from the following procedure: the hindleg of a dog was removed and then perfused with 10% dextran solution, which in four animals reduced the average interstitial fluid pressure to −27 mm Hg. Then the leg was perfused with saline solution having no colloid osmotic pressure until the interstitial fluid pressure rose to +10 to +20 mm Hg. During these procedures the weight of the leg was recorded continuously, and the changes in weight were assumed to reflect mainly the changes in interstitial fluid volume. Figure 3 illustrates four records of the interstitial fluid pressure plotted against leg weight

changes. Note that so long as the interstitial fluid pressure was in the negative range, very slight increases in interstitial fluid volume caused very rapid increases in interstitial fluid pressure. However, at a level of pressure approximately equal to atmospheric pressure, a sudden inflection occurred in each curve, showing that large volumes of fluid were then required to cause even slight further increases in interstitial fluid pressure. Then, after severe pitting edema had occurred, the interstitial fluid pressure began to rise again. At this time the skin became hard like a tightly filled bladder, showing obvious tension in the skin.

To express this pressure-volume curve in another way, the compliance of the tissues was very slight while the interstitial fluid pressure was negative, having a compliance of only 0.4 ml/mm Hg per 100 g of tissue. Once the interstitial fluid pressure reached atmospheric pressure level, the compliance suddenly increased an average of 24-fold, up to 9.6 ml/mm Hg per 100 g of tissue. Finally, when extremely severe edema had developed, the compliance once again decreased while the skin developed marked tension.

A similar pressure-volume curve has been shown to occur in almost any balloon structure (47, 74, 75). That is, when a balloon is collapsed because of negative intraluminal pressure the compliance becomes very slight; the remaining compliance is caused by spaces in folds of the balloon walls that can be collapsed further only with very negative pressure. On the other hand, when the balloon is progressively filled, the pressure at first, while the walls of the balloon are flaccid, equals atmospheric pressure and remains almost exactly at this level until the walls begin to stretch; that is, the compliance is very high. Then, when the balloon walls do begin to stretch, the compliance falls again. Similar pressure-volume curves have also been measured for the heart (10, 75) and for veins (3).

Therefore, the pressure-volume curve of the tissue spaces fits the concept that normal tissue spaces are mainly collapsed and that the solid elements of the tissues

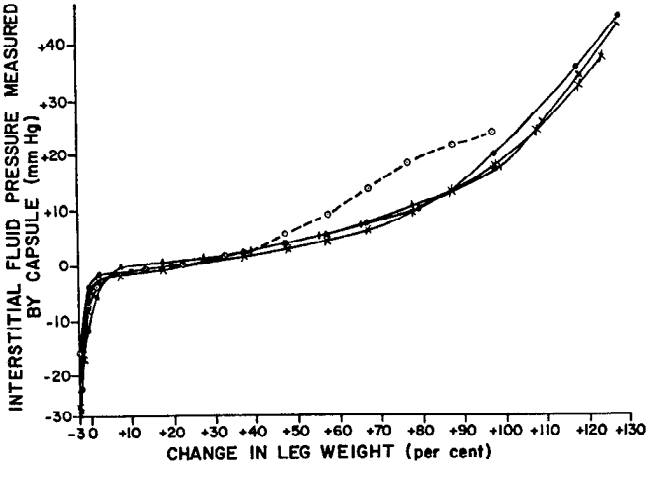

FIG. 3. Relationship of interstitial fluid pressure to change in leg weight during progressive increase in interstitial fluid pressure. Each curve represents results from a separate leg. [From Guyton (47).].

are compacted upon each other. It fits also with measurements from implanted capsules showing that in normal tissues the measured pressures are normally subatmospheric while in edematous tissues they are either atmospheric or supra-atmospheric. And, finally, an experiment by Scholander et al. (116), in which the wick technique for measuring interstitial fluid pressure was used in animals subjected to progressive dehydration, demonstrated that the interstitial fluid pressure falls very little at first but then falls abruptly to very low values in the terminal stages of dehydration. They explained this phenomenon on the basis of progressive compaction of the tissues until finally it became almost impossible to remove further fluid from the interstitial spaces without tremendous further decreases in pressure (116); this phenomenon is also predicted from the pressure-volume curves measured by the capsule technique (47).

CAUSE OF NEGATIVE INTERSTITIAL FLUID PRESSURE—ROLE OF LYMPHATIC SYSTEM AND CAPILLARY MEMBRANE DYNAMICS

Several clues related especially to function of the lymphatic system offer a good explanation for the mechanism of negative interstitial fluid pressure.

First, measurements from several different laboratories have shown that the pumping action of the lymphatics can cause negative pressures in lymphatics, average values having been measured in large lymphatics of −2 mm Hg by Allen (4) and −0.7 mm Hg by Blocker et al. (9).

Second, measurements of interstitial fluid pressure by the capsule technique in an awake and active animal average −6 to −7 mm Hg, but after anesthetization to immobilize the animal, the pressure rises during the next 3–8 hr to approach −2 to −3 mm Hg, usually stabilizing at this level (47). If the animal then begins to awaken and to strain at its bounds, the pressure becomes more negative once again, sometimes falling as much as 1 mm Hg/10 min. Therefore, physical motion of the tissues seems to be an important factor in development and maintenance of negative pressure.

Third, 30 years ago Parsons and McMaster (101) showed that lymph flow in the rabbit's ear stops and fluid begins to accumulate in the tissues of the ear if the isolated ear is perfused with blood propelled by nonpulsatile pressure. However, changing to pulsatile pressure immediately causes fluid to begin moving once more. This experiment demonstrated that transmission of vascular pulsation into the tissues causes movement of fluid into the lymphatics.

Fourth, recent electron-micrographic studies of lymphatics have demonstrated that the lymphatic capillaries have valvular structures all the way to their very tips (17, 23, 83). This operates in the following manner: the endothelial cells of the terminal lymphatic capillaries are not tightly bound to each other; instead, where two endothelial cells come in contact, one of them usually overlaps the other. The inside overlapping edge of the cell is free to flap back and forth and, therefore, to act as a valve. Also, the outside surfaces of the endothelial cells are anchored to the surrounding tissues by filaments that prevent the endothelial wall of the lymphatic capillary from collapsing inward. Thus, any time a lymphatic capillary is com-

pressed, the internal flaps of the endothelial cells close and fluid in the capillary is squeezed into a larger lymphatic vessel. Then, when the capillary recoils from the compression, the endothelial flaps open and allow fluid to move into the capillary. This recoil seems to be caused mainly by recoil of the surrounding tissue, with consequent pull on the endothelial cells by the anchoring filaments, because destruction of the filaments with hyaluronidase causes permanent collapse of the capillaries (16). Thus, motion in the tissue causes a pumping effect at the very tip of the lymphatics.

Finally, evidence is accumulating that some terminal lymphatics undergo continual rhythmical contraction, which could add further to the pumping action of the lymphatics. Such lymphatic contraction has been demonstrated many times in the lymphatics of the bat's wing (135, 142) and of the mesentery (96, 121, 134) and there is evidence that the terminal lymphatics of the intestinal villi also provide lymphatic pumping from the very tips of the lymphatics (27).

Putting all these factors together, it appears that the lymphatic system can pump fluid from the tissue spaces. The degree of negativity of the pressure that can be pumped in the tissues theoretically is determined by three factors: *1*) the tightness of anchoring of the endothelial cells to the surrounding tissues; *2*) the amount of tissue motion; and *3*) the degree of recoil turgor of the surrounding tissues, which dilates the lymphatics during the dilatation cycle of the pumping mechanism. Since the recoil turgor of most tissues is not great, it is to be expected that the interstitial fluid pressure could not be very negative, only a few millimeters Hg, barely enough to keep the tissue spaces reasonably dry of free fluid.

An objection that has been raised to the lymphatic pumping mechanism for maintaining negative interstitial fluid pressure has been that the quantity of lymph flow is very slight, much too slight to carry away more than a small part of the necessary fluid to maintain negative interstitial fluid pressure. However, it is not necessary to carry away large amounts of fluid but only to carry away protein, because if lymph flow is enough to keep the protein concentration in the tissue spaces at a low value, then the colloid osmotic pressure of the plasma can cause absorption of water and small-molecular-weight solutes directly into the blood capillaries. Therefore, the main function of the lymphatics in maintaining negative interstitial fluid pressure is theoretically to remove protein from the tissue spaces, while the blood capillaries perform the function of removing excess fluid by the process of osmosis.

If the blood capillaries are to reabsorb enough water to maintain a negative interstitial fluid pressure, it is necessary that they operate mainly in an absorptive capacity rather than in a filtration capacity. Much evidence indicates this to be true, including the following: *1*) the total surface area of the venous capillaries is far greater than that of the arterial capillaries (137); *2*) the permeability of the venous capillary is much greater than that of the arterial capillaries (111, 138, 139, 145); *3*) isogravimetric and isovolumetric estimations of *functional capillary pressure* indicate this to be 7–8 mm Hg less than the previous estimations of mean capillary pressure by the capillary pipette method (49, 71, 106); and *4*) rhythmic contraction of the precapillary sphincters and meta-arterioles closes off the capillaries to arterial

pressure during a major part of the time (18, 97, 141), leaving the capillaries exposed only to venous pressure and thus causing the average capillary pressure to be much lower than would otherwise be the case. Thus, there is no lack of evidence in modern capillary literature for the concept that the capillaries operate at a very low "functional" average capillary pressure.

Relationship Between Interstitial Fluid Pressure and Lymph Flow

Another study that strongly supports the ability of the lymphatics to pump fluid from the tissues even in the face of a negative interstitial pressure is that of Taylor et al. (130) and Gibson and Gaar (37) in which the relationship between lymph flow and interstitial fluid pressure as measured by the capsule technique was determined. This study showed that lymph flow increases 12- to 50-fold when interstitial fluid pressure rises from its control level of about −6 mm Hg up to 0 mm Hg. Once the pressure rises above 0 mm Hg, however, no further increase in lymph flow occurs even when interstitial fluid pressure increases to as high as +6 or +7 mm Hg. The explanation given for these effects was that when the interstitial fluid pressure is very negative (−6 mm Hg or more), the lymphatic pump simply cannot pump with enough suction to remove more than minute quantities of fluid from the tissues. Yet, as the interstitial fluid pressure rises to approach zero, entry of fluid into the lymphatic capillaries becomes progressively greater and the rate of lymph flow increases correspondingly. On the other hand, when the interstitial fluid pressure rises above zero, the positive pressure in the interstitial spaces not only increases the fluid pressure at the input pores of the lymphatic capillaries but also increases by an equal amount the pressure on the outsides of the lymphatic vessels themselves; the two pressures, therefore, nullify each other, one promoting lymph flow and the other opposing lymph flow. Consequently, an increase in interstitial fluid pressure above atmospheric pressure level would not be expected to increase lymph flow, which was the experimental effect observed by Taylor et al. (130) and also the effect predicted earlier in a systems analysis by Guyton and Coleman (52, 53). The same effect has been demonstrated for blood flow in the veins of the abdomen when the abdominal pressure increases (50) and for blood flow in the veins of the skull when cerebrospinal fluid pressure rises (113). Finally, the mechanics of this effect have been explored and demonstrated in detail in models by Rodbard (108–110). Thus, the mechanism suggested by the Rodbard hypothesis seems to cause autoregulation of lymph flow once the interstitial fluid pressure has risen into the positive range.

QUANTITATIVE ANALYSIS OF SAFETY FACTOR AGAINST EDEMA

One of the important consequences of the pressure-volume curve illustrated in Figure 3 is that so long as the interstitial fluid pressure remains slightly less than atmospheric pressure, the solid elements of the tissues should be at least partially compacted together and there should be no evidence of free, mobile edema fluid. On the other hand, once the interstitial fluid pressure should rise even slightly into

the positive range, free, mobile fluid ought to collect in the tissues and continue to collect until definitive edema should result. Furthermore, the great change in the tissue spaces from very low compliance to very high compliance when the pressure rises from negativity to positivity indicates that there is a very critical pressure level at which edema (defined as excess free, mobile fluid in the tissue spaces) begins, namely when the fluid pressure beneath the skin becomes greater than atmospheric pressure.

Yet, it was pointed out earlier that there is a large safety factor against edema. For instance, the venous pressure must be increased to 11–15 mm Hg before edema will occur (78); a vacuum of 15–22 mm Hg must be applied to the outer surface of the arm before edema will occur (Guyton, unpublished observations); and the colloid osmotic pressure of the blood usually must be decreased 15–22 mm Hg below normal before edema will occur (8, 67). Thus, on the average, there seems to be a safety factor of about 15 mm Hg before edema will ensue. We now need to consider quantitatively the different factors that provide this safety factor. At least three effects enter into this.

1) The normal negative interstitial fluid pressure of −6 mm Hg must be overcome before edema will ensue. This can account for 6 mm Hg of the safety factor against edema.

2) Burgen and Francombe (14), using radioactive albumin to detect albumin concentration in the interstitial fluid, demonstrated that as the tissue spaces begin to fill with fluid so that the edema state is approached, lymphatic removal of protein from the spaces markedly decreases tissue protein concentration. This fits the finding of Gibson and Gaar (37) that even before detectable edema occurs lymph flow increases 12- to 50-fold as interstitial fluid pressure rises. This effect obviously greatly reduces protein concentration in the tissue spaces and thereby also reduces interstitial fluid colloid osmotic pressure. Assuming that the colloid osmotic pressure were 5 mm Hg to begin with, this probably can be reduced to as low as 1–2 mm Hg [values actually measured in edema fluid of cardiac edema patients (80)], which gives another contribution to the safety factor against edema of about 3 mm Hg.

3) The final effect contributing to the safety factor against edema is the lymphatic pumping of fluid from the tissue spaces. By utilizing three different sets of data it is possible to calculate how much safety factor can be caused by this pumping mechanism. These are: *a*) the data of Taylor et al. (130), which show an average of about 20-fold increase in lymph flow between normal tissue conditions and the immediately pre-edematous condition; *b*) the capillary membrane permeability coefficient, as measured in limbs by a number of authors (22, 78, 100), averaging about .01 ml/mm Hg per min per 100 g of tissue; and *c*) the normal rate of lymph flow from a moving limb, averaging about 0.003 ml/min per 100 g of tissue (68). The equation required for this calculation is the thermodynamic equation for fluid filtration at the capillary membrane:

$$J/K = P_c - P_i + \pi_i - \pi_p \qquad (4)$$

At normal lymph flow (which also equals the normal *net* outflow of fluid from the capillary) the factor J/K equals 0.3 mm Hg, which is also the normal net pressure differential between the inside and outside of the capillary. When lymph

flow increases 20-fold, J/K becomes 6 mm Hg, which represents the net pressure difference that develops across the capillary membrane as a result of net flow of fluid outward into the tissue spaces and then into the lymphatics. Because of this pressure drop across the capillary membrane, the capillary pressure can rise an additional 6 mm Hg before edema will ensue, providing still a third contribution to the safety factor against edema.

Adding the three factors (the normal negative interstitial fluid pressure factor of 6 mm Hg, the tissue protein washout factor of 3 mm Hg, and the lymphatic pump factor of 6 mm Hg), the total safety factor against edema should be approximately 15 mm Hg, a value near that measured in the three different types of experiments explained above.

Similar types of studies in the lungs, where the capillary pressure is probably less than that in peripheral tissues (1, 34), indicate a safety factor against edema of 20–28 mm Hg (33, 39, 55, 59, 84, 125).

INTERSTITIAL FLUID SPACES AS AN OVERFLOW RESERVOIR FOR THE CIRCULATORY SYSTEM

As blood volume increases, the mean systemic pressure also increases, which in turn tends to increase all other pressures throughout the circulatory system, including capillary pressure (44, 45). However, once the capillary pressure has risen an amount equal to the safety factor against edema, there comes a critical point at which the tissue spaces will accept almost unlimited quantities of fluid because of the sudden increase in tissue space compliance at interstitial fluid pressures above atmospheric pressure (47). Consequently, with hardly any additional rise in capillary pressure, fluid pours into the tissues, creating the state of edema. Looking at this phenomenon from the point of view of the circulation instead of that of the tissue spaces, this ability of the tissues to store large quantities of fluid in the form of edema represents a safety valve to prevent further increases in intravascular pressures. The drastic effects of too high intravascular pressures can be demonstrated by infusing fluid into the circulation more rapidly than it can transude into the tissue spaces, in which case the heart is likely to fail and capillaries or other vessels are likely to rupture (54). These observations support further the very logical organization of the interstitial fluid system, having as its primary goal the ability to prevent edema except under unusual circumstances but, secondarily, the ability to allow rapid overflow from the circulatory system when the circulatory system reaches the upper limits of safe pressures.

MOBILITY OF FLUID THROUGH TISSUE SPACES

If it is true that the solid elements of the tissues are normally compacted upon each other, then the spaces through which fluid can flow should theoretically be very minute. Also, if a major share of these spaces is filled with gel, this would further decrease the ease of fluid flow through the tissues. On the other hand, in the edematous state the spaces are greatly enlarged, so that fluid flow should occur

very easily. In a study that supports this concept two perforated capsules were implanted 2.5 cm apart, and fluid flow from one to the other at a constant pressure difference was measured (57). When a pressure difference of 1 mm Hg was established, with one capsule pressure measuring −5 mm Hg and the other −6 mm Hg, the fluid flow from one capsule to the other was almost unmeasurable, only 2.4×10^{-6} cm²/sec per mm Hg. Then the tissue was made edematous by injecting minute quantities of fluid into the surrounding tissues. When the pressure was +1 mm Hg in one capsule and 0 mm Hg in the other, flow from one capsule to the other increased suddenly to 10,000 times the flow in normal tissues. Similar experiments were performed by inserting two 10-cm-long multiply-perforated catheters into tissues 2.5 cm apart and then measuring conductivity of fluid through the tissues from one catheter to the other. In this case, as the pressure was increased from a negative value of −6 mm Hg up to +1 mm Hg, there occurred at atmospheric pressure level a flow rate increase averaging 250,000-fold, again illustrating sudden opening of large tissue spaces.

Pressure-Volume Curves for Free and Nonmobile Interstitial Fluids

Utilizing the above data, it has been possible to estimate the relative sizes of the tissue spaces in which free fluid moves from one point to another. This was done by assuming that Poiseuille's law holds for the very slow flow that does occur in these spaces, in which case the diameters of the spaces would increase according to the fourth root of the increased conductivity and the cross-sectional areas and volumes would increase according to the square root of conductivity. Utilizing these data and the pressure-volume curves determined in Figure 3, it has been possible also to calculate absolute quantities of mobile (or "free" fluid) in tissues (57). This calculation gives a value for mobile fluid less than 1% of the total fluid in normal tissue spaces. Figure 4 gives the calculated relationships between interstitial fluid pressure on the one hand and fluid volumes, both nonmobile and free-fluid volumes, on the other hand. The free fluid is defined in this instance as the fluid that is mobile, and it does not include nonmobile sequestered pockets of free fluid. The top curve represents the total fluid volume in the interstitial fluid spaces of man as calculated from *a*) the pressure-volume curve of Figure 3 and *b*) the assumption that the average baseline total interstitial fluid volume (nonmobile plus free fluid) of man at an interstitial fluid pressure of −6 mm Hg is 12 liters [based on indicator dilution measurements (119)]. The difference between the top curve and the second curve represents the quantity of free fluid as determined from the fluid conducitivity experiments just described. The lower curve depicts the remaining fluid, the nonmobile fluid in the interstitial spaces, much of it presumably in the form of gel and the remainder located in very small crevices of the tissue spaces or in minute cavities within the gel. Since low-molecular-weight substances diffuse through 0.5% hyaluronic acid solutions at least 95% as well as through free fluids (82) nutrients and excreta can diffuse through the nonmobile fluid between capillary and tissue cells with complete ease, even in the absence of a large amount of free fluid.

Possible physiologic roles of tissue gel. One can propose at least two theoretically

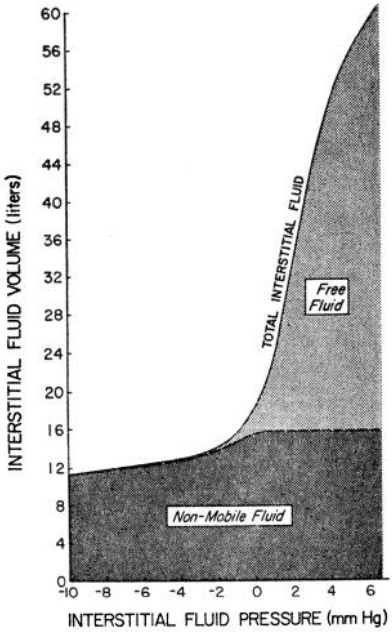

FIG. 4. Effect of interstitial fluid pressure on total interstitial fluid volume, interstitial free fluid volume, and interstitial nonmobile fluid volume. Methods used for determining these volumes explained in text.

important physiologic roles for tissue gel. One would be to prevent flow of interstitial fluid from one level of the body to another. In normal tissues, significant flow does not occur, which is the effect that would be expected from the experiments quoted above (57) on fluid conductivity in normal tissue. In edema, on the other hand, fluid flow to dependent tissues is well known. Also, the phenomenon of "pitting" is a manifestation of facile fluid flow in tissues.

A second theoretical importance of gel would be to keep adjacent cells partially separated from each other. If they were not separated, those cells that lie more than one cell thickness away from capillaries would receive nutrition only through interposed cells. Some nutrients do not pass through cell membranes with sufficient ease for the secondary cells to receive adequate nutrition under such conditions. Therefore, one can show mathematically that there is optimum spacing between cells for maximal delivery of nutrition to cells. If the degree of spacing is too little, nutrition suffers because of cell barriers; if too great, it suffers because of increased distance from the capiliary to the outlying cells. One could speculate that the normal quantity of gel in tissues is controlled in some way at least partially related to these requirements for nutrient diffusion.

CONTROL OF INTERSTITIAL FLUID VOLUME

Another corollary of these new concepts concerning tissue fluid dynamics is that they provide a very simple mechanism for control of interstitial fluid volume: whenever excess free fluid appears in the interstitial spaces, the interstitial fluid pressure rises rapidly, and the lymphatic mechanism discussed earlier automatically

removes the excess free fluid. The pressure-volume curves of Figure 3 show that only very small quantities of free fluid must enter the normally compacted tissue spaces for the pressure to rise several millimeters Hg. In turn, this rise in interstitial fluid pressure toward atmospheric pressure causes rapid lymphatic flow, with correspondingly rapid removal of proteins from the interstitial spaces. The lowered colloid osmotic pressure of the tissue spaces then allows the colloid osmotic pressure of the plasma in the capillaries to cause reabsorption of the remaining excess fluid. To state this another way, the free-fluid component of the interstitial fluids is controlled essentially to a minimal value and remains near this minimal value all the time except when extracellular fluid edema occurs. Therefore, from the point of view of free fluid, the interstitial fluid spaces seem normally to remain essentially in a dry state (57).

However, there still remains a large quantity of fluid in the nonmobile state, which is relatively stable in quantity either because it is present as gel (26) or because it is sequestered in small crevices by surface forces (92).

The volume of nonmobile fluid, therefore, seems to be the major factor determining the normal interstitial fluid volume. This volume is determined at least partially by the amounts of hyaluronic acid, chondroitin sulfate, collagen fibers, and other intercellular substances secreted into or formed in the interstitial spaces. The greater the quantities of these substances, the greater also becomes the quantity of nonmobile or bound fluid in the tissue spaces. An interesting example occurs in myxedema, in which large quantities of hyaluronic acid are present in the interstitial spaces (6, 120). As a result, the interstitial fluid volume is far greater than normal, but the fluid is nonmobile as evidenced by the fact that these persons do not have pitting edema.

REFERENCES

1. AGOSTONI, E., AND J. PIIPER. Capillary pressure and distribution of vascular resistance in isolated lung. *Am. J. Physiol.* 202: 1033–1036, 1962.
2. AGOSTONI, E., A. TAGLIETTI, AND I. SETNIKAR. Absorption force of the capillaries of the visceral pleura in determination of the intrapleural pressure. *Am. J. Physiol.* 191: 277–282, 1957.
3. ALEXANDER, R. S. The participation of the venomotor system in pressor reflexes. *Circulation Res.* 2: 405–409, 1954.
4. ALLEN, L. Volume and pressure changes in terminal lymphatics. *Am. J. Physiol.* 123: 3–4, 1938.
5. ANAS, P., W. A. NEELY, AND J. D. HARDY. Interstitial fluid pressure changes in endotoxin shock. *Surgery* 63: 938–941, 1968.
6. ASBOE-HANSEN, G. Hormone control of connective tissue. *Federation Proc.* 25: 1136–1140, 1966.
7. BAUER, W., M. W. ROPES, AND H. WAINE. The physiology of articular structures. *Physiol. Rev.* 20: 272–312, 1940.
8. BENNHOLD, H., H. PETERS, AND E. ROTH. Über einen Fall von Completter Analbumin-aemia ohne wesentlische klinische Krankheitszeichen. *Verhandl. Deut. Ges. Innere Med.* 2: 630–634, 1954.
9. BLOCKER, T. G., JR., J. R. SMITH, E. F. DUNTON, J. M. PROTAS, R. M. COOLEY, S. R. LEWIS, AND E. J. KIRBY. Studies of ulceration and edema of the lower extremity by lymphatic cannulation. *Ann. Surg.* 149: 884–896, 1959.
10. BRECHER, G. A., AND P. M. GALLETTI. Functional anatomy of cardiac pumping. In: *Handbook of Physiology. Circulation.* Washington, D. C.: Am. Physiol. Soc., 1963, sect. 2, vol. II, p. 759–798.
11. BURCH, G. E. Factors influencing edema formation in the eyelids of man. *J. Clin. Invest.* 18: 487, 1939.
12. BURCH, G. E. Formation of edema in the eyelids of man. Influence of local tissue pressure, skin distensibility, lymph flow, intraorbital pressure gradient and venous pressure. *Arch. Internal Med.* 65: 477–498, 1940.
13. BURCH, G. E., AND W. A. SODEMAN. The estimation of the subcutaneous tissue pressure by a direct method. *J. Clin. Invest.* 16: 845–850, 1937.
14. BURGEN, A. S. V., AND W. H. FRANCOMBE. The role of plasma colloid osmotic pressure in the regulation of extracellular fluid. In: *Proc. Intern. Union Physiol. Sci., 22nd, Leiden.* 1962, vol. 2, p. 130.
15. CALNAN, J. S., G. CHESHOLM, J. J. PFLUG, P. FORD, AND P. J. L. HOLT. What is tissue fluid? *Clin. Sci.* 38: 16P, 1970.

16. CASLEY-SMITH, J. R. Electron microscopical observations on the dilated lymphatics in oedematous regions and their collapse following hyaluronidase administration. *Brit. J. Exptl. Pathol.* 48: 680–686, 1967.
17. CASLEY-SMITH, J. R. The functioning of the lymphatic system under normal and pathological conditions: its dependence on the fine structures and permeabilities of the vessels. In: *Progress in Lymphology*, edited by A. Ruttiman. New York: Hafner, 1967, p. 348–359.
18. CHAMBERS, R., AND B. W. ZWEIFACH. Intercellular cement and capillary permeability. *Physiol. Rev.* 27: 436–463, 1947.
19. CHASE, W. H. Extracellular distribution of ferrocyanide in muscle. *Arch. Pathol.* 67: 525–532, 1959.
20. CHVAPIL, M. *Physiology of Connective Tissue.* London: Butterworths, 1967.
21. CLARK, E. R., AND E. L. CLARK. Further observations on living lymphatic vessels in the transparent chamber in the rabbit's ear—their relation to the tissue spaces. *Am. J. Anat.* 52: 273–305, 1933.
22. COBBOLD, A., B. FOLKOW, I. KJELLMER, AND S. MELLANDER. Nervous and local chemical control of precapillary sphincters in skeletal muscle as measured by changes in filtration coefficient. *Acta Physiol. Scand.* 57: 180–192, 1963.
23. COLLIN, H. B. The ultrastructure of conjunctival lymphatic anchoring filaments. *Exptl. Eye Res.* 8: 102–105, 1969.
24. DAY, T. D. The permeability of interstitial connective tissue and the nature of the interfibrillary substance. *J. Physiol. (London)* 117: 1–8, 1952.
25. FERNANDO, N. V. P., G. A. VAN ERKEL, AND H. Z. MOVAT. The fine structure of connective tissues. IV. The intercellular elements. *Exptl. Mol. Pathol.* 3: 529–545, 1964.
26. FESSLER, J. H. A structural function of mucopolysaccharide in connective tissue. *Biochem. J.* 76: 124–132, 1960.
27. FLOREY, H. Observations on the contractility of lacteals. Part I. *J. Physiol. (London)* 62: 267–272, 1927.
28. FLORY, P. J. *Principles of Polymer Chemistry.* Ithaca: Cornell Univ. Press, 1953.
29. FLOYER, M. A. The mechanism underlying the response of the hypertensive subject to a saline load. *Expansion Sci. Franc.* 1: 440–448, 1966.
30. FLOYER, M. A. Discussion. In: *Ciba Symp. Circulatory and Respiratory Mass Transport*, edited by G. E. W. Wolstenholme and J. Knight. London: Churchill, 1969, p. 21–22.
31. FRIEDERICI, H. H. R. Freeze-etch observations on interstitial connective tissue. *J. Ultrastruct. Res.* 24: 269–285, 1968.
32. FUND, Y. C., B. W. ZWEIFACH, AND M. INTAGLIETTA. Elastic environment of the capillary bed. *Circulation Res.* 19: 441–461, 1966.
33. GAAR, K. A., JR., A. E. TAYLOR, L. J. OWENS, AND A. C. GUYTON. Effect of capillary pressure and plasma protein on development of pulmonary edema. *Am. J. Physiol.* 213: 79–82, 1967.
34. GAAR, K. A., JR., A. E. TAYLOR, L. J. OWENS, AND A. C. GUYTON. Pulmonary capillary pressure and filtration coefficient in the isolated perfused lung. *Am. J. Physiol.* 213: 910–914, 1967.
35. GAUER, O. H., J. P. HENRY, AND C. BEHN. The regulation of extracellular fluid volume. *Ann. Rev. Physiol.* 32: 547–595, 1970.
36. GERSH, I., AND H. R. CATCHPOLE. The nature of ground substance of connective tissue. *Perspectives Biol. Med.* 3: 282–319, 1960.
37. GIBSON, H., AND K. A. GAAR, JR. Dynamics of the implanted capsule. *Federation Proc.* 29: 319, 1970.
38. GILMORE, J. P. Influence of tissue pressure on renal blood flow autoregulation. *Am. J. Physiol.* 206: 707–713, 1964.
39. GORLIN, R., B. M. LEWIS, F. W. HAYNES, R. J. SPIEGEL, AND L. DEXTER. Factors regulating pulmonary "capillary" pressure in mitral stenosis. IV. *Am. Heart J.* 41: 834–854, 1951.
40. GOTTSCHALK, C. W. A comparative study of renal interstitial pressure. *Am. J. Physiol.* 169: 180–187, 1952.
41. GRANGER, H. J., A. E. TAYLOR, AND A. C. GUYTON. Quantitative analysis of the permeability characteristics of membranes isolated from chronically implanted subcutaneous capsules. *Microvasc. Res.* 2: 240, 1970.
42. GREEN, J. A., J. LUCAS, AND M. A. FLOYER. The effect of the kidney in altering the response of the circulation to fluid loading. *Clin. Sci.* 38: 4P, 1970.
43. GREGG, D. E., AND R. W. ECKSTEIN. Measurements of intramyocardial pressure. *Am. J. Physiol.* 132: 781–790, 1941.
44. GRODINS, F. S. *Control Theory and Biological Systems.* New York: Columbia Univ. Press, 1963, p. 161
45. GUYTON, A. C. *Circulatory Physiology: Cardiac Output and Its Regulation.* Philadelphia: Saunders, 1963.
46. GUYTON, A. C. A concept of negative interstitial pressure based on pressures in implanted perforated capsules. *Circulation Res.* 12: 399–414, 1963.
47. GUYTON, A. C. Interstitial fluid pressure: II. Pressure-volume curves of interstitial space. *Circulation Res.* 16: 452–460, 1965.
48. GUYTON, A. C. Pressure-volume relationships in the interstitial spaces. *Invest. Ophthalmol.* 4: 1075–1084, 1965.
49. GUYTON, A. C. Interstitial fluid pressure-volume relationships and their regulation. In: *Ciba Symp. Circulatory and Respiratory Mass Transport*, edited by G. E. W. Wolstenholme and J. Knight. London: Churchill, 1969, p. 14–20.
50. GUYTON, A. C., AND L. H. ADKINS. Quantitative aspects of the collapse factor in relation to venous return. *Am. J. Physiol.* 177: 523–527, 1954.
51. GUYTON, A. C., G. G. ARMSTRONG, AND J. W. CROWELL. Negative pressure in interstitial spaces. *Physiologist* 3: 70, 1960.
52. GUYTON, A. C., AND T. G. COLEMAN. Long-term regulation of the circulation: interrelationships with body fluid volumes. In: *Physical Bases of Circulatory Transport: Regulation and Exchange*, edited by E. B. Reeves and A. C. Guyton. Philadelphia: Saunders, 1967, p. 179–201.
53. GUYTON, A. C., AND T. G. COLEMAN. Regulation of interstitial fluid volume and pressure. *Ann. N. Y. Acad. Sci.* 150: 537–547, 1968.
54. GUYTON, A. C., J. E. LINDLEY, R. N. TOUCHSTONE, C. M. SMITH, AND H. M. BATSON, JR. Effect of massive transfusion and hemorrhage on blood pressure and fluid shifts. *Am. J. Physiol.* 163: 529–538, 1950.

55. GUYTON, A. C., AND A. W. LINDSEY. Effect of elevated left atrial pressure and decreased plasma protein concentration on the development of pulmonary edema. *Circulation Res.* 7: 649–657, 1959.
56. GUYTON, A. C., J. W. PRATHER, K. SCHEEL, AND J. McGEHEE. Interstitial fluid pressure: IV. Its effect on fluid movement through the capillary wall. *Circulation Res.* 19: 1022–1030, 1966.
57. GUYTON, A. C., K. SCHEEL, AND D. MURPHREE. Interstitial fluid pressure: III. Its effect on resistance to tissue fluid mobility. *Circulation Res.* 19: 412–419, 1966.
58. GUYTON, A. C., A. E. TAYLOR, AND H. J. GRANGER. Analysis of types of pressure in the pulmonary interstitial spaces—interstitial fluid presrue, solid tissue pressure, and total tissue pressure. In: *Symp. European Thoracic Soc., Pisa, Italy.* Basel: Karger. In press.
59. HADDY, F. J., A. L. FERRIN, D. W. HANNON, J. F. ALDEN, W. L. ADAMS, AND I. D. BARNOFSKY. Cardiac function in experimental mitral stenosis. *Circulation Res.* 1: 219–225, 1953.
60. HEDBYS, B. O. The role of polysaccharides in corneal swelling. *Exptl. Eye Res.* 1: 81–91, 1961.
61. HEDBYS, B. O. Corneal resistance to the flow of water after enzymatic digestion. *Exptl. Eye Res.* 2: 112–121, 1963.
62. HEDBYS, B. O., AND S. MISHIMA. Flow of water in corneal stroma. *Exptl. Eye Res.* 1: 262–275, 1962.
63. HEDBYS, B. O., S. MISHIMA, AND D. M. MAURICE. The imbibition pressure of the corneal stroma. *Exptl. Eye Res.* 2: 99–111, 1963.
64. HELDT, T. J., AND J. C. MOLONEY. Negative pressure in epidural space; preliminary studies. *Am. J. Med. Sci.* 175: 371–376, 1928.
65. HENDERSON, Y., A. W. OUGHTERSON, L. A. GREENBERG, AND C. P. SEARLE. Muscle tonus, intramuscular pressure and the venopressor mechanism. *Am. J. Physiol.* 114: 261–268, 1936.
66. HOPKINSON, B. R., J. R. BORDER, W. C. HEYDEN, AND W. G. SCHENK, JR. Interstitial fluid pressure changes during hemorrhage and blood replacement with and without hypotension. *Surgery* 64: 68–74, 1968.
67. HUMPHREY, J. H. Serum albumin concentration in analbuminaemia and its immunological significance. *Nature* 187: 304–305, 1960.
68. JACOBSSON, S., AND I. KJELLMER. Flow and protein content of lymph in resting and exercising skeletal muscle. *Acta Physiol. Scand.* 60: 278–285, 1964.
69. JOHNSON, J. R., AND J. R. DI PALMA. Intramyocardial pressure and its relation to aortic blood pressure. *Am. J. Physiol.* 125: 234–243, 1939.
70. JOHNSON, P. C. Origin, localization, and homeostatic significance of autoregulation in the intestine. *Circulation Res.* 15, Suppl. 1: 225–233, 1964.
71. JOHNSON, P. C. Effect of venous pressure on mean capillary pressure and vascular resistance in the intestine. *Circulation Res.* 16: 294–300, 1965.
72. KATCHALSKY, A. Polyelectrolyte gels. *Progr. Biophys.* 4: 1–59, 1954.
73. KATCHALSKY, A., AND P. F. CURRAN. *Nonequilibrium Thermodynamics in Biophysics.* Cambridge: Harvard Univ. Press, 1965.
74. KING, A. L., AND R. W. LAWTON. Elasticity of body tissues. In: *Medical Physics,* edited by O. Glasser. Chicago: Year Book, 1950, vol. II, p. 303–316.
75. KING, A. L., AND R. W. LAWTON. The elasticity of soft body tissues. *Sci. Monthly* 71: 258–260, 1950.
76. KIRK, E. S., AND C. R. HONIG. An experimental and theoretical analysis of myocardial tissue pressure. *Am. J. Physiol.* 207: 361–367, 1964.
77. KJELLMER, I. An indirect method for estimating tissue pressure with special reference to tissue pressure in muscle during exercise. *Acta Physiol. Scand.* 62: 31–40, 1964.
78. KROGH, A., E. M. LANDIS, AND A. H. TURNER. The movement of fluid through the human capillary wall in relation to venous pressure and to the colloid osmotic pressure of the blood. *J. Clin. Invest.* 11: 63–95, 1932.
79. LADEGAARD-PEDERSEN, H. J. Measurement of the interstitial pressure in subcutaneous tissue in dogs. *Circulation Res.* 26: 765–770, 1970.
80. LANDIS, E. M. Capillary pressure and capillary permeability. *Physiol. Rev.* 14: 404–481, 1934.
81. LANDIS, E. M., AND J. R. PAPPENHEIMER. Exchange of substances through the capillary walls. In: *Handbook of Physiology. Circulation.* Washington, D. C.: Am. Physiol. Soc., sect. 2, vol. II, 1963, p. 961–1034.
82. LAURENT, T. C., I. BJORK, A. PIETRUSZKIEWICZ, AND H. PERSSON. On the interaction between polysaccharides and other macromolecules. II. The transport of globular particles through hyaluronic acid solutions. *Biochim. Biophys. Acta* 78: 351–359, 1963.
83. LEAK, L. V., AND J. F. BURKE. Ultrastructural studies on the lymphatic anchoring filaments. *J. Cell Biol.* 36: 129–149, 1968.
84. LEVINE, O. R., R. B. MELLINS, R. M. SENIOR, AND A. P. FISHMAN. The application of Starling's law of capillary exchange of the lung. *J. Clin. Invest.* 46: 934–944, 1967.
85. MATHEWS, M. B. The interaction of collagen and acid mucopolysaccharides. A model for connective tissue. *Biochem. J.* 96: 710–716, 1965.
86. MAYERSON, H. S., AND G. E. BURCH. Relationships of tissue (subcutaneous and intramuscular) and venous pressures to syncope induced in man by gravity. *Am. J. Physiol.* 128: 258–269, 1940.
87. McDONALD, D. A. Hemodynamics. *Ann. Rev. Physiol.* 30: 525–556, 1968.
88. McMASTER, P. D. Intermittent take-up of fluid from the cutaneous tissue. *J. Exptl. Med.* 73: 67–84, 1941.
89. McMASTER, P. D. The pressure and interstitial resistance prevailing in the normal and edematous skin of animals and man. *J. Exptl. Med.* 84: 473–494, 1946.
90. McMASTER, P. D. The relative pressures within cutaneous lymphatic capillaries and the tissues. *J. Exptl. Med.* 86: 293–308, 1947.
91. McMASTER, P. D., AND R. J. PARSONS. Physiological conditions existing in connective tissue. I. The method of interstitial spread of vital dyes. *J. Exptl. Med.* 69: 247–264, 1939.
92. McMASTER, P. D., AND R. J. PARSONS. The movement of substances and the state of the fluid in the intradermal tissue. *Ann. N. Y. Acad. Sci.* 52: 992–1003, 1950.
93. MEYER, B. J., A. MEYER, AND A. C. GUYTON. Interstitial fluid pressure: V. Negative pressure in the lungs. *Circulation Res.* 22: 263–271, 1968.
94. MEYER, K., E. DAVIDSON, A. LINDKER, AND

P. HOFFMAN. The acid mucopolysaccharides of connective tissue. *Biochim. Biophys. Acta* 21: 506–518, 1956.

95. MEYERS, D. B., T. C. HIGHTON, AND D. G. RAYNS. Acid mucopolysaccharides closely associated with collagen fibrils in normal human synovium. *J. Ultrastruct. Res.* 28: 203–213, 1969.
96. MISLIN, H., AND R. SCHIPP. Structural and functional relations of the mesenteric lymph vessels. In: *Progress in Lymphology*, edited by A. Ruttiman. New York: Hafner, 1967, p. 360–365.
97. NICOLL, P. A., AND R. L. WEBB. Vascular patterns and active vasomotion as determiners of flow through minute vessels. *Angiology* 6: 291–310, 1955.
98. OGSTON, A. G. On water binding. *Federation Proc.* 25: 986–989, 1966.
99. OGSTON, A. G., AND T. F. SHERMAN. Effects of hyaluronic acid upon diffusion of solutes and flow of solvent. *J. Physiol. (London)* 156: 67–74, 1961.
100. PAPPENHEIMER, J. R., AND A. SOTO-RIVERA. Effective osmotic pressure of the plasma proteins and other quantities associated with the capillary circulation in the hind limbs of cats and dogs. *Am. J. Physiol.* 152: 471–491, 1948.
101. PARSONS, R. J., AND P. D. McMASTER. The effect of the pulse upon the formation and flow of lymph. *J. Exptl. Med.* 68: 353–376, 1938.
102. PERMUTT, S., P. CALDINI, H. N. BANE, P. HOWARD, AND R. L. RILEY. Liquid pressure versus surface pressure of the esophagus. *J. Appl. Physiol.* 23: 927–933, 1967.
103. PRESTON, B. N., M. DAVIES, AND A. G. OGSTON. The composition and physicochemical properties of hyaluronic acids prepared from ox synovial fluid and from a case of mesothelioma. *Biochem. J.* 96: 449–474, 1965.
104. QUINTARELLI, G. Methods for the histochemical identification of acid mucopolysaccharides: a critical evaluation. In: *The Chemical Physiology of Mucopolysaccharides*, edited by G. Quintarelli. Boston: Little, Brown, 1968, p. 199–215.
105. REEVES, B. Negative pressures in knee joints. *Nature* 212: 1046, 1966.
106. RICHARDSON, D. R., J. W. PRATHER, AND B. W. ZWEIFACH. Comparison of techniques for measuring mean capillary pressure in perfused organs. *Physiologist* 13: 293, 1970.
107. ROBERTSON, G. A. Methodology of the Guyton capsule. *Biorheology* 6: 59, 1969.
108. RODBARD, S. Autoregulation in encapsulated passive soft-walled vessels. *Am. Heart J.* 65: 648–655, 1963.
109. RODBARD, S. Mechanical properties of a permeable flexible "capillary." *Physiologist* 12: 341, 1969.
110. RODBARD, S., AND K. KURAMOTO. Transmural pressure and vascular resistance in soft-walled vessels. *Am. Heart J.* 66: 786–791, 1963.
111. ROUS, P., H. P. GILDING, AND F. SMITH. The gradient of vascular permeability. *J. Exptl. Med.* 51: 807–830, 1930.
112. RUSZNYAK, I. M. FOLDI, AND G. SZABO. *Lymphatics and Lymph Circulation* (2nd ed.). Oxford: Pergamon, 1967.
113. SAGAWA, K., O. CARRIER, AND A. C. GUYTON. Elicitation of theoretically predicted feedback oscillation in arterial pressure. *Am. J. Physiol.* 203: 141–146, 1962.
114. SALISBURY, P. F., C. E. CROSS, AND P. A. RIEBEN. Intramyocardial pressure and strength of left ventricular contraction. *Circulation Res.* 10: 608–623, 1962.
115. SCHOLANDER, P. F. Osmotic mechanism and negative pressure. *Science* 156: 67–69, 1967.
116. SCHOLANDER, P. F., A. R. HARGENS, AND S. L. MILLER. Negative pressure in the interstitial fluid of animals. *Science* 161: 321–328, 1968.
117. SCHOLANDER, P. F., AND M. DE OLIVEIRA PEREZ. Sap tension in flooded trees and bushes of the Amazon. *Plant Physiol.* 43: 1870–1873, 1968.
118. SCHUBERT, M. Intercellular macromolecules containing polysaccharides. *Biophys. J.* 4, Suppl.: 119–138, 1964.
119. SHIRES, T., J. WILLIAMS, AND F. BROWN. Simultaneous measurement of plasma volume, extracellular fluid volume and red blood cell mass in man, utilizing I^{131}, $S^{35}O_4$ and Cr^{51}. *J. Lab. Clin. Med.* 55: 776–783, 1960.
120. SISSON, J. C. Hyaluronic acid in localized myxedema. *J. Clin. Endocrinol.* 28: 433–436, 1968.
121. SMITH, R. O. Lymphatic contractility. A possible intrinsic mechanism of lymphatic vessels for the transport of lymph. *J. Exptl. Med.* 90: 497–509, 1949.
122. SODEMAN, W. A., AND G. E. BURCH. The tissue pressure in subcutaneous edema. *Am. J. Med. Sci.* 194: 846–850, 1937.
123. SPICER, S. S., R. G. HORN, AND T. J. LEPPI. Histochemistry of connective tissue mucopolysaccharides. In: *The Connective Tissue*, edited by B. M. Wagner and D. E. Smith. Baltimore: Williams & Wilkins, 1967, p. 251–303.
124. STARLING, E. H. On the absorption of fluids from the connective tissue spaces. *J. Physiol. (London)* 19: 312–326, 1895.
125. STAUB, N., H. NAGANO, AND M. PEARCE. Pulmonary edema in dogs especially the sequence of fluid accumulation in lungs. *J. Appl. Physiol.* 22: 227–240, 1967.
126. STROMBERG, D. D., AND C. A. WIEDERHIELM. Effects of oncotic gradients and enzymes on negative pressures in implanted capsules. *Am. J. Physiol.* 219: 928–932, 1970.
127. STROMME, S. B., J. E. MAGGERT, AND P. F. SCHOLANDER. Interstitial fluid pressure in terrestrial and semiterrestrial animals. *J. Appl. Physiol.* 27: 123–126, 1969.
128. SWANN, H. G., A. V. MONTGOMERY, J. C. DAVIS, JR., AND E. R. MICKLE. Method for rapid measurement of intrarenal and other tissue pressures. *J. Exptl. Med.* 92: 625–636, 1950.
129. TANFORD, C. *Physical Chemistry of Macromolecules*. New York: Wiley, 1961.
130. TAYLOR, A. E., K. A. GAAR, JR., AND H. GIBSON. Effect of tissue pressure on lymph flow. *Biophys. J.* 10: 45A, 1970.
131. THURAU, K. W. C. Autoregulation of renal blood flow and glomerular filtration rate, including data on tubular and peritubular capillary pressure and vessel wall tension. *Circulation Res.* 15, Suppl. I: 132–141, 1964.
132. WASTESON, A., AND B. OBRINK. Demonstration of an interaction between collagen and chondroitin sulfate. *Biochim. Biophys. Acta* 170: 201–204, 1968.
133. WAUGH, W. H. Circulatory autoregulation in the fully isolated kidney and in the humorally supported,

isolated kidney. *Circulation Res.* 15, Suppl. I: 156–169, 1964.

134. WEBB, R. L. Observations on the propulsion of lymph through the mesenteric lymphatic vessels of the living rat. *Anat. Record* 57: 345–350, 1933.
135. WEBB, R. L., AND P. A. NICOLL. Behavior of lymphatic vessels in the living bat. *Anat. Record* 88: 351–367, 1944.
136. WELLS, H. S., J. B. YOUMANS, AND D. G. MILLER, JR. Tissue pressure (intracutaneous, subcutaneous, and intramuscular) as related to venous pressure, capillary filtration, and other factors. *J. Clin. Invest.* 17: 489–499, 1938.
137. WIEDEMAN, M. P. Architecture of the terminal vascular bed. In: *Physical Bases of Circulatory Transport: Regulation and Exchange*, edited by E. B. Reeve and A. C. Guyton. Philadelphia: Saunders, 1967, p. 307–312.
138. WIEDERHIELM, C. A. Transcapillary and interstitial transport phenomena in the mesentery. *Federation Proc.* 25: 1789–1798, 1966.
139. WIEDERHIELM, C. A. Analysis of small vessel function. In: *Physical Bases of Circulatory Transport: Regulation and Exchange*, edited by E. B. Reeve and A. C. Guyton. Philadelphia: Saunders, 1967, p. 313–326.
140. WIEDERHIELM, C. A. Dynamics of transcapillary fluid exchange. *J. Gen. Physiol.* 52: 29–63, 1968.
141. WIEDERHIELM, C. A. Microvascular, lymphatic, and interstitial pressures in the bat wing. *Proc. Intern. Union Physiol. Sci.* 7: 468, 1968.
142. WIEDERHIELM, C. A. The interstitial space and lymphatic pressures in the bat wing. In: *The Pulmonary Circulation and Interstitial Space*, edited by A. P. Fishman and H. H. Hecht. Chicago: Univ. of Chicago Press, 1969, p. 29–40., 1951, p. 51–100.
143. WIEDERHIELM, C. A., D. D. STROMBERG, AND D. R. LEE. Oncotic pressure in minute fluid samples and tissues. *Federation Proc.* 29: 319, 1970.
144. WINTON, F. R. Intrarenal pressure and renal blood flow. In: *Third Conference on Renal Function.* New York: Josiah Macy, Jr. Found., 1951, p. 51–100.
145. ZWEIFACH, B. W., AND M. INTAGLIETTA. Mechanics of fluid movement across single capillaries in the rabbit. *Microvasc. Res.* 1: 83–101, 1968.