Interstitial Fluid Pressure: II. Pressure-Volume Curves of Interstitial Space

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In a previous study we found the interstitial fluid pressure, as measured in tissue cavities created by implanting perforated capsules, to be —6 to —7 mm Hg in most normal tissues, though it rose to positive values in edema. Very slight changes of interstitial fluid volume changed the interstitial fluid pressure markedly in normal tissues but only slightly in edematous tissues. The present study is an attempt to quantitate earlier findings by recording pressure-volume curves for the interstitial spaces. Also, a physical model was constructed to exhibit some of the physical principles that govern the nature of the pressure-volume relationships of the interstitial spaces, and curves determined in this model are compared with those from the interstitial spaces themselves.

Methods

In nineteen dogs, ranging in weight between 15 and 20 kg, perforated capsules 1.5 cm in diameter, of the type described in the previous paper, were implanted subcutaneously, one in each lower leg and one in each side of the anterior abdominal wall. Each capsule was perforated with several hundred holes approximately 1 mm in diameter. The surgical wounds were allowed to heal for three to four weeks before the intracapsular pressures were measured.

These measurements were made by inserting a number 27 needle through the skin and into the cavity of the capsule. The needle was connected to a catheter 60 cm long, I.D. 1 mm, which led to a Statham low pressure transducer. The zero pressure reference level was set at the midlevel of the capsule. Pressure-volume curves were estimated in four different ways in dogs anesthetized with pentobarbital, 30 mg/kg, as follows:

(1) Isolated Leg Method

Four hindlegs were removed from two animals at the hip joint, and each was perfused with solutions of different colloid osmotic pressures in order to change the interstitial fluid volume. Leg weights and interstitial capsule pressures were measured at frequent intervals.

(2) Elevated Venous Pressure Method

In six dogs, a pneumatic cuff was placed around the upper thigh of the hindleg and inflated until the venous pressure in the lower leg rose to 50 to 70 mm Hg as measured by a mercury manometer. Fluid transuded continuously into these legs for the next few hours while intracapsular pressures were recorded.

(3) Infusion Method

Tyrode's solution was infused in massive volumes into 11 intact dogs, up to 50% of the dog's own initial weight, in order to produce edema. Capsule pressures were measured as the edema developed and progressed.

(4) Dextran Method

Standard volumes of 20% dextran in Tyrode's solution were injected intravenously, and the respective pressure changes in the capsule were recorded. By estimating the quantity of fluid removed from the interstitial spaces by the dextran solution, it was possible to calculate a pressure-volume curve.

Additional details of these methods will be described in connection with results.

Results

CONTROL STUDIES

The pressures measured in the different capsules prior to determining the pressure-volume curves ranged between —4 and —9 mm Hg, in agreement with a much larger series of measurements previously reported. Measurements were made both in awake and...
in anesthetized animals. Immediately after anesthetization the pressure was within 1 mm Hg of the pressure in the awake animal. However, during hours of reduced activity under anesthesia, the pressure gradually rose toward the zero level at a rate averaging about 1 mm per hour, indicating that immobilization is associated with progressive loss of the negative pressure. Activity of the animal, such as resisting restraint or shivering during pentobarbital anesthesia, caused the pressure to become more negative at a rate of about 1 mm per 10 minutes of activity, this rate varying obviously with the degree of activity.

PRESSURE-VOLUME CURVES IN ISOLATED LEGS

At the time that each hindleg was removed at the hip joint, a tourniquet was placed around the cut upper thigh to prevent loss of fluid either from the blood vessels or from the interstitial fluid spaces. Catheters were inserted in the femoral artery and vein distal to the tourniquet so that the vascular system could be perfused. At the beginning of each experiment, the leg was weighed on a chemical balance, and the weight of the portion of the leg distal to the tourniquet was calculated on the basis of diameter measurements and position of the tourniquet.

The pressures in the lower leg capsules before the four legs were removed were —5, —6, —8, and —6 mm Hg. After removal of the legs from the animals, the pressures were still within 1 mm Hg of these values.

Each leg was then perfused for 30 minutes with Tyrode's solution containing 10% dextran, while the weight of the leg was recorded on the chemical balance. The weight of the leg gradually declined, presumably because interstitial fluid was removed by the colloid osmotic pressure of the dextran solution. To make precise measurements of leg weight, the perfusion was stopped periodically, the blood vessels allowed to empty, and the leg weight allowed to stabilize for about two minutes. At the end of 30 minutes of intermittent perfusion in this manner, the weights of the legs had decreased an average of 3%, and the pressures recorded from the capsules were —17, —20, —26, and —27 mm Hg, averaging —22 mm Hg.

Once the pressure had been made very negative by osmotically dehydrating the tissues, each leg was then perfused with Tyrode's solution minus the dextran. Immediately, the recorded weight of the leg began to rise. Capsular pressures and leg weights were again measured intermittently in the same manner as described above. The results were plotted as the four pressure-weight curves shown in figure 1. In the higher pressure ranges, above +10 mm Hg, it was necessary to occlude partially the venous outflow to achieve sufficient accumulation of fluid in the tissues. The results in figure 1 are reported in terms of per cent change in weight of the calculated portion of the leg beyond the tourniquet. This portion averaged 86% of total leg weight. A broken line was used for a portion of one of the curves to indicate that pressure measurements made for part of that curve were still falling very rapidly at the end of two minutes of stopped perfusion. The pressure in this particular capsule always rose to equal venous pressure when fluid was being perfused through the leg, which indicated that the measuring needle had ruptured a vessel in the capsule; this presumably accounted for the higher values and the failure of the pressure to become steady at the end of two minutes of stopped perfusion.

Compliance of the Interstitial Spaces

At an interstitial fluid pressure of —7 mm Hg the compliance (dV/dP), as measured from the curves in figure 1, averaged 4 ml increase in fluid volume per kg of leg weight per mm Hg rise in pressure. At +1 mm Hg interstitial fluid pressure it was 96 ml/kg/mm Hg. Thus, in the positive pressure range associated with edema, the compliance of the tissues was about 24 times as great as it was in the normal negative pressure range.

"Delayed Compliance" of the Interstitial Spaces

In the lower pressure ranges (below +3 mm Hg), the pressures recorded in the capsule 15 seconds, 30 seconds, 1 minute, and 2 minutes, after perfusion had been stopped, were all the
same within the limits of accuracy of measurement. However, in higher pressure ranges, particularly when the pressure had reached +10 mm Hg or greater, the capsule pressure fell very rapidly during the first two minutes, and was still falling at a rate of about 10% per minute at the time that each measurement was made, even though the weight of the leg remained exactly constant. This effect we interpreted as "delayed compliance." We discounted the possibility that this continuing fall in pressure could have resulted from failure of capsule pressure to reach equilibrium with the interstitial fluid pressure because a previous study had shown that capsule pressures measured in edematous tissues come to equilibrium with needle pressures measured in the same tissue within thirty seconds after the pressure is momentarily elevated by compressing the skin.1 Furthermore, at the end of each experiment the pressures recorded after the last perfusion continued to fall for at least an hour, reaching about one-half the final values reported in figure 1 in about 20 minutes.

Estimations of Edema in the Interstitial Spaces

Edema of the limb could not be observed until the leg weight had increased to approximately 10% above normal. At this point the concavities of the skin, e.g., where the skin creases inward around the tendons, had begun to fill out. When the leg weight had increased to approximately 25% above normal, the edema was classified as approximately 2+ using the usual clinical scale. At 40% increase in weight the edema was 3+, and above 60% it was 4+. Note that the capsule pressure at 60% averaged only +6 mm Hg.

**ESTIMATED PRESSURE-VOLUME CURVES DURING TRANSUDATION OF FLUID PRODUCED BY ELEVATED VENOUS PRESSURE**

A pneumatic cuff was placed around the thigh of a dog's hind limb, and the venous pressure was recorded from a catheter inserted in a lower leg vein through which a continuous drip of saline flowed at a rate of 5 to 10 drops per minute to prevent clotting. The
Increase of interstitial fluid pressure over a period of six to nine hours in three hind limbs of dogs following increase in venous pressure up to 60 mm Hg.

Pressure in the cuff was raised suddenly to levels such that venous pressure rose to 50 mm Hg in one experiment, 60 mm Hg in four experiments, and 70 mm Hg in one experiment. This pressure rose from zero to its final level in approximately 30 seconds and was maintained at this final level for 6.5 to 13 hours while the pressure within the lower leg capsule was measured continuously.

Figure 2A presents three of the curves recorded when the venous pressure was elevated to 60 mm Hg and held at this level throughout the remainder of the experiment. Initial capsule pressures were -5, -6, and -10 mm Hg respectively. Note that these pressures rose very rapidly at first but much more slowly after the first hour or so.

The results from all six experiments were then translated into an estimated pressure-volume curve in the following way. First, the total volume of fluid accumulation in the leg was estimated by measuring the circumference of the leg at four equidistant levels before and after the experiment. The per cent change in volume (ΔV) was calculated by assuming that the volume in each segment of the leg is proportional to the square of the circumference. Second, it was assumed that the rate of transudation of fluid out of the capillaries into the interstitial spaces was proportional to the increase of the pressure gradient across the capillary wall. This increase in pressure gradient, in turn, was assumed to equal 80% of the increase in venous pressure minus the increase in pressure measured in the capsule. Using these assumptions and the total ΔV as determined above, the time scale of the curve was converted to a Δvolume scale. As will be shown, even serious error in these assumptions would exert only slight effects on the general characteristics of the calculated pressure-volume curves.

Using the calculated values for ΔV and the measured values for capsule pressure, the average pressure-volume curve of figure 2B was derived. The shaded area shows standard errors of the means up to a volume increase of approximately 100% and a pressure increase up to approximately 5 mm Hg. Only two experiments went beyond this pressure level, for which reason only the average is presented in the higher range to the right. Figure 2B shows also the estimated grades of edema, +1 to +4, during the different stages of the experiment, based on the usual clinical scale for reporting edema.

PRESSURE-VOLUME CURVES DETERMINED BY INFUSING TYRODE’S SOLUTION INTO THE WHOLE ANIMAL

Tyrode’s solution was infused intravenously...
Progressive change of interstitial fluid pressure in two dogs while large volumes of Tyrode's solution were injected intravenously. The upper curve is from a normal dog, and the lower curve is from a dog whose interstitial spaces had been dehydrated previously by infusing 8 ml/kg of 20% dextran solution intravenously.

into six dogs at rates of 10 to 14 ml/kg/min in amounts up to half the initial body weight. Pressure was measured in leg capsules and also in capsules in the abdominal wall. Pressures in eleven separate capsules were recorded as the volume of infused fluid increased. The interstitial spaces of three of the animals were dehydrated immediately prior to infusing the fluid by injecting intravenously 8 ml/kg of 20% dextran in Tyrode's solution.

Figure 3A illustrates two typical experiments; the upper curve was recorded from an animal not dehydrated with dextran, and the lower curve was recorded from an animal that had been dehydrated. Figure 3B presents an algebraically averaged curve derived from all eleven recordings and shows also the standard errors of the means.

ESTIMATION OF THE PRESSURE-VOLUME CURVE BY INJECTING STANDARD VOLUMES OF DEXTRAN SOLUTION

In our previous paper on negative interstitial fluid pressure, we reported that interstitial fluid pressure decreased when concentrated dextran solution was injected intravenously. Figure 4 in that paper showed that the decrease of interstitial fluid pressure following injection of a given amount of dextran solution was considerably greater when the initial interstitial fluid pressure was very negative than when the pressure was only slightly negative. It was reasoned that this difference might result from a difference in compliance of the interstitial fluid spaces at the two pressure levels and that, by use of appropriate calculations, one could utilize this type of experiment to estimate the pressure-volume curve of the spaces.

A standard volume, 8 ml/kg, of Tyrode's solution containing 20% dextran, was injected into each experimental animal while pressures were recorded from the abdominal and leg capsules. The amount of fluid that this quantity of dextran solution would remove from the interstitial space was estimated in the following way. A 20% dextran solution has a colloid osmolar concentration approximately four times that of plasma. Therefore, injection of 8 ml of such a solution could remove up to 24 ml of fluid from the interstitial fluids before the dextran osmolar concentration would fall to equal that of plasma. Such complete removal does not actually occur, because the capillary pressure almost certainly rises as fluid is drawn into the circulation, and the interstitial pressure, as measured by the cap-
PRESSURES IN INTERSTITIAL SPACES

FIGURE 4A

Effect of injecting a standard volume of dextran solution (8 ml/kg of 20% dextran) into dogs. It is presumed that each injection removed 12 ml of fluid per kilogram of animal weight from the interstitial spaces for reasons discussed in the text. Ordinate represents ratio of change of pressure to change of calculated volume of the interstitial fluid spaces. Abscissa represents the interstitial fluid pressure immediately prior to injecting the standard volume of dextran solution.

sule, decreases at the same time. How much effect these changes of pressure have on the amount of fluid removed from the interstitial spaces by the dextran solution is unknown. Yet, to determine the shape of the pressure-volume curve from this procedure, we need make only the assumption that the amount of fluid removed from the interstitial spaces did not vary greatly following each standard injection of dextran solution. As a first approximation, we assumed that half as much fluid was removed from the interstitial spaces as one could expect on the basis of the osmotic factors above. Using this approximation, we calculated that for each 8 ml/kg of the dextran solution injected, 12 ml of interstitial fluid would be removed.

The decrease of capsule pressure following injection of the standard volume of dextran solution was recorded in 32 instances. Some of these injections were made in animals that presented normal control values, i.e., negative capsule pressures. In other animals, Tyrode's solution was infused intravenously until the capsule pressure rose to some higher value (up to +3 mm Hg) before the dextran solution was injected. Figure 4A shows the results of these measurements. The pressure on the abscissa is the initial capsule pressure before injection of the dextran solution, and the ordinate represents the decrease in pressure for each ml of fluid calculated to have been removed from the interstitial spaces per kilogram of body weight. This value is the reciprocal of the compliance of the tissue spaces. Note that when the interstitial fluid pressure was already very negative, the injection of dextran solution caused far greater fall of capsule pressure than when the interstitial fluid pressure was at some higher level.

The four points on the zero ordinate were corroborated by eight additional experiments in which larger quantities of dextran solution were infused into edematous animals recording positive capsule pressures. The four points at very high ordinate values were also corroborated by four additional experiments using either larger or smaller quantities of dextran solution. Some of the data from these additional experiments were illustrated in figure 4 of a previous study.1

Figure 4B gives the integral of the curve shown in figure 4A. Mathematically, this integral is the calculated pressure-volume curve of the interstitial spaces. Note the correspond-
Diagram at left illustrates a model of the interstitial spaces when they were filled with excess fluid. Diagram at right illustrates the model when the pressure in the spaces between the simulated cells was made negative by applying negative pressure to the central perforated tube.

ence between this curve and the first portions of the curves in figures 1 and 2B.

PRESSURE-VOLUME CURVE OF A PHYSICAL MODEL SIMULATING THE INTERSTITIAL SPACES

Figure 5A illustrates a physical model of the interstitial fluid spaces. The upper part of the figure shows the model when excess fluid was present in the spaces, and the lower part shows the model when fluid was removed from the spaces by applying a negative pressure.

The model consisted of a 10-inch rubber bag from an anesthesia machine. The bag was covered with two layers of vinyl plastic sheeting 6 mils in thickness. The bag was filled with 1- to 2-inch balloons and cotton, and a 1-cm perforated plastic tube extended through its center. The balloons, which simulated cells in the tissues, were barely filled with water, without any excess stretching of their walls. The cotton was distributed between the balloons to simulate fibers in the intercellular spaces. The perforated plastic tube provided a means for adding or removing fluid from the spaces and for measuring pressure. The rubber bag and vinyl plastic simulated the skin; the rubber gave the simulated skin the property of elasticity, and the vinyl gave it the property of plasticity.

The curve drawn through the dots in figure 5B illustrates the pressure-volume curve of the model obtained by changing the volume of "interstitial fluid" in the bag and measuring pressure. This figure also shows, for comparison, the average pressure-volume curve of the four experiments on isolated hind limbs illustrated in figure 1. Note that the curve from the model is very similar to that from the isolated limb. By changing the physical characteristics of the model, e.g., the thickness of the plastic on the outside, the amount of cotton in the spaces, the degree of filling of the balloons, etc., the compliances of the model in different pressure ranges could be changed easily. However, the general shape of the curve always remained the same, with very low compliances in the negative pressure range, very high compliances in the range slightly above zero pressure, and intermediate compliances in the higher pressure range.

The model also behaved in the same way as the isolated limb with respect to "delayed compliance." When the pressure was highly positive, particularly above +10 mm Hg, an
increase of fluid volume elevated pressure markedly at first, but the pressure gradually fell toward lower values during the next few minutes.

Another similarity between the model and tissue can be seen in the right-hand diagram of figure 5A. When the pressure was negative in the spaces between the balloons, the balloons were pressed tightly against each other and assumed the multifaceted shapes of cells, while the interstitial spaces were reduced to very minute interstices.

Discussion

The real importance of the pressure-volume curves recorded in these experiments is not their precise quantitative values but instead their shapes. All the curves, recorded by four different procedures from animal tissues and also from a physical model of the interstitial spaces, demonstrated a very low compliance of the interstitial spaces so long as the capsule pressure was in the negative pressure range but, yet, a compliance that increased many fold once the capsule pressure rose above the surrounding atmospheric pressure.

The shape of the curves explains why the interstitial spaces appear to have minimal fluid in them under normal conditions when the capsule pressure is negative and also explains why they collect tremendous quantities of fluid in edema when the capsule pressure rises above atmospheric pressure. These findings support the long recognized "safety factor" that protects against the development of edema. It is known, for instance, that the plasma protein concentration in an otherwise normal person must fall to less than two-thirds normal before he begins to develop edema. Yet, on the basis of the physiological principles of capillary exchange dynamics, we also know that this much decrease in plasma protein concentration should increase the interstitial fluid pressure many millimeters of mercury. The curves of the study show that the capsule pressure must rise about 7 mm Hg from its normal negative value of —7 mm Hg before the tissues begin to collect large quantities of fluid. Thus, the low compliance of the tissues in the negative pressure range allows large changes in interstitial fluid pressure without marked changes in interstitial fluid volume. Yet, the existence of a critical plasma protein concentration below which edema will develop would also be predicted from the pressure-volume curves, because once the interstitial fluid pressure has risen only slightly into the positive range, the very large compliance of the tissues in this range would be expected to allow considerable collection of fluid with only slight additional increase in interstitial fluid pressure.

At least two other mechanisms probably also contribute to the safety factor. First, it has been believed for a long time that when tissue fluid volume increases, the lymphatic flow also increases and removes most of the accumulated protein from the interstitial spaces, thereby reducing the interstitial colloid osmotic pressure. This will reduce the tendency for fluid to transude into the interstitial spaces. Burgen has recently provided convincing evidence that this mechanism does indeed operate in the tissues and provides as much as 4 to 5 mm Hg additional safety factor before edema will develop. Second, increased return of fluid volume via the lymphatics can perhaps also play a role in opposing the development of edema. Unfortunately, the quantitative importance of this mechanism is unknown.

It is obvious from the results of this study that the pressure-volume curves recorded in the isolated limbs are quantitatively more exact than those recorded in the other three types of experiments because conditions could be very exactly controlled in the isolated limbs. Yet, it was also important to record the pressure-volume curves in the other three ways as well, because these curves showed that the principles demonstrated in isolated limbs also apply to the intact animal.

Finally, the validity of the curves obviously depends on the validity of the capsule method for measuring interstitial fluid pressure. The validity of measurements of interstitial fluid pressure by this means was discussed at length in a previous paper.
Summary

Pressure-volume curves of the interstitial fluid spaces were estimated using four different methods. Interstitial fluid pressure was measured from implanted perforated capsules while interstitial fluid volume was varied (a) by perfusing the isolated hind limb of the dog with several types of fluid and at different perfusion pressures, (b) by elevating the venous pressure so that fluid would transude into the interstitial compartment, (c) by infusing large quantities of Tyrode's solution into the whole animal, and (d) by intravenous infusion of concentrated dextran solution. In all these studies, the compliance of the interstitial fluid system was found to be very low when the interstitial fluid pressure was negative but very high when the pressure rose slightly above atmospheric pressure. A physical model of the interstitial spaces was also constructed. Pressure-volume curves recorded from this model demonstrated a pattern of compliance changes similar to that shown by the interstitial pressure-volume curves recorded from dogs. These experiments give further support to the concept that the interstitial fluid pressure is normally negative but becomes positive as edema fluid accumulates.

References

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