A Concept of Negative Interstitial Pressure
Based on Pressures in Implanted Perforated Capsules

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Interstitial pressure has usually been measured by inserting a very small needle into a tissue and then determining the minimum pressure required to make fluid flow from the needle into the tissue. Unfortunately, even when extremely small needles are used for this purpose, such as 25 to 30 gauge, the external diameter of the needle is still some 300 to 500 times as large as the widths of the tissue spaces. The insertion of such needles into the tissues must distort the spaces greatly and could easily give a false measure of the interstitial pressure. For this reason, we attempted to find another method for measuring interstitial pressure and developed a technique using perforated capsules implanted in the tissues. After such capsules have remained in place for two to three weeks they become filled with interstitial fluid, and the intracapsular pressure can be measured by inserting a minute needle through the skin and then through one of the capsule perforations into the inner cavity.

During the last two years we have implanted approximately 200 such capsules in different tissue spaces of dogs. Contrary to general belief regarding interstitial pressure, we have found that the pressure inside these capsules is normally negative rather than positive. On the other hand, in edematous tissues, the measured pressures have always been positive. This paper presents a series of different experiments performed to determine whether the pressure measured inside the capsule is in reality equal to the pressure in the surrounding interstitial spaces. It also explains why we have come to the conclusion that the normal interstitial pressure, at least in many areas of the body, is negative rather than positive.

Methods

Two to six capsules were implanted in each of 55 dogs, and were located in two or more of the following tissues: (a) subcutaneous tissue of the lower leg, (b) subcutaneous tissues of the abdomen, (c) subcutaneous tissue of the axillary space, (d) deep thigh muscle, (e) retroperitoneal space beneath the abdominal muscles, and (f) scrotum after removal of a testicle. Four different types of capsules were implanted: 1) a cylindrical methacrylate tube 2.5 cm in length by 0.8 cm diameter, closed at both ends, and perforated by approximately 100 1-mm holes, 2) a capsule similar to the first but 1.4 cm in diameter and perforated by approximately 200 holes, 3) a 2-cm celluloid ball perforated by approximately 200 1-mm holes (illustrated in fig. 1), and 4) a standard celluloid ping pong ball 3 cm in diameter and perforated by approximately 250 1-mm holes. The results were almost identical for all these capsules except that the small capsules filled rapidly with ingrowing tissue which limited their usefulness.

The capsules were implanted with appropriate sterile technique, and the animals were given penicillin and streptomycin for the first week to prevent infection. Even so, some of the capsules still became infected with resultant inflammation and edema around the capsules. The results were quite different in these capsules from those which did not become infected, as will be explained later.

The pressure inside the capsule was measured by inserting a 25 or 27 gauge syringe needle through the skin, then through one of the perforations of the capsule, and thence into the cavity. The needle was connected to a Statham strain gauge by means of a 2 mm O.D., 60 cm long polyvinyl tube hardened in gasoline for 24 hours until the tube had become almost brittle. The displacement of the recording system was 0.12...
condition of the implanted capsules

Ordinarily, after the capsules were implanted, all the air was absorbed and replaced by interstitial fluid within the first four to seven days (as determined by paracentesis), and overt signs of inflammation from the surgery disappeared within 10 to 12 days. Capsules removed at this time showed the following internal appearance: Fibrous tissue had grown through the perforated holes to the inside of the capsule, and the entire wall of the cavity was lined with a continuous tissue approximately 1 to 2 mm in thickness. During the ensuing weeks, the tissue continued to grow, often reaching a thickness of 5 or more mm. In the 0.8 cm capsules, the entire cavity became completely obliterated by tissue within approximately four weeks. The cavities of the 1.4 cm capsules became completely obliterated within two to three months, while the cavities of most of the 2 cm and 3 cm capsules usually remained patent during the entire course of the experiments, which lasted up to four months. Histological study of the tissues lining the capsules revealed that, along with the ingrowth of fibrous tissue, new blood vessels also developed. Thus, the fluid inside the cavity was not in contact with the capsule itself but instead was in a true tissue space lined on all sides by tissue supplied with a vascular system. The capsule itself was embedded in the surrounding tissues and served as a rigid support to keep the cavity from collapsing.

pressures measured in the capsules following initial implantation

Pressures in nine capsules were measured either daily, every other day, or every three days during the recovery period following implantation. During the first week following implantation, the measured pressure was always positive, ranging between +1 and +5 mm Hg. This was true whether the capsule had filled with fluid or was still partially filled with air. During the next week, i.e., from one to two weeks following implantation, the pressures were very erratic, sometimes measuring...
NEGATIVE INTERSTITIAL PRESSURE

TABLE 1

Pressures in mm Hg Referred to Atmospheric Pressure at the Mid-level of Each Capsule

<table>
<thead>
<tr>
<th>Area</th>
<th>Mean ± SD (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal subcut. space</td>
<td>-2.8 ± 1.8 (29)</td>
</tr>
<tr>
<td>Abdominal subcut. space</td>
<td>-2.3 ± 0.7 (6)</td>
</tr>
<tr>
<td>Upper leg subcut. space</td>
<td>-3.5 ± 2.1 (5)</td>
</tr>
<tr>
<td>Lower leg subcut. space</td>
<td>-0.8 ± 1.1 (18)</td>
</tr>
<tr>
<td>Scrotum</td>
<td>-5.5 ± 1.3 (8)</td>
</tr>
<tr>
<td>Thigh muscle</td>
<td>-6.6 ± 1.4 (3)</td>
</tr>
</tbody>
</table>

Table 1: Pressures in mm Hg Referred to Atmospheric Pressure at the Mid-level of Each Capsule

Mean ± standard deviation (number observations)

-5.5 ± 1.3 (8)
—7.1 ± 2.8 (10)
—6.3 ± 1.2 (4)
—6.6 ± 1.4 (3)

negative and sometimes positive, ranging from —3 mm Hg to +3 mm Hg. After 14 days, however, the pressures were almost always in the negative range and became progressively more and more negative during the ensuing few weeks.

PRESSURES MEASURED IN CAPSULES IN NORMAL TISSUES TWO TO FOUR WEEKS FOLLOWING IMPLANTATION

Pressures were measured in 56 capsules two to four weeks following implantation. These measurements were made both in awake dogs and in dogs anesthetized with sodium pentobarbital with no discernible difference in results. The capsules had produced no evident surrounding inflammation nor edema, and they had not been punctured previously. The average values of these measurements are listed in Table 1, column a. There was a distinct tendency for the pressure measurements to be less negative in the lower leg than elsewhere. Of the entire 56 measurements, 49 were negative and only 7 positive, two of which were in the abdomen, one in the thigh muscle, and four in the lower leg.

PRESSURES MEASURED IN CAPSULES IN NORMAL TISSUES FOUR OR MORE WEEKS FOLLOWING IMPLANTATION

In our eagerness to complete the experiments we most often made our first pressure measurements only 25 to 35 weeks following capsule implantation. Once the capsule had been entered by a needle, infection or hemorrhage into the capsule often precluded further use of the capsule for several weeks. However, 25 separate pressure measurements were made under one of the following two conditions:

(1) at least four weeks following implantation of the capsule without any disturbance of the capsule whatsoever during the intervening time, or
(2) at least four weeks following implantation and at least three weeks following the last puncture of the capsule by a needle.

The results are listed in Table 1, column b.

The average negativity of all of the 25 measurements was —6.4 ± 2.0 mm Hg. There was no statistically significant difference between the values in the different tissues. Even though the number of these measurements is far less than the number of measurements made during the first few weeks following implantation of the capsule, these undoubtedly represent more nearly the true equilibrium pressures that would be obtained in capsules left in the tissues indefinitely. Indeed, pressures measured in three capsules that had been implanted four months prior to measurement gave almost identical values.

REEQUILIBRATION OF CAPSULAR PRESSURE FOLLOWING ADDITION OF FLUID TO OR SUBTRACTION OF FLUID FROM THE CAPSULE

When a minute amount of fluid was injected into a capsule, the pressure rose immediately, but, over a period of 5 to 15 minutes, it always returned asymptotically back to the normal value. Removal of a small amount of fluid caused the reverse effect. Figure 2 illustrates typical pressure recordings following injection and removal of fluid from a capsule. In the upper record, the control pressure was —6 mm Hg; then 1.5 cubic millimeters of normal saline were injected into the capsule. The pressure rose immediately from —6 to +3.5 mm
Reequilibration of pressure in the capsule after the fluid volume in the capsule was changed. In the upper record 1.5 mm$^3$ of fluid was injected into the capsule. In the lower record 1.0 mm$^3$ of fluid was removed from the capsule. Note the asymptotic approach of the pressure back toward its original level.

Hg and then fell back to the original control value with a half-time averaging approximately two minutes (somewhat more rapid than this at first and slower toward the end). The lower record illustrates the effect of removing 1.0 cubic millimeter of fluid from the capsule, which caused immediate reduction in pressure from −6 mm Hg to −12 mm Hg, followed by return of the pressure toward the normal value, again with a half-time of approximately two minutes. The same results were recorded in over 500 measurements. The half-times of approach ranged between one and three minutes. This asymptotic reappraoch of the pressure back to its original value following addition or subtraction of fluid from the capsule illustrates that the measured pressure was not the result of some peculiar eneystment of the fluid in such a way as to cause the negative pressures.

Rate of Fluid Exchange Between the Capsule Cavity and the Tissues

Observing the upper curve of figure 2 once again and remembering that 1.5 mm$^3$ of normal saline was injected to cause this rise in pressure, we can calculate from the rate at which the pressure returned toward the control value that the average rate of fluid removal from the capsule cavity was 0.11 mm$^3$ per minute per mm Hg. This was a typical value for all the experiments (average = 0.09 ± 0.02 mm$^3$/min per mm Hg in 18 experiments); it indicates the magnitude of fluid exchange into and out of the capsule during non-equilibrium states.

Interstitial Pressures Measured by the Usual Needle Technic

Tissue pressures were measured in over 300 instances in many different subcutaneous tissues of the body by the usual needle technique, using essentially Swann's method$^6$ as outlined under “Methods.” In general, we were able to duplicate the results found by previous workers when we copied their procedures.$^1$ The pressures usually measuring +1 to +3 mm Hg but occasionally measuring as low as minus 4 mm Hg in such areas as the axillary space and in the space behind the Achilles tendon where the skin is pulled inward forming a concavity.

Demonstrations that the Intracapsular Pressure Obeys Starling’s Law of the Capillaries and That the Needle Pressure Does Not Obeys This Law

Starling's law of the capillaries states that if the effect of lymphatic flow is neglected and if equilibrium conditions prevail, the mean capillary pressure plus the tissue colloid osmotic pressure will equal the plasma colloid osmotic pressure plus the interstitial fluid pressure.$^8$ Therefore, if one of the other factors besides interstitial fluid pressure should be changed, interstitial fluid pressure should automatically change in an appropriate direction to reestablish equilibrium because of osmosis at the capillary wall. Three different experiments were performed to show that changes in mean capillary pressure or in plasma colloid osmotic pressure will affect the intracapsular pressure as predicted by Starling’s law of the capillaries. These experiments were (a) alteration of the capillary pressure by venous compression, (b) alteration of the capillary pressure by arterial compression, and (c) alteration of the plasma colloid osmotic pressure by intravenous injec-
Effect of Increased Venous Pressure on Capsule and Needle Pressures

Figure 3A illustrates the beginning of an experiment in which the venous pressure was suddenly elevated to 50 mm Hg. The method was the following: Needle and capsule pressures were recorded from the subcutaneous tissue of the lower leg of a dog, and venous pressure was recorded from a catheter in a vein in the same lower leg. A blood pressure cuff placed around the upper leg was suddenly inflated until the pressure in the vein rose to 50 mm Hg. The venous pressure was maintained at this level for the remainder of the experiment (a total duration of 13 hours) while both capsule and needle pressures were recorded continuously. Figure 3A shows the first 2½ hours of the recording. Immediately after elevating the pressure in the cuff, artifacts were present in both the capsule and needle recordings. However, within a few minutes the recordings became stable and thereafter recorded true pressures. The capsule pressure prior to inflating the cuff was approximately −7 mm Hg. This rose immediately and rapidly after inflating the cuff and continued to rise more and more slowly throughout the experiment. On the other hand, the needle pressure (as represented by the plateau levels of the upper recording) was +2.5 mm Hg immediately before inflating the cuff and rose to +2.7 mm Hg during the first half hour after inflating the cuff. Two hours later it had fallen to +2.2 mm Hg. Thus, while the capsule pressure was rising as a result of elevated venous pressure in the leg, the needle pressure actually fell.

Figure 3B illustrates the complete sequence of events throughout the 13 hours of this experiment. At the end of six hours the capsule pressure had risen to over +2 mm Hg and now equalled the needle pressure. From there...
Results of the entire 13-hour experiment, showing that after six hours the capsule pressure had risen to equal the needle pressure and that edema was apparent at this time. Further development of edema was associated with parallel increases in capsule and needle pressures.

The needle pressure rose in step with the capsule pressure. Also, at approximately the same time that capsule pressure first equaled needle pressure, frank pitting edema was first observed. These results indicate that the needle pressure and capsule pressure record essentially the same value once sufficient free fluid is available in the tissues, whereas, as long as the tissue spaces are essentially dry, the capsule indicates different values from the needle. This experiment showed also that the capsule pressure changed in accordance with Starling's law of the capillaries during the entire experiment, whereas it was only in edematous tissues that the needle pressure changed in a manner predicted by Starling's law of the capillaries. This same experiment was repeated seven times in different dogs with the same basic results.

Effect of Arterial Occlusion on Capsule Pressure

If the artery to a leg is compressed, capillary pressure should fall, and the colloid osmotic pressure of the plasma proteins should produce transfer of fluid out of the tissue spaces of the leg into the blood stream, thereby causing the interstitial fluid pressure to fall. In six experiments two-minute occlusion of the femoral artery reduced capsule pressure by an average of 1.4 mm Hg, which was approximately the same initial rate of change in capsule pressure in the opposite direction that occurred when the venous pressure was raised 10 to 20 mm Hg.

Effect on Capsule and Needle Pressures of (a) Dehydrating the Tissues by Intravenous Injection of Concentrated Dextran Solution and of (b) Rehydrating the Tissues by Intravenous Injection of Saline

Figure 4 illustrates capsule and needle pressure recordings, showing a control capsule pressure of $-4$ mm Hg and a control needle pressure (as represented by the plateaus) of $+2$ mm Hg. Three separate injections, 50 ml each, of 20% dextran solution were administered intravenously. The effect of each injection, theoretically, should have tended to cause (1) osmosis of fluid into the capillaries because of increased plasma colloid osmotic pressure and (2) filtration of fluid out of the capillaries because of increased blood volume. However, the dextran concentration was cal-
NEGATIVE INTERSTITIAL PRESSURE

Effects on capsule and needle pressures caused by saline injected 10 ml at a time into the lower leg of a dog. Three minutes were allowed between each injection.

...lated to be great enough and the volume small enough for the osmosis of fluid into the capillary to far outweigh the hydrostatic factor, thereby dehydrating the tissues. Following each of the injections, the capsule pressure fell to a lower level. This was true particularly with the third injection at the beginning of which the capsule pressure had already fallen to a level at which one would expect the tissues to be already almost totally dehydrated; removal of very slight additional fluid from the already dehydrated tissues as a result of osmosis into the circulatory system should cause an extreme fall in interstitial fluid pressure. The record illustrates such a fall from —6 to —13 mm Hg, a total fall of 7 mm Hg as a result of one 50-ml injection of concentrated dextran solution. Following the administration of dextran, isotonic saline was then injected in three separate quantities of 150 milliliters each. This should have increased the capillary hydrostatic pressure and decreased the plasma colloid osmotic pressure, both of which effects would be expected to cause fluid transudation into the tissues. The figure illustrates a rise in intracapsular pressure to a new plateau following each injection. All these effects on capsule pressure were in accordance with Starling's law of the capillaries, and they were repeated with different variations in eight experiments in four dogs. Note, however, that the needle pressure measurements failed to decrease during the dehydration phase of the experiment following the dextran injections; indeed, they actually rose. Also, they failed to change following the injections of saline. Therefore, in this experiment, the needle measurements again failed to obey the predictions of Starling's law of the capillaries. This is in contrast to the intracapsular pressure measurements which did obey the predictions.

EFFECT ON CAPSULE AND NEEDLE PRESSURES OF EDEMA CAUSED BY LOCAL INJECTION OF FLUID

Figure 5A illustrates the effect of progressive injection of saline on both needle and capsule pressure measured in the subcutaneous tissue of the lower leg. In this experiment, needle pressure was measured approximately 1 inch away from the capsule, and the saline was injected 1 inch away from both the needle and capsule. The fluid was injected in 10 ml increments and allowed 3 minutes after each increment for diffusion through the tissue spaces before the pressures were recorded. Note that the initial capsule pressure was —2 mm Hg, while the initial needle pressure was +1.5 mm Hg. As edema began to appear, the capsule pressure rose to equal the needle pressure, and the two pressures thereafter remained almost exactly equal as the edema
Effect of continuous intravenous infusion of Tyrode solution into a 14 kilogram dog over a period of approximately two hours. Approximately three-fourths of the animal's own weight in fluid was infused. Note the steady rise in capsular pressure during the infusion and the rapid decrease in capsular pressure following the end of the infusion.

became very severe. At the termination of the experiment, the diameter of the leg had been increased to 1.7 times normal, illustrating the extreme degree of edema caused in the experiment.

Figure 5B illustrates an experiment in which both needle and capsule pressures were measured in a leg made locally edematous. The leg was swollen to almost double normal diameter, saline having been injected half an hour earlier. It is particularly noteworthy that the pressure recorded by both the needle and capsule was only 4 mm Hg.

At this point the edematous leg was squeezed 2 inches away from the capsule and the needle, which increased the pressure throughout the edematous area and caused the capsule pressure to rise instantly to 15 mm Hg. At the same time, the needle pressure began to rise and 1 minute later reached the same pressure level as that recorded in the capsule. Two cycles of this experiment are illustrated in the figure. The results were repeated again and again, the capsule pressure always rising instantaneously when the pressure on the tissues was increased, and the needle pressure eventually reaching the elevated pressure even though it was slow to come to equilibrium.

These experiments on local edema were repeated in nine different animals, and the results were always the same. One especially notable feature common to all of the experiments was that after the edema had been present for as long as one-half to one hour, the diameter of the lower leg could be as large as 1.5 to 2 times normal (the volume of the leg much more than 2 times normal), thus representing extreme edema, and yet the pressure recorded from the capsule or from the needle would be only 3 to 5 mm Hg. This was illustrated by the control pressure levels in figure 5B in which the edema was of this degree.

GENERALIZED EDEMA CAUSED BY INTRAVENOUS INFUSION

Figure 6 illustrates a pressure recording from a capsule in the subcutaneous tissue of the abdomen during the infusion of Tyrode solution. The initial capsule pressure was −3.2 mm Hg. This rose progressively to +7.5 during 2½ hours of the experiment while Tyrode solution was infused at a rate of 4 liters per hour. At the end of this time, approximately ¼ of the animal’s own body weight of Tyrode solution had been infused. Most of the fluid had become ascites, but a reasonable proportion of it had appeared in the form of cutaneous edema.

In the earlier stages of the experiment, needle pressures were too erratic to be completely trustworthy. However, once the tissue spaces began to expand so that some extracapillary pressure had been reached, needle pressures were steady and illustrated the extreme degree of generalized edema.
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fluid was obviously present in the tissues, then
the needle pressures could be reproduced
within 1/5 mm Hg, and they varied from the
intracapsular pressure by no more than 1
mm Hg.

These experiments, with essentially identical
results, were repeated in seven animals. Needle
pressure measurements were erratic under
normal conditions, but once edema appeared,
they invariably became equal to the capsule
pressures and thereafter paralleled the cap-
sule pressures almost exactly despite as much
as 10 mm Hg change.

INTERSTITIAL PRESSURES IN EDEMA

In edema caused by excess interstitial fluid
volume, the relationship between pressure and
the degree of edema depended to a great ex-
tent on the duration of the edema. To correl-
ate the degree of edema with capsular and
needle pressure, the edema was graded by the
usual clinical method of subjective grading,
using the +1 to +4 scale. In the experiments
described above in which local edema was cre-
ated by rapidly injecting saline into the leg,
the relationships between pressure and degree
of edema during the first minute of the devel-
ooping phase of the edema were roughly the
following: +1 edema, 5 to 10 mm Hg; +2, 8
to 15 mm Hg; +3, 12 to 20 mm Hg; +4, 15
to 25 mm Hg. However, after the edema had
lasted for thirty minutes or more, and espe-
cially when the edema was in the resorbing
stage, the relationship between pressure and
degree of edema was more tightly fixed, and
correlated as follows: +1 edema, 1 to 4 mm Hg;
+2, 3 to 7 mm Hg; +3, 5 to 10 mm Hg; +4, 8 to 13 mm Hg.

Because this grading was purely subjective,
any statistical analysis that we might make
of the results would be valueless. Neverthe-
less, it was obvious that the greater the degree
of inflammatory edema, the greater was the
pressure.

EDEMA CAUSED BY INFLAMMATION

About 20 capsules became infected and the
surrounding tissues edematous following im-
plantation, and another 25 became infected
following repeated pressure measurements. In
some instances the capsules sloughed out, but
in others intramuscular administration of
streptomycin and penicillin caused healing.

Figure 7 illustrates the course of pressure
changes following infection of a capsule, with
subsequent recovery from the infection.

The pressure ranges in the capsules were
roughly the following for the different degrees
of edema: +1, 1 to 4 mm Hg; +2, 3 to 7 mm
Hg; +3, 5 to 10 mm Hg; +4, 8 to 13 mm Hg.

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any statistical analysis that we might make
of the results would be valueless. Neverthe-
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of inflammatory edema, the greater was the
pressure.

EFFECT OF TISSUE MOVEMENT ON
THE CAPSULE PRESSURE

During the course of the experiments, spon-
taneous movements often developed and af-
fected the pressure measurements. These were
1) restless movements, 2) deep and gasping
breathing, and 3) shivering. In all these in-
stances, the capsule pressure fell 1 to 2 mm
Hg to a new plateau over a period of 15 to 30
minutes after onset of the movements.

EXPERIMENTS TO DETERMINE WHETHER
CAPSULE PRESSURE EQUALS THE
SURROUNDING INTERSTITIAL
FLUID PRESSURE

Theoretically, it would be possible for the
fluid to be encapsulated inside the capsule in
such a manner that its pressure would be dif-
ferent from that in the surrounding inter-
stitial fluid spaces. In this case the trans-
capillary dynamics of the tissue lining the
capsule should determine the level of the
capsule pressure. On the other hand, a second
possibility is that fluid might flow so readily
between the capsule cavity and the surround-
ning tissue spaces that the pressure inside the
capsule would be more nearly a measure of the
surrounding interstitial fluid pressure. Our
initial concept was that the first of these mech-
anism was by far the more predominant, but
experiments to prove this showed the opposite
to be true.

Let us assume that the measured capsule
pressure is some algebraic average of the two
Pressures discussed above. If this be true, the following formula will hold:

\[ \text{Measured pressure} = \text{XI} + \text{YE} \]

in which I equals the pressure that would be caused by transcapillary dynamics within the capsule assuming there is no exchange between the capsular cavity and the surrounding tissue spaces, X is the fraction of the measured pressure caused by these transcapillary dynamics, E is the extracapsular interstitial fluid pressure, and Y is the fraction of the measured pressure caused by this pressure.

Using this equation, we can roughly estimate from the following experiments the degree to which the measured capsular pressure approximates each of the two pressures discussed above.

**Movement of Evans Blue Out of the Cavity into the Surrounding Tissues**

Approximately 0.1 ml of concentrated Evans blue dye solution was injected into each of three capsules, and movement of the dye into the surrounding tissue spaces was studied. A slight pressure, about 10 mm Hg, was applied intracapsularly through a needle and maintained for one hour. At the end of this time the capsules were excised. In each instance the dye had moved into all areas of the intracapsular tissue and had appeared in the tissues outside the capsule adjacent to the holes. Thus, it was shown that fluid could flow through the tissue spaces from the cavity of the capsule into the surrounding tissues.

Since Evans blue dye combines with tissue fluid proteins, it is reasonable also to suppose that proteins could move through the same tissue spaces.

These initial experiments did not shed any light on the relative importance of X and Y in the above equation, but they did show that fluid flow between the capsule and interstitial spaces undoubtedly played at least some part in determining the measured intracapsular pressure.

**Reequilibration of Intracapsular Pressures in Dead Dogs**

In six separate experiments the rates of reequilibration of the intracapsular pressure after injection of a small amount of fluid into or removal from the capsule was measured in a live dog and then again in the same dog after it was sacrificed. For a period of at least an hour following death, the rates of reequilibration were so nearly the same as before death that we could not discern a difference within the limits of experimental error. This was the effect to be expected if the capsular pressure were determined by extracapsular interstitial fluid pressure but not the effect to be expected if intracapsular capillary dynamics determined the pressure, for, if blood were not flowing through the vessels in the capsule, any significant amount of fluid transfer across the capillary membrane should have altered the capillary colloid osmotic pressure so much that the rates of reequilibration should have changed. These experiments, therefore, indicated that Y in the above formula approached unity while X approached zero.

**Effect of Intracapsular Protein Concentration on the Intracapsular Pressure**

One-tenth ml of capsular fluid was removed from each of two 3-cm capsules, and the protein concentrations were measured. These aver-
aged 1.96 grams per cent (1.93 and 1.99 respectively). Following this, the fluid in the capsule cavity was washed out with plasma that had been obtained under sterile conditions from the same animal. Following this exchange, the total protein in the two capsules had risen to an average of 3.6 grams per cent. Ten minutes were allowed for the pressures in the capsules to reequilibrate. In neither instance had the equilibration pressure changed from the control values within the limits of measurement of the method.

These experiments were repeated six additional times in smaller capsules from which it was not possible to obtain sufficient initial intracapsular fluid to make satisfactory protein measurements but in which it was possible to wash out more thoroughly the capsular cavities with plasma, increasing the intracapsular protein concentration up to an average value of 5.0 grams per cent. In these experiments, there was also no measurable rise in intracapsular pressure.

Our first thought regarding the failure of the increased protein to increase intracapsular pressure was that the protein might not have had time to penetrate the intracapsular tissue enough to affect local capillary dynamics. Therefore, the pressure measurements were continued for a period of two to three hours in each experiment, and still no pressure rise occurred. Also, in one dog the protein concentrations were measured four times intermittently for a period of a month. At the end of one month, the protein concentration was still 4.1 grams per cent, and the recorded pressure was —6 mm Hg. Thus, in these experiments we were not able to find a measurable rise in intracapsular pressure.

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Effect of Elevated Venous Pressure on the Course of Intracapsular Pressure Changes

If intracapsular capillary dynamics were the main determinant of intracapsular pressure, a sudden rise in capillary pressure should cause the intracapsular pressure to rise and approach asymptotically a level equal to the control pressure plus the rise in capillary pressure. Furthermore, the rate of fluid transfer through the capillary membrane at any given instant should be proportional to the gradient between the actual pressure in the capsule and the asymptotic pressure level. The reason for this is that the plastic capsule is a rigid structure with a modulus of volume elasticity that remains relatively constant within the pressure ranges recorded in these experiments.

On the other hand, if the capsule pressure is determined mainly by the pressure in the extracapsular interstitial fluid spaces, then the modulus of volume elasticity of these spaces rather than the modulus of elasticity of the capsule should govern the rate of pressure rise in the capsule. The dehydration studies using dextran and overhydration studies caused by saline infusion described earlier in this paper demonstrated that the extracapsular fluid spaces definitely do not have a constant modulus of volume elasticity but instead have a very high modulus in the negative tissue pressure range and a very low modulus in the positive tissue pressure range. This should cause the pressure curve to rise very rapidly when the tissue pressure is in the negative range but extremely slowly when in the positive range.

Referring back to figure 3, we see that a sudden rise in venous pressure of 50 mm Hg raised capsule pressure very rapidly in the negative pressure range and then very slowly indeed in the positive pressure range. Furthermore, when free fluid was present in the tissues the intracapsular pressure measured exactly the same as that measured by a needle in the surrounding tissue spaces. If intracapsular capillary dynamics were determining the capular pressure, the intracapsular pressure should have risen to values far greater than the surrounding tissue pressure, but it did not.

In this particular experiment let us assume that the capillary pressure rose three-fourths as much as the venous pressure, or 37.5 mm Hg.
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The rate of rise of intracapsular pressure, if it were determined by intracapsular transcapillary dynamics, should have been only 1.4 times as rapid when the intracapsular pressure was —7 mm Hg as when it was +4 mm Hg. Instead, the actual difference in these two rates was 186 times, which is a very large difference. If we assume that the extracapsular tissue pressure remained constant during the experiments, the value of Y in this instance amounts by calculation to about 59% and the value of X to about 1%. However, since the extracapsular pressure was rising, the value of Y would lie between the limits of 99% and 100%, and the value of X would lie between the limits of 1% and 0%.

**Rapidity of Equilibration of Intracapsular Pressure Under Different Conditions**

When extensive extracellular fluid edema is present, the pressure measured by a needle in the tissues is equal to the pressure in the capsule, as has been discussed at several points earlier in the paper. Furthermore, as shown in figure 2B a sudden increase in pressure on the outside of the capsule causes a very rapid rise in pressure on the inside, having a half-time of only 3 to 4 seconds. This is the effect that one would expect if fluid should flow very readily between the extracapsular interstitial spaces and the intracapsular space. If the interstitial spaces become less edematous, the rapidity of transfer between the extracapsular spaces and capsule should become progressively reduced because of increased resistance to fluid flow through the spaces. The slowness of reequilibration (half-time of 1 or more minutes) when a minute amount of fluid is injected into or removed from a capsule located in non-edematous tissue, which was discussed earlier, fits with this prediction.

On the other hand, if fluid flow through the tissue spaces played no role in reequilibration of intracapsular pressure, reequilibration should occur only after a reasonable quantity of fluid had moved across the capillary membranes within the capsule. If we assume that the rate of fluid transfer across the capillary membranes remains relatively constant for a given pressure differential, as seems to have been true in Pappenheimer’s studies,11 the half-time of reequilibration should likewise change very little in different pressure ranges, which was not the effect observed. Thus, here again one finds that the value of Y approaches unity while the value of X approaches zero.

**Summary of Experiments to Determine What Pressure is Measured by the Capsule**

Though all the experiments we have done so far indicate that the measured capsule pressure is probably determined mainly by extracapsular interstitial fluid pressure, undoubtedly intracapsular capillary dynamics also play a at least some role. Therefore, our failure to find any positive value for X in the above equation was probably caused by experimental methods not sensitive enough to estimate the small value. At any rate, the results are consistent with the idea that the rate of exchange of fluid between the capsule and the extracapsular tissue spaces for a given pressure gradient is far greater than the rate of exchange through the intracapsular capillary membrane.

**Discussion**

What is the Significance of the Capsule Pressure? All capsule pressures measured in uninfected capsules that had been implanted at least 4 weeks were negative, averaging —6.4 mm Hg. On the other hand, in more than 20 experiments on progressive edema the capsule pressure always became positive whenever edema developed, and the pressure rose in direct proportion to the degree of edema. When the tissues were edematous, the pressures were the same, within the limits of error of measurement, when measured by either the needle technique or the capsule, but in non-edematous tissue, the needle measurements were erratic and usually positive in contrast to the negative pressures recorded in the capsules.

A most important question to be answered is: which more nearly measures the true interstitial fluid pressure—the needle or the capsule? The diameters of the needles (500×)
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are 500 to 900 times as great as the widths of the largest interstitial spaces so that insertion of the needle into the tissue would be expected to distort the spaces very severely and cause rupture of local blood vessels. Indeed, Walls found in his needle pressure studies that red blood cells were always found in the fluid in the needle tip upon removal from the tissue. Therefore, it is doubtful that the pressure measured by the needle is a true measure of the pressure of the interstitial spaces, which is a conclusion reached long ago by many others (reviewed in detail by Rusznjak, Foldi, and Szabo).

Unfortunately, needle pressure measurements in the interstitial spaces without distorting the tissues and without rupturing the tissues have never been made. The only close approach to this was a series of studies in mice made by McMaster in which he studied normal and edematous tissues using a #30 platinum needle inserted subcutaneously under observation with a microscope to prevent rupture of vessels. McMaster found that fluid was intermittently drawn into normal tissues from the needle but in edematous tissues flowed in the opposite direction. Though he observed only fluid movement and did not measure pressures, this demonstration of fluid absorption into normal subcutaneous tissue does indicate that the interstitial pressures could easily be negative rather than positive.

Implantation of a capsule is followed at first by inflammation around the capsule, and the pressure measured in the capsule for the first week to ten days following implantation is approximately the same as that measured in the needle. Thereafter, as the inflammation recedes, the capsule pressure becomes negative. Is this negative pressure the result of some tissue reaction that causes "exudation" to be applied to the fluid in the cavity, or is the fluid in the cavity in reasonable communication with the fluid surrounding the capsule so that the capsule pressure is a measure of the surrounding interstitial fluid pressure? In the present experiments several separate types of evidence showed that the capsule cavity is in communication with the fluid surrounding the capsule and that the intracapsular pressure is probably determined mainly by the extravascular interstitial fluid pressure and only slightly by intracapsular transcapillary dynamics. This evidence need not be repeated here, but, on the basis of the findings, we believe the pressure inside the capsule to be a reasonably true measure of the surrounding interstitial fluid pressure.

Perhaps most important of all the experiments supporting the concept that the capsular pressure is a measure of interstitial fluid pressure were the several different demonstrations that intracapsular pressure obeys Starling's law of the capillaries. To the best of our knowledge, no other measured tissue pressure in non-edematous tissue has ever been shown to do this. Certainly, pressures measured by a needle do not fulfill this criterion, which was demonstrated repeatedly in these studies.

Another consideration that adds circumstantial support to the concept of negative interstitial fluid pressures in normal tissues is that pressure is negative in almost all tissue fluid cavities of the body. For instance, the intrapleural space, the pericardial space (E. H. Wood, personal communication), and the joint cavities all have negative pressure. Especially interesting are the joint cavities, for their linings are only an aggregation of normal connective tissue elements, forming no impervious membrane. Therefore, it must be assumed that the fluids of the joint cavities are continuous with the fluids of the surrounding tissues; if this is so, one would suspect the pressure in the cavities to be a measure also of the pressure in the surrounding interstitial spaces.

Also, in the pulmonary circulatory system, it is almost certain that negative pressure exists in the interstitial spaces between the capillaries and alveolar membranes. Experiments have shown that progressive elevation of the capillary pressure from its normal value of approximately 5 mm Hg up to values of approximately 24 mm Hg causes no transu-
ulation of fluid out of the capillaries into the pulmonary tissue spaces.17-21 Yet, when the pulmonary capillary pressure rises above the critical value of 24 mm Hg, fluid transudation occurs in direct proportion to the additional rise in capillary pressure. Thus, there is a safety factor of approximately 19 mm Hg. Because this is the result of osmotic force developed at the capillary membrane, the 19 mm Hg presumably represent the amount of negative pressure in the space between the capillary and alveolar membrane. It is believed that this negative pressure removes fluid from the alveoli, thus keeping the alveoli "dry.",21-22

It is not meant to imply in this paper that negative interstitial pressure necessarily exists in all normal tissues of the body. For instance, encapsulated organs, such as the kidney or eye, or tissues in firm cavities, such as the central nervous system, certainly have different fluid dynamics from those in loose tissue. Particularly surprising were the negative capsule pressures that we measured in the muscles, which lie in tight sheaths. The capsules implanted in the muscles tended to work their way to the edge of the muscle and to lie against the sheath, making it doubtful whether we were truly measuring intramuscular pressure. Yet, they were still in the sheath, showing that negative pressures can develop even in this compartment.

**Mechanism of Negative Interstitial Pressure.** Let us make two initial assumptions: first, that the average capillary pressure throughout the tissues is considerably lower than the plasma colloid osmotic pressure (this will be discussed later); second, that there is continual movement in almost all tissues caused either by gross movement of the body or by local intratissue movements resulting from (a) pulsations of the arteries, (b) contraction of contractile cells in the tissues or vessels, etc. From these assumptions, which are not unreasonable, we can surmise that interstitial pressures could be negative as follows: Because plasma colloid osmotic pressure is higher than capillary pressure, the sum of these two forces would be a net force tending to cause movement of fluid into the capillary. If this force should be greater than the tissue colloid osmotic pressure, the interstitial fluid pressure would have to be negative to balance all the forces. Therefore, to make this mechanism work, all we would have to explain would be some means by which the proteins that normally leak into the interstitial spaces could be removed. To explain this, we could invoke the lymphatic pump mechanism. That is, tissue movement could cause intermittent positive pressure (even though the mean pressure remained negative) in local tissue areas or in the lymphatics, thereby continually forcing the proteins out of the interstitial spaces and thus keeping the protein concentration in the interstitial fluid at a low value.

In support of this mechanism are the following observations: 1) At the onset of increased tissue movement, such as at the onset of heavy breathing or shivering, the pressure in a capsule located in the moving tissue usually became a mm Hg or so more negative within 10 to 30 minutes. 2) Parsons and McMaster observed in 1938 that arterial pulsation promotes lymphatic flow, indeed that almost no flow occurs in the absence of the pulsation,22 and this result was supported by Cressman and Blalock's23 demonstration that arterial pressure pulsations are transmitted into the lymphatics. Therefore, gross body movements might not be necessary to keep the lymphatic pump operative. 3) Blocker and coworkers have recently observed that pressures measured in the lymphatics of normal limbs are always slightly negative, indicating that the lymphatics are capable of removing fluid from the tissues even in the presence of a negative pressure.24 Allen has also demonstrated negative lymphatic pressure under some conditions.25

One of the assumptions made above in explaining this possible mechanism for the development of negative interstitial fluid pressure was that the mean capillary pressure is considerably lower than the plasma colloid osmotic pressure. Yet, most definitive studies
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on this proposition, particularly those by Landis, have shown only slight differences between the plasma colloid osmotic pressure and the capillary pressure. However, two newer studies indicate that the disparity between plasma colloid osmotic pressure and capillary pressure could be much greater than has been realized. First, several studies of the capillary circulation have demonstrated that most capillaries have a pre-capillary sphincter at their arterial ends and that under resting conditions of the tissue this sphincter remains completely closed much more than half of the time. If this should be the general case throughout most of the tissues of the body, then the mean pressure in the tissue capillaries should be considerably closer to the pressure in the venous ends of the capillaries (averaging 12 mm Hg in Landis' studies in human skin) than to that in the arterial ends (averaging 32 mm Hg). This would cause a low mean capillary pressure which in turn would favor development of negative interstitial pressures. Second, Hansen has recently suggested, on the basis of in vitro osmometer studies, that whole blood has a far greater colloid osmotic pressure than does plasma. This effect, if true, could help account for the difference between plasma colloid osmotic pressure and capillary pressure. However, this effect would not be necessary, because a low capillary pressure by itself could easily account for sufficient difference. Putting all of these considerations together, it is not unreasonable to suggest that we might have an adequate mechanism for the development of negative interstitial fluid pressures at least in many tissues of the body.

Summary

Over 200 perforated plastic capsules were implanted in different tissues of the dog, and the wounds were allowed to heal. After one month, pressures measured by inserting a needle through the skin and then through a perforation of the capsule into its cavity were always negative in normal tissues, averaging -6.4 mm Hg. The pressure was always positive in edematous tissues. Evidence is presented to indicate that the pressure measured in the capsule is equal to, or nearly equal to, the interstitial fluid pressure in the tissue spaces surrounding the capsule. On the other hand, pressure measurements made by a needle technique failed except in rare instances to give negative values in normal tissues but in edematous tissues gave almost exactly the same values as those recorded by the capsules. Also, pressures measured by the capsules changed in accordance with Starling's law of the capillaries when (a) venous pressure was raised, (b) when arterial pressure was lowered, (c) when the tissues were dehydrated by intravenous infusion of dextran, or (d) when the tissues were hydrated by intravenous infusion of saline. Pressures measured by a needle technique failed to change in accordance with this law in these same experiments. Therefore, it is concluded that needle pressure determinations in non-edematous tissue do not measure the interstitial fluid pressure.

References

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