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EFFECTIVE OSMOTIC PRESSURE OF THE PLASMA PROTEINS AND OTHER QUANTITIES ASSOCIATED WITH THE CAPILLARY CIRCULATION IN THE HINDLIMBS OF CATS AND DOGS

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The principal factors regulating the exchange of fluid between capillaries and tissue spaces were outlined by Starling (1) following his experiments on the absorption of fluid from the connective tissue spaces. Starling's experiments, performed on perfused hindlimbs of dogs, gave rise to one of the most widely recognized hypotheses in physiology and medicine. According to the 'Starling Hypothesis', the direction and rate of fluid transfer between plasma and tissue fluids are determined by three factors; a) the hydrostatic pressures on each side of the capillary membranes, b) the protein osmotic pressures of plasma and tissue fluids acting across the capillary membranes, and c) the physical properties of the capillary membranes considered as mechanical filters.

Convincing quantitative evidence in support of the Starling theory was obtained by Landis (2) from direct measurements of the hydrostatic pressure and rates of fluid movement in individual capillaries of the frog's mesentery. In this preparation the rate of fluid movement across the capillary membrane is, on the average, proportional to the difference between the mean hydrostatic pressure in the capillary and the protein osmotic pressure of the plasma as measured in vitro. This behavior represents a special case of the Starling theory in which tissue pressure opposing filtration is negligible and the capillary membranes are almost completely impermeable to the plasma proteins; it implies that the protein concentration in tissue fluid immediately outside the capillary wall is too low to play a significant role in determining the osmotic balance.

No such direct measurements have been made in mammalian capillaries. Circumstantial evidence, reviewed by Landis (3), has in general lent support to the

1 Fellow of the Rockefeller Foundation.

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Starling theory, but the quantitative relations between capillary pressure, effective protein osmotic pressure and rate of fluid exchange have never been clearly demonstrated in the mammalian circulation. In the present paper we shall describe methods for measuring these variables in the isolated perfused hindlimbs of cats and dogs. Quantitative evidence will be given that the Starling theory is in fact applicable to the perfused hindlimb and with a precision rarely encountered in biological preparations.

METHODS

A. GENERAL. The hindlimbs of cats or dogs were amputated and suspended from a sensitive recording balance. They were supplied with blood from a pump-lung circulation under conditions such that the arterial perfusion pressure, the venous pressure and the protein osmotic pressure of the plasma could be independently adjusted to desired constant values. The rate of filtration of fluid from blood to tissues was recorded as the rate of gain of weight, a technique which has been employed in several previous investigations (4-6); conversely, absorption of fluid from tissues to blood was recorded as a loss of weight. The blood flow was measured and recorded continuously. From these quantities it is possible, by the methods described below, to determine the mean hydrostatic pressure in the capillaries, the effective osmotic pressure of the plasma proteins, the filtration coefficient of the capillary membranes and certain other quantities important to the fluid exchange.

B. DETAIL. 1. Perfusion. The technique of perfusion was similar to that described by Whittaker and Winton (7). A diagram of the perfusion circuit is shown in figure 1.

2. Preparation of blood for perfusion. Three hundred to 500 cc. of perfusion fluid were used for each experiment. The blood was drawn from the carotid arteries of one or more animals not more than three days prior to the experiment. For experiments with cats the blood was heparinized; with dogs the blood was usually defibrinated. When a high protein osmotic pressure was required, the plasma was separated from the cells and placed in cellophane bags in front of a fan. Loss of water by evaporation from the surface of the cellophane occurred at a rate sufficient to double the concentration of protein in about four hours. The bags were then sealed close to the liquid level and the concentrated plasma dialyzed against cold Ringer's solution (0.90% NaCl, 0.042% KCl, 0.024% CaCl₂, 0.020% NaHCO₃) until ionic equilibrium was established as indicated by the electrical conductivity. The pooled plasma concentrates from several cats were required for experiments involving high protein pressures. Low protein pressures were obtained by diluting whole blood with Ringer's solution. It was noted that the protein osmotic pressure of plasma obtained by 'bleeding out' from the carotid arteries was considerably less than normal, the average pressure being 15 mm. Hg. Presumably this was the result of dilution of the plasma with tissue fluid during the bleeding process.

The corpuscular concentration was adjusted to desired levels by the addition of red cells to the previously prepared plasma. The whole fluid was filtered
through glass wool and two layers of linen cloth before admission to the perfusion reservoir.

Fig. 1. Diagram of the perfusion circuit and weight-recording system. The pump-lung-hindlimb circulation is similar to that employed by Whittaker and Winton (7). The leg is mounted horizontally on the balance with the ventral surface facing upwards. The balance beam axle rotates on ball bearings. The beam is loaded with a spring which lengthens 0.21 mm. per gram; optical and electrical amplification of this displacement allow full scale deflection (50 mm.) of the recording oscillograph for a change of one gram on the balance. Temperature of blood in the arterial cannula is measured by thermometer (T) and controlled by heating coil (H).

3. Operative procedures. All experimental animals were anesthetized with nembutal (40 mgm. per kgm.). The right hindleg from a small dog (5 to 6 kgm.) or a large cat (2.8 to 4 kgm.) was separated from the body at the hip, all visible blood vessels being ligatured except the femoral artery and vein. Small bleeding
points were seared with a cautery. The operation required one and one-half to two hours. The femoral vein was cleared of connective tissue as far down as the saphenous branch; this was necessary to prevent compression of the vein from the weight of unsupported muscles pulling on the tissue immediately adjacent to the vein.

During this operation the lungs were removed from a second animal and the pump-lung circulation started about one-half hour before the preparation of the leg for perfusion was complete. Arterial and venous perfusion cannulae of sizes appropriate to the individual vessels were fitted to the perfusion apparatus and calibrated as described in section 4 below. The femoral artery and vein were then cut and the leg transferred to the perfusion apparatus; transfer from the natural to the artificial circulation required about two minutes.

The circulation through the leg prepared in this way was such that in the first hours of perfusion all the perfusing blood left the leg via the femoral vein. There was no detectable drainage from the lymphatics. Towards the end of each experiment, however, a small amount of blood (less than two per cent of total flow) left the leg by oozing from small vessels which had been cut during the operation. The protein content and corpuscular concentration of this blood were indistinguishable from those of venous blood in the two instances in which these quantities were compared. When dye was injected into the arterial perfusion cannula the whole leg rapidly became colored up to the cut surfaces of the skin and muscle.

4. Pressures. The mean arterial and venous pressures were determined with mercury and blood manometers, respectively. The manometers were connected to the perfusion circuit at some distance from the arterial and venous perfusion cannulae so that it was necessary to correct for the pressure drop across the tubing and cannulae in order to obtain the true pressure in the artery and vein at each flow rate. Since a change of 0.5 mm. Hg in mean capillary pressure produced an easily detectable change in rate of fluid movement, it was necessary to devote considerable attention to these corrections. The pressure-flow characteristics of each cannula were obtained before and after each experiment by substituting a short length of wide bore tubing for the leg and determining the pressure difference across the cannulae at various flow rates in the range used during the experiment. Pressure-flow diagrams were then constructed for each cannula and the appropriate corrections for each flow rate during the experiment derived from the smoothed curves. The true zero of each manometer was determined by extrapolating the indicated pressure-flow diagram to zero flow. All pressures are referred to the level of a horizontal plane bisecting the leg. The uncertainty in determining this level, and hence the absolute value of all hydrostatic pressures, was about ±0.5 mm. Hg.

5. Blood flow. The rate of venous outflow was measured at intervals by a stopwatch and measuring cylinder; it was also recorded simultaneously by a Gaddum (8) outflow recorder.

6. Weight. The center of the balance beam was fitted with a steel axle which rotated on ball bearings. One arm of the balance was loaded with a helical spring.
which lengthened 0.21 mm. per gram. The main weight of the limb was balanced with calibrating weights, as indicated in figure 1, and changes in weight were recorded from the small off-balance excursions of the spring-loaded arm. The excursions were amplified approximately 100-fold and were recorded on the kymograph as follows: one arm of the balance was provided with a shutter which interrupted light focussed on a photo tube (Cetron CE-2). The output of the photo tube was led through an impedance changer (6SJ7) and transconductance stage (6AG7) to a Weston Model 30 moving coil relay modified as described by Winton (9) to operate as a recording oscillograph. Stabilizing devices were employed for all voltage supplies so that at equilibrium temperatures the overall drift in the recording system corresponded to a change of less than two mgm. per minute on the balance. The sensitivity of the system was controlled electrically and was generally operated so that one gram on the balance produced a deflection of 20 to 30 mm. on the kymograph. The deflection of the recorder was linearly related to the weight and was calibrated at intervals during the experiment by adding gram weights to the balance. The rubber tubing which connected the perfusion cannulae on the balance to the perfusion system did not interfere with the measurements, owing to the small displacement of the balance (0.4 mm.) required for full scale deflection of the recorder. The leg itself lay horizontally with the flat dorsal surface supported by a malleable coarse mesh wire screen which was attached to the balance arm by means of three chains of adjustable length.

7. Temperature control. Blood in the arterial cannula was maintained at 37.5°C. at all flows by means of a heater coil (H, fig. 1) operated from a manually controlled variable transformer.

8. pH. The pH of plasma or of protein solutions was measured with a glass electrode (Beckman).

9. Protein osmotic pressure. In vitro measurements of protein osmotic pressure were made with a Hepp osmometer (10) as modified by Brown (11). In our hands multiple determinations of osmotic pressure performed on samples of the same protein concentration agreed within a standard deviation of ±0.7 mm. Hg. The absolute values of protein osmotic pressure found at any given protein concentration and pH agreed with the values calculated from the data of Scatchard, Batchelder and Brown (12, 13) as indicated in table 1. Calculation of the molecular weight of albumin from the observed osmotic pressures of bovine albumin lead to a value of 69,000, which is in close agreement with values obtained by independent methods (14). The measurements were made against 0.15 molar NaCl at room temperature with the plasma or protein samples in equilibrium with ambient air. Since the samples in vivo were equilibrated with a pCO₂ of 40–45 mm. Hg, the pH was higher in the osmometer than in the capillary circulation. The effects of a change in pH from 7.2 to 7.7 may be predicted from the equation of table 1; they were too small to be detected with our apparatus and are not considered in the final figures. However, all data were corrected to 37°C., the observed values being multiplied by the factor 310 ÷ (273 + t°C.). The actual temperature in the capillaries during the perfusion was less than 37°C. by
an amount which varied with the rate of blood flow; an error not exceeding +2 per cent may arise from neglect of this factor.

EXPERIMENTAL

1. The effects of arterial pressure on the limb weight. Figure 2 shows the effects of variations in arterial pressure on the limb weight and blood flow, the venous pressure and the protein pressure remaining constant. The arterial pressure was initially set to maintain the limb at constant weight. A sudden rise from 91 to 110 mm. Hg produced an initial increment of weight followed by a slow steady gain in weight. Presumably the initial rise is a result of an increased vascular volume beyond the arterial system, for it is abolished if the arterioles are constricted with adrenaline. Nor is it present if the capillary pressure is maintained constant by simultaneous lowering of the venous pressure as shown in figure 4. The slow steady gain in weight (+0.26 grams/min.) is presumably a result of filtration of fluid from blood to tissues. When the arterial pressure was restored to its initial value, the vascular volume was also restored, following which the leg weight remained constant at a new level corresponding to the amount of fluid which had been filtered. The arterial pressure was then lowered below the value necessary to maintain constant weight. Exactly analogous changes of weight resulted except that they were of opposite sign. The slow steady loss of weight

TABLE 1. COMPARISON OF PROTEIN CONCENTRATION WITH PROTEIN OSMOTIC PRESSURE

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>PROTEIN CONCENTRATION</th>
<th>PROTEIN OSMOTIC PRESSURE, MM. Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cat plasma</td>
<td>4.8</td>
<td>13.6 ± .7</td>
</tr>
<tr>
<td>2. Bovine albumin pH 5.7</td>
<td>4.1</td>
<td>14.7 ± 2.1</td>
</tr>
<tr>
<td>3. Cat plasma</td>
<td>5.2</td>
<td>16.2 ± .6</td>
</tr>
<tr>
<td>4. Cat plasma</td>
<td>6.0</td>
<td>20.8 ± .8</td>
</tr>
<tr>
<td>5. Cat plasma + bovine albumin</td>
<td>8.7</td>
<td>45.3 ± .8</td>
</tr>
</tbody>
</table>

1 Calculated from protein nitrogen with exception of sample 2 which was estimated from the density. We are indebted to Dr. Hegsted and members of the Department of Nutrition, Harvard Medical School, for performing the Kjeldahl analyses.

2 Calculated from the equation of Brown, Batchelder and Scatchard (12) as modified by Scatchard (15):

$$
\pi = \frac{288(1 - 0.64g)c}{1 - (0.4 + 0.9pH)c}
$$

where $\pi =$ protein osmotic pressure, mm. Hg; $c =$ protein concentration, g./cc; $g =$ ratio of globulin to total protein. We assumed that in normal cat plasma $g = 0.42$, corresponding to an $A:G$ ratio of 1.4. Electrophoretic studies of Deutsch and Goodloe (16) indicate that the actual $A:G$ ratio in cat plasma is 0.7. However, a large proportion of the globulin fractions are proteins of low molecular weight which produce the osmotic equivalent of a higher $A:G$ ratio. Kjeldahl analyses of the above samples after precipitation with Na$_2$SO$_4$ yielded ‘apparent $A:G$ ratios’ of 1.3-2.0.
Fig. 2. The effects of arterial pressure on limb weight. A rise of arterial pressure above the isogravimetric value (91 mm. Hg) produced a sudden increase in limb weight (increased vascular volume) followed by a slow rate of gain of weight of +0.26 gram per minute (filtration rate). Exactly analogous changes, but of opposite sign, followed a fall in arterial pressure below the isogravimetric value (absorption rate -0.28 gram per minute). The time required to complete changes of vascular volume is variable and must be considered in determining the final slope. Slight alterations in venous pressure occurred owing to the changed pressure drop across the venous cannula at each different rate of blood flow; corrections for this variation are made as described in text. The arterial pressure scale is corrected for the pressure drop across the arterial cannula.

Fig. 3. The effects of venous pressure on limb weight. A rise of venous pressure produced an initial small increment in limb weight (vascular volume) followed by a slow sustained rate of gain of weight of +0.17 gram per minute (filtration rate). Exactly analogous changes, but of opposite sign, followed a fall in venous pressure (absorption rate -0.21 gram per minute). The arterial and protein osmotic pressures remained constant. The time required to complete changes of vascular volume is variable and must be considered in determining the final slope. Note that the alterations in venous pressure, although small, produced detectable and reversible changes of blood flow as well as easily measurable rates of fluid transfer.

Fig. 4. The isogravimetric state. This record illustrates the method of obtaining isogravimetric values for arterial pressure and blood flow. The tendency for each stepwise decrement in arterial pressure to cause absorption (as in fig. 2) is compensated for by increasing the venous pressure until the limb weight remains constant. In order to maintain the weight as constant as illustrated it was necessary to adjust the venous pressure within 0.3 mm. Hg and the arterial pressure within 2 mm. Hg. The isogravimetric capillary pressure and postcapillary resistance to blood flow are determined from the isogravimetric blood flow and the isogravimetric venous pressure as illustrated in figure 5. Indicated pressures must be corrected for the pressure drop across the cannulae at each rate of blood flow. Note that there are no changes of vascular volume such as those found with an uncompensated change of either arterial or venous pressures (figs. 2 and 3). This suggests that the principal site of change in vascular volume is the capillary bed; alternatively, changes in volume on the arterial side of the capillary circulation must be exactly counterbalanced by the changes in volume on the venous side of the capillary circulation. (-0.28 grams/min.) is presumably a measure of the rate of absorption of fluid from tissues to blood.
DISCUSSION

In the intact animal changes of arterial pressure are generally associated with complex changes in mean capillary pressure owing to simultaneous alterations in vascular tone. It is therefore not surprising that in the whole animal a rise of arterial pressure may cause filtration (17), absorption (18) or no detectable change in fluid balance (19). The results illustrated in figure 2 and in the remaining sections of this paper clearly demonstrate the quantitative relations between arterial pressure and rate of filtration or absorption when all other known factors are maintained constant.

2. The effects of venous pressure on the limb weight. Figure 3 shows the effects of variations in venous pressure on the limb weight, the arterial and protein pressures being maintained constant. The effects are similar to those produced by arterial pressure except that the changes of venous pressure required to produce comparable rates of filtration or absorption are one-fifth to one-tenth as great (compare fig. 3 with fig. 2). Thus an easily measurable rate of filtration is brought about by a rise of 4 mm. Hg in the venous pressure and a detectable effect results from a change of 0.5 mm. Hg or less.

3. The ‘isogravimetric state.’ It will be clear from paragraphs 1 and 2 above that there exist an infinite number of pairs of values of arterial and venous pressures at which the leg will remain at constant weight. Thus the tendency to absorb, caused by a given reduction in arterial pressure, can be counterbalanced by raising the venous pressure until no net transfer of fluid occurs between blood and tissue (constant weight). Such an experiment, in which seven pairs of ‘isogravimetric’ arterial and venous pressures were obtained, is illustrated in figure 4. It is evident that if the arterial pressure is progressively lowered and the venous pressure raised to maintain constant weight there will come a point at which the venous pressure will equal the arterial pressure. Under these conditions there will be no pressure drop along the vascular tree and both the arterial and venous pressures will equal the capillary pressure. Since, by the terms of the experiment, there is no net transfer of fluid, this value of capillary pressure (isogravimetric capillary pressure) is equal and opposite to the sum of all pressures opposing filtration.

As pointed out by Green (20) the mean capillary pressure will be slightly less in the upper (ventral) half of the preparation than in the lower half. There will therefore be some transfer of fluid in the isogravimetric state even when the arterial and venous pressures are nominally equal. The average thickness of the hindlimb is about 2.5 cm. The mean capillary pressure in the top half is therefore about 0.5 mm. Hg less than in a horizontal plane bisecting the leg. This difference is by no means trivial since a change of 0.5 mm. Hg in mean capillary pressure regularly produces a detectable change in rate of fluid exchange in this preparation (section 2, fig. 9, table 3). In the isogravimetric state we therefore suppose that absorption occurs in the upper half of the preparation at a net rate of about 6 mgm. per minute per 100 grams tissue, while an equal rate of filtration takes place in the lower half even when arterial and venous pressures are nominally equal.

The isogravimetric capillary pressure cannot be obtained directly by equalizing arterial and venous pressures without disturbing the physiological properties of the capillaries because, when the arterial and venous pressures are equal, there is
FIG. 5. THE DETERMINATION OF ISOGRAVIMETRIC QUANTITIES. Each point on the graph was obtained as illustrated in figure 4—the blood flow was altered by varying the arterial pressure and the venous pressure adjusted to maintain constant weight. At zero flow the pressure is everywhere equal along the vascular tree and, by the terms of the experiment, this pressure is equal and opposite to the sum of all pressures opposing filtration. At all finite values of blood flow the pressure at the arterial end of the capillary bed is greater than at the venous end; however, no net transfer of fluid occurs and the mean capillary pressure is presumably the same at all blood flows. The resistance to blood flow from the effective midpoint of the capillaries to the vein is, by definition, equal to the slope of the observed line; in contrast to precapillary resistance it is independent of rate of flow.

FIG. 6. THE RESISTANCE TO BLOOD FLOW from the femoral artery to the effective midpoint of the capillary circulation (precapillary resistance) increases as the flow is reduced by lowering the arterial pressure. This phenomenon may be a result of anomalous viscous flow of blood in the arterioles; the effect is diminished when the arterioles are dilated (21) or when most of the corpuscles are removed (1, 21) as described in text. The resistance to flow on the venous side of the capillary circulation (postcapillary resistance) is substantially independent of the flow rate.
no blood flow. However, its value may be estimated by plotting the difference between isogravimetric arterial and venous pressures (abscissa) against the isogravimetric arterial or venous pressures (ordinates) and extrapolating to zero pressure difference. The intercept on the ordinate is then the isogravimetric capillary pressure.

**FIG. 8.** THE EFFECTIVE OSMOTIC PRESSURE OF THE PLASMA PROTEINS in the hindlimb capillaries of cats and dogs. The mean hydrostatic pressure in the capillaries required to prevent net transfer of fluid is slightly less than the (in vitro) osmotic pressure of the plasma proteins over the entire range of protein concentrations so far investigated. The mean difference between these two quantities is 1.7 mm. Hg as compared with a standard error of measurement of 0.54 mm. Hg. In all probability this difference arises from a small concentration (0.7 ± 0.2%) of protein in tissue fluid bathing the external surfaces of the capillaries as explained in the text. At normal plasma protein pressures (23-27 mm. Hg) the effective osmotic pressure acting across the capillary membranes is 93 ± 1.5 per cent of the protein pressure as measured across a collodion membrane in vitro.

**FIG. 9.** FLUID EXCHANGE IN THE HINDLIMB OF A CAT. The rate of fluid exchange is simply proportional to the difference between the mean hydrostatic pressure in the capillaries \((pC = Q_t + pV)\) and the sum of all pressures opposing filtration (isogravimetric capillary pressure, \(pC_i\)); it is independent of the absolute value of these quantities. The results of figures 8 and 9, taken together, show that the Starling Hypothesis may be applied with some precision to the hindlimbs of cats and dogs perfused under the specialized conditions of these experiments.

In practice this method of determining the isogravimetric capillary pressure is not the most precise one owing to the fact that the extrapolation is alinear. This alinearity will be discussed in greater detail below; it arises from the complex relations between pressure and flow which obtain when whole blood is employed for perfusion. The difficulty may be avoided by considering the relations between isogravimetric blood flow and isogravimetric venous pressure in the following analysis.
4. The determination of capillary quantities. Definition of symbols: the following notation will be employed in the remaining sections of this paper.

\[ p_A = \text{arterial pressure, mm. Hg} \]
\[ p_V = \text{venous pressure, mm. Hg} \]
\[ p_C = \text{mean capillary pressure, mm. Hg} \]
\[ p_r = \text{osmotic pressure of plasma proteins, mm. Hg} \]
\[ p_t = \text{osmotic pressure of proteins in tissue fluid, mm. Hg} \]
\[ Q = \text{blood flow, cc./min.} \]
\[ r_a = \frac{(p_A - p_C) + Q}{Q} = \text{resistance to blood flow from artery to effective midpoint of capillaries (pre-capillary resistance).} \]
\[ r_v = \frac{(p_C - p_V) + Q}{Q} = \text{resistance to blood flow from effective midpoint of capillaries to vein (post-capillary resistance).} \]
\[ i = \text{subscript referring to isogravimetric conditions.} \]
\[ Z = \text{rate of filtration (+) or absorption (-), grams/min.} \]

Rearranging the definition of \( r_v \) we have, in the isogravimetric state,

\[ p_C + p_V = Q \frac{r_v}{Q} + p_V \]

In order to solve for \( p_C \), plot \( Q_i \) (abscissa) against \( p_V \) (ordinate) from data similar to that shown in figure 4. Extrapolate to zero flow; when \( Q_i = 0, p_C = p_V \). Such a plot is shown in figure 5. It is seen that the relation between \( Q_i \) and \( p_V \) is linear over a wide range of values and that the extrapolation to zero flow may be made with an uncertainty of less than 1 mm. Hg. The slope of the line is \( r_v \), which is constant and independent of flow.

Presumably both filtration and absorption occur at all finite values of blood flow in the isogravimetric state. Thus when \( p_V \) is small and \( Q_i \) large (fig. 5), the pressure drop along the capillaries is also large; filtration occurring at the arterial ends or along the entire length of some capillaries is counterbalanced by equal absorption at the venous ends or along the entire length of other capillaries. But as the flow is reduced the pressure drop in the capillaries is also reduced until, at zero flow, the pressure is everywhere equal along the capillary and no fluid exchange takes place along its length. It is of importance for the subsequent development in this paper to note that the value of \( r_v \) is unaltered by the changes of flow or of filtration and absorption.

A similar analysis can be made on the arterial side of the capillary circulation using the definition \( p_C = p_A - Q r_a \). In this case, however, it is found experimentally that the relation between \( Q_i \) and \( p_A \) is not linear. Since \( p_C \) is constant (isogravimetric conditions) it follows that \( r_a \), the precapillary resistance to blood flow, must vary with flow. This is illustrated in the experiment of figure 6 in which the precapillary resistance was more than doubled as the flow was decreased by reducing the perfusion pressure. The postcapillary resistance \( (r_v) \), determined simultaneously, remained constant.

**Discussion**

This increase in precapillary resistance at low flows is not unexpected. It was found by Pappenheimer and Maes (21) and by Green et al. (22) that the overall resistance to blood flow \( (r_a + r_v) \) increases greatly at low flows and that the effect is most prominent in constricted vessels. The former workers attributed the
effect to the anomalous viscous properties of the blood, for it was not present when Ringer's solution was substituted for blood. The data now presented (fig. 6) show that the entire effect is localized in blood vessels proximal to the effective midpoint of the capillaries. It may be inferred that the blood vessels on the venous side of the capillary circulation have dimensions such that anomalous flow effects are too small to be of significance; in this respect they resemble fully dilated arterioles. Six of the present series of experiments have been conducted with plasma containing relatively few corpuscles (hematocrit about ten per cent). Under these conditions the precapillary resistance \((r_a)\) varied only slightly with changes of flow, thus confirming and extending the earlier observations with Ringer's solution.

While the resistance to blood flow is not the principal subject of this paper, it may be pointed out that the resistances to flow on either side of the capillary circulation have not previously been measured, although they are major factors determining the mean capillary pressure and the rate of fluid transfer as pointed out by Bayliss and Starling (23). The dependence of mean capillary pressure on arterial and venous tone may be stated quantitatively by combining the definitions of \(r_a\) and \(r_v\) given above and solving for \(pC\).

\[
pC = \frac{\frac{r_v}{r_a} pA + PV}{1 + \frac{r_v}{r_a}}
\]

It is seen that at any given values of arterial and venous pressures the mean capillary pressure depends solely on the ratio of the postcapillary to precapillary resistances to blood flow. Since \(r_v\) and \(r_a\) are now measurable the way is open for the quantitative study of factors affecting the mean capillary pressure.

5. Comparison of the isogravimetric capillary pressure with the osmotic pressure of the plasma proteins. The isogravimetric capillary pressure determined as described above is equal to the sum of all pressures opposing filtration. It is of interest to compare its value with the osmotic pressure of the plasma proteins as determined with an artificial semipermeable membrane in vitro. Figure 7 shows the effects of alterations in protein concentration on isogravimetric quantities in one experiment. It is seen that the protein osmotic pressure as measured in vitro \((\pi_p)\) was approximately 2 mm. higher than the isogravimetric capillary pressure at each of the two protein concentrations employed. Note that the postcapillary resistance to blood flow \((r_v)\) remained approximately constant throughout the measurements; alterations in postcapillary resistance, produced by the infusion of drugs or other procedures, do not affect the value of \(pC\) if the protein concentration is constant.

The results of 22 such comparisons of \(pC\) with \(\pi_p\) are summarized in figure 8. The upper (broken) line represents the theoretical values which would obtain if the isogravimetric capillary pressure were equal and opposite to the (in vitro) protein pressure. The lower solid line is the best straight line drawn through the experimental points (method of least squares). It is seen that the capillary hydrostatic pressure in the isogravimetric state is, on the average, one to 2 mm.
Hg less than the protein pressure as measured in vitro over the range of protein pressures so far investigated. Thus,

\[ pC_i = 0.95\pi_p - 0.56 \quad S.D. = \pm 0.6 \text{ mm. Hg} \]

\[ S.E. = S.D. \div \sqrt{n} = 0.34 \text{ mm. Hg.} \]

If no other forces (e.g., tissue pressure) were involved, the results of figure 8 could be explained on the basis of a concentration of protein in tissue fluid sufficient to exert an osmotic pressure of 1 mm. Hg in the lower range of protein concentrations and 2 mm. Hg in the higher range. It may be noted that tissue pressure would act to increase \( pC_i \) with respect to \( \pi_p \). Thus if the protein concentration were reduced to zero we should expect that a positive hydrostatic pressure in the capillaries would be required to counterbalance tissue pressure. Actually the extrapolation to zero protein pressure (fig. 8) intercepts the capillary pressure axis at a negative value (-0.6 mm. Hg), a result which would be difficult to explain in terms of tissue pressure but which would be expected if a small concentration of protein remained in the tissue fluid. Further evidence will be given below (Section 6B) that the tissue pressure is in fact negligible under the conditions of these experiments and we make the provisional conclusion that the isogravimetric capillary pressure is equal and opposite to the effective osmotic pressure of the plasma proteins.

\[ pC_i = (\pi_p - \pi_i) = \text{effective protein pressure across capillary membranes.} \]

If this conclusion is correct we have for normal plasma \( (\pi_p = 25 \text{ mm. Hg}) \)

\[ \frac{pC_i}{\pi_p} = \left( \frac{0.95 - 0.56}{25} \right) = 0.93, \text{ whence the effective osmotic pressure of the plasma proteins within the living capillaries is 93 per cent (standard error \( \pm 1.5 \) per cent) of the osmotic pressure measured with a collodion membrane in vitro. The mean value of \( \pi_i \) under these conditions is 1.7 \( \pm \) 0.4 mm. Hg, a pressure which would be expected in fluid containing 0.8 \( \pm \) 0.2 grams plasma protein per 100 cc. This estimate of protein in tissue fluid is too high if albumin and low molecular weight globulins constitute the principal protein fractions of the capillary filtrate. Electrophoretic studies of serum exudates (24) and of lymph (25) suggest that a partial sieving action does occur, and in this case our estimate of protein in fluid bathing the capillary walls would be closer to 0.7 \( \pm \) 0.2 per cent.

The close correspondence between isogravimetric capillary pressure and plasma protein pressure is maintained only in the first few hours of perfusion and the data given above were obtained in this period. After 4 to 6 hours the isogravimetric capillary pressure diminishes; in a typical experiment it remained within 90 to 95 per cent of the plasma protein pressure for the first four hours of perfusion, but fell to 70 per cent in the fifth hour and to 30 per cent after six hours of perfusion. Presumably this is a quantitative expression of failure of the capillary membranes to retain plasma protein after prolonged perfusion.

DISCUSSION

The isogravimetric capillary pressure is, on the average, 1.7 \( \pm \) 0.4 mm. Hg less than the osmotic pressure of the plasma proteins and this value is equivalent to
a protein concentration of 0.7 ± .2 per cent in fluid bathing the external surfaces of the capillaries. Inspection of table 2 shows that this concentration is slightly greater than estimates of protein in capillary filtrate (26) or in lymph obtained during venous congestion (27). On the other hand it is considerably less than the protein concentration in small samples of lymph collected during massage of the resting dog's leg (28). It is clear that these results do not confirm the view, originally expressed by Starling (1) and repeatedly stated by Drinker (29–31), that the composition of tissue fluid is identical with that of lymph. Indeed, it appears unlikely that the protein concentration of tissue fluid is at any time uniform throughout the interstitial spaces. The diffusion coefficients of the plasma proteins are such that relatively large concentration gradients are possible be-

### Table 2. Protein Composition of Tissue Fluids

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Protein</th>
<th>Number of Observations</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Capillary filtrate in human forearm during venous congestion (60 mm. Hg)</td>
<td>$c = 0.3\pm0.3%$</td>
<td>4</td>
<td>(26)</td>
</tr>
<tr>
<td>2. Lymph from dog's leg during venous congestion (40 to 60 mm. Hg)</td>
<td>$c = 0.2%$</td>
<td>1</td>
<td>(27)</td>
</tr>
<tr>
<td>3. Average tissue fluid bathing external capillary surface in perfused limbs of cats and dogs</td>
<td>$\pi = 1.4\pm0.4$ mm. Hg, $c = 0.7\pm0.2%$</td>
<td>22</td>
<td>Present data</td>
</tr>
<tr>
<td>4. Lymph obtained during massage of quiescent limb (dog)</td>
<td>$\pi = 17.5\pm.8$ mm. Hg, $c = 1.9\pm0.2%$</td>
<td>8</td>
<td>(28)</td>
</tr>
</tbody>
</table>

1 This value is larger than would be predicted from the observed protein concentration. The osmotic pressure of a 1.9 per cent solution of pure albumin in .15 molar NaCl at pH 7.3 is 6.1 mm. Hg (13) and that of a 1.9 per cent solution of plasma proteins is less than 5.0 mm. Hg.

tween tissue fluid undergoing absorption at the venous end of the capillaries and tissue fluid composed of freshly formed capillary filtrate at the arterial end of the capillary. Even if all filtration and absorption processes were stopped, some 20 minutes would be required to reach 90 per cent equalization of protein concentration over a distance of 50 microns. As pointed out by Landis et al. (26), the protein content of lymph may lie anywhere between that of capillary filtrate (as in venous congestion) and that of tissue fluid immediately adjacent to capillary areas taking part in the absorptive process. On this hypothesis our estimate of protein in tissue fluid represents an average concentration lying between these two extremes.

6. Filtration and absorption. A. The filtration coefficient. Once having determined $r_V$ in the isogravimetric state, it is possible to compute the value of the mean hydrostatic pressure in the capillaries when the arterial or venous pressures are altered so as to produce net filtration or absorption as in figures 2 and 3. Thus the value of $r_V$ was found to be independent of the blood flow (figs.
PROTEIN OSMOTIC PRESSURE IN MAMMALIAN CAPILLARIES

5, 6 and 7) and of changes in filtration or absorption of fluid along the capillary wall (section 4). The mean hydrostatic pressure in the capillaries following an uncompensated change of arterial or venous pressure is then given by the relation

\[ p_C = Q r_v + p_V \]

where \( r_v \) is obtained in the isogravimetric state but \( Q \) and \( p_V \) are no longer isogravimetric quantities.

The mean pressure head across the capillary membranes available for filtration or absorption is \( p_C - p_{C_i} \); for the latter term is equal to the sum of all pressures opposing filtration. Figure 9 shows the relation between the pressure head across the membranes and the rates of net filtration (+ \( F \)) or net absorption (− \( F \)). The rate of fluid exchange is simply proportional to the pressure difference across the capillary membranes. Black circles are points obtained with undiluted blood with a measured osmotic pressure of 16.0 mm. Hg. The crossed points were obtained after concentrated bovine albumin was added until the protein osmotic pressure was 50.1 mm. Hg. The two sets of points do not differ significantly.

### TABLE 3. FILTRATION COEFFICIENTS OF PERFUSED HINDLIMBS

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>FILTRATION COEFFICIENT ( K ) − GRAMS/MIN/MM Hg/100 GRAMS TISSUE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Cats (14)</td>
<td>0.012</td>
</tr>
<tr>
<td>Dogs (4)</td>
<td>0.014</td>
</tr>
</tbody>
</table>

The rate of fluid exchange is therefore independent of the absolute values of capillary and protein pressures and depends only on the difference between the mean capillary pressure (\( p_C \)) and the effective protein osmotic pressure (\( p_{C_i} \)). Thus,

\[ \pm F = K(p_C - p_{C_i}) \]

where the proportionality factor (\( K \)) may be termed the ‘filtration coefficient’ of the capillary membranes. The mean value of \( K \) in the hindlimbs of 14 cats was 0.012 grams per minute per mm. Hg pressure difference across the capillary membranes per 100 grams tissue. The value was not significantly different in the hindlimbs of four dogs (table 3).

### DISCUSSION

The results of figure 9 show that the rate of flow of fluid passing in either direction across the capillary membranes is simply proportional to the difference between the mean hydrostatic pressure in the capillaries and the sum of all pressures opposing filtration. Evidence has been given above that the latter term is equal to the effective osmotic pressure of the plasma proteins. These results, taken together, show that the Starling Hypothesis may be applied with some precision to the hindlimbs of cats and dogs perfused under the specialized conditions of these experiments.
The values of table 3 may be compared with estimates of filtration rate in the intact human forearm. Landis and Gibbon (32) found that a rise in venous pressure of 10 cm. H$_2$O produced, on the average, a filtration of 0.033 cc. per 100 cc. forearm per minute. Assuming that a rise in venous pressure of 10 cm. H$_2$O produces an increment of 8 cm. H$_2$O in mean capillary pressure, the 'filtration coefficient' of the human forearm would be 0.0055 gram/min/mm. Hg/100 grams tissue or less than half that of the isolated perfused hindlimb of the cat or dog. This difference may be an expression of a smaller capillary surface per unit volume of tissue in the human forearm, a difference which is suggested also by the absolute value of the blood flow, which is generally reported to be less per unit tissue volume in the human forearm (33–35) than in the extremities of dogs or cats (36–38).

Proportionality between pressure head and flow is characteristic of the viscous flow of fluids through artificial porous membranes. From this point of view it will be interesting to determine if the filtration coefficient of the capillary membranes varies inversely with the viscosity at different temperatures as found with artificial porous membranes (39).

B. THE EFFECTS OF PROLONGED FILTRATION AND ABSORPTION. The results illustrated in figures 1 and 2 show that net filtration or absorption of small quantities of fluid (less than one gram per 100 grams tissue) occur at a constant rate following an increment or a decrement in mean capillary pressure. Evidence has been given by Krogh, Landis and Turner (40) and by Landis and Gibbon (32) that continued net filtration increases tissue pressure in the human forearm, thereby causing a progressive decrease in the rate of filtration following an increment in venous pressure. Continued net absorption, on the other hand, must eventually be limited by the volume and protein content of the fluid originally present in the tissue spaces, a limitation which was clearly envisioned by Starling (1) but which has never been evaluated experimentally.

Figure 10 shows the relations between the volume of tissue fluid and the total pressure opposing filtration (isogravimetric capillary pressure) in an isolated hindlimb of a dog. The design of the experiment was as follows. The tissue fluid was first concentrated by allowing net absorption to take place; capillary pressure was then increased above the isogravimetric value and 68 grams of fluid filtered from plasma to tissue fluid. The filtration process was interrupted at intervals in order to obtain isogravimetric values. Approximately five hours were required to complete the experiment.

It is seen that the isogravimetric capillary pressure obtained at minimum limb weight was only two-thirds of the plasma protein pressure. Presumably this is a result of the previous prolonged absorption with resulting concentration of protein in tissue fluid and diminution of effective osmotic pressure of plasma proteins. Filtration of 15 grams of fluid into the tissue spaces diluted the tissue fluid proteins by an amount sufficient to increase the isogravimetric pressure to 92 ± 8 per cent of the plasma protein pressure. Further filtration of 33 grams did not increase this value significantly and in this latter range of tissue fluid volumes the rate of filtration remained constant following an increment in capillary pressure.
In another experiment of this type filtration was continued until 175 grams of fluid had been filtered into the tissue spaces (65 per cent of the initial weight of the limb). Conditions were made such that the osmotic pressure of the proteins was only a small fraction of the total filtration pressure by diluting the plasma with a large volume of Ringer's solution and raising the mean capillary pressure to an abnormally high value. The results are summarized in table 4.

It is seen that the filtration rate remained constant until 90 grams of fluid had been filtered. The limb appeared moderately edematous at this time.

**FIG. 10.** The effects of prolonged filtration in previously dehydrated limb. The isogravimetric capillary pressure rises rapidly at small tissue fluid volumes but remains relatively constant over a wide range of higher tissue fluid volumes. Presumably this is the result of dilution of protein in tissue fluid with capillary filtrate, which must contain protein in concentration less than that of tissue fluid at the end of the filtration period (0.5%). The smooth curve and the tissue fluid volume scale are calculated from the relation $V = (G_o + [P]_f \cdot \Delta W) / [P]_t$, as explained in the text.

**FIG. 11.** The effective protein osmotic pressure relative to true protein osmotic pressure following the addition of bovine albumin to the perfusing blood. Bovine albumin, unlike concentrated homologous plasma, is not retained by the capillary membranes in the hindlimbs of cats or dogs perfused under the conditions of these experiments.

Further filtration occurred at a progressively slower rate and this we attribute to developing tissue pressure for the protein pressure opposing filtration remained a small fraction of the mean capillary pressure. Evidently the isolated hindlimb differs from the human forearm in that tissue pressure does not become a significant factor opposing filtration until the limb is grossly edematous.

The results illustrated in figure 10 may be employed to estimate the volume of tissue fluid and the protein composition of the filtrate. Thus,

$$V = \frac{G_o + [P]_f \cdot \Delta W}{[P]_t}$$

where $V =$ volume of tissue fluid, $G_o =$ total quantity of protein in tissue fluid at start of filtration, $[P]_f =$ concentration of protein in capillary filtrate, $[P]_t =$ concentration of protein in tissue fluid $= \psi(\pi_p - pC_i)$, $\psi =$ relation between...
protein concentration and protein osmotic pressure given in table 1 and $\Delta W =$ increase in weight of limb during filtration.

This equation was employed to construct the 'tissue fluid volume' scale on the abscissa of figure 10. The protein concentration in capillary filtrate corresponding to the calculated curve is 0.3 per cent; this value best fits the observed data but other values, within the range 0.2–0.4 per cent, lie within the range of analytical errors.

**DISCUSSION**

Starling (1) considered the protein in tissue fluid to be an important factor in the regulation of the fluid exchange. To quote (p. 394): "With diminished capillary pressure there will be an osmotic absorption of salt solution from the extravascular fluid, until this becomes richer in proteids; and the difference between its (proteid) osmotic pressure and that of the intravascular plasma is equal to the diminished capillary pressure." A consideration of figure 10 shows that concentration of extravascular protein does in fact occur, but only after relatively large quantities of tissue fluid have been absorbed. In the intact animal the effective osmotic pressure would be diminished by dilution of the plasma proteins long before the concentration of protein in tissue fluid became a significant factor. It therefore appears likely that the principal osmotic factor regulating the fluid exchange normally involves changes in plasma protein concentration rather than changes in the protein concentration of extravascular fluid.

7. *Foreign protein.* In four experiments (three cats, one dog) bovine albumin was added to the perfusion reservoir. Two preparations of albumin were employed, a) Armour Co. 'Fraction V' which is said to contain about three per cent of impurities in the form of globulin fractions and acetate buffer; b) crystalline bovine albumin prepared by the Armour Co. The albumin was added in a 25 per cent solution titrated to pH 7.4. The results were similar for both preparations.

Figure 11 shows the effective osmotic pressure relative to plasma protein pressure before and after the administration of albumin. One hour after the addition
of albumin the effective osmotic pressure across the capillary membrane was only 50 per cent of the protein pressure, a value which would obtain if the protein in tissue fluid were sufficient to exert an osmotic pressure of 20 mm. Hg (equivalent to 4.9 per cent albumin). Evidently purified or crystalline bovine albumin, unlike concentrated homologous plasma, is not retained by the capillary membranes in the hindlimbs of cats and dogs perfused under the conditions of these experiments. The reasons for this leakage are not clear; Heyl, Gibson and Jane-way (41) found that crystalline bovine albumin is as effective as reconstituted human plasma in the restoration of blood volume following hemorrhage in humans.

Despite the large and progressive leakage of bovine albumin through the capillary membranes, the filtration coefficient remained unaltered. This is illustrated in figure 9 in which the net rate of fluid exchange for any given difference between mean capillary pressure and effective osmotic pressure was the same before and after albumin was added, although the effective osmotic pressure after the addition of albumin diminished progressively from 40 to 15 mm. Hg. The filtration coefficient was similarly unaffected by the leakage of protein which occurred at the end of each experiment as described in section 5 above. Evidently the permeability of the capillary wall to fluid is not necessarily related to the permeability to protein in the mammalian circulation.

SUMMARY

1. Methods are described for determining the following quantities in isolated perfused hindlimbs of cats and dogs.
   a) Mean capillary pressure (accuracy ±0.5 mm. Hg),
   b) Total pressure opposing net filtration of fluid from plasma to tissues (isogravimetric capillary pressure),
   c) Net rate of fluid transfer across capillary membranes,
   d) Resistance to blood flow from femoral artery to effective midpoint of capillary circulation and from capillary circulation to the femoral vein.

2. The rate of net fluid exchange between plasma and tissue spaces may be delicately adjusted over a wide range of values by varying the arterial pressure (fig. 2), the venous pressure (fig. 3) or the protein osmotic pressure of the plasma. At any given protein pressure there are an infinite number of pairs of values of arterial and venous pressures at which no net transfer of fluid occurs; two or more pairs of such values define the mean hydrostatic pressure in the capillaries (fig. 5).

3. The mean hydrostatic pressure in the capillaries at which no net transfer of fluid takes place \( (pC_i) \) is \( 93 \pm 1.5 \) per cent of the normal osmotic pressure of the plasma proteins \( (\pi_c) \). In the range of plasma protein pressures, 8 to 32 mm. Hg, the relation is given by

\[
pC_i = 0.95\pi_c - 0.56
given by (fig. 8).

Reasons are given for believing that \( pC_i \) is equal and opposite to the effective osmotic pressure of the plasma proteins.

4. The mean pressure head available for net fluid transfer across the capillary
membranes is the mean capillary pressure minus the isogravimetric capillary pressure \((pC - pC_1)\). The rate of filtration or absorption is accurately proportional to this pressure head and is independent of the absolute protein or capillary pressures over a wide range of values (fig. 9). The proportionality constant is termed the ‘filtration coefficient’; its mean value in the hindlimbs of 16 cats was 0.012 grams per minute per mm Hg pressure difference across the capillary membrane per 100 grams tissue (table 3).

5. Absorption of fluid following a decrement in capillary pressure continues at a constant rate until the proteins in tissue fluid become sufficiently concentrated to oppose continued absorption. However, the quantity of protein present in tissue fluid is so small that a large volume of tissue fluid must be absorbed before the protein osmotic pressure of tissue fluid is significantly increased. It therefore appears likely that the principal osmotic factor regulating the fluid exchange normally involves changes in plasma protein concentration rather than changes in the composition of tissue fluid.

6. Filtration of fluid following an increment in capillary pressure continues at a constant rate until the limb becomes grossly edematous (table 4). In contrast to the intact human forearm, tissue pressure does not limit the rate of filtration over a wide range of tissue fluid volumes.

7. Crystalline bovine albumin, unlike concentrated homologous plasma, is not retained by the capillary membranes in the hindlimbs of cats or dogs perfused under the conditions of these experiments (fig. 11).

8. The resistance to blood flow from the femoral artery to the effective midpoint of the capillary circulation increases greatly when the blood flow is diminished by lowering the perfusion pressure (fig. 6). The effect is perhaps a result of anomalous viscous flow of blood in the arterioles; it is diminished or absent when the blood contains less than ten per cent red cells. The resistance to blood flow on the venous side of the capillary circulation is independent of flow even at normal corpuscular concentrations (figs. 5, 6 and 7). It is suggested that the dimensions of the venules are such as to minimize the effects of anomalous flow.

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