THE CAPILLARY PRESSURE IN FROG MESENTERY AS DETERMINED BY MICRO-INJECTION METHODS

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For the purpose of studying some phases of capillary permeability the introduction of substances into single capillaries, using the micro-injection technique described by Chambers (1922), was attempted at the suggestion of Prof. M. H. Jacobs. It was first intended to study the rate of passage of certain substances through the capillary wall but the extreme variability of the results and the apparent importance of the condition of the circulation generally and locally has made it seem necessary to devise a method for determining the pressure in the particular capillary or capillary net during the period of injection.

The several indirect methods used to measure capillary pressure have produced a wide range of values, the determinations made for the capillaries of human skin alone varying from 75 cm. of water (v. Recklinghausen, 1906) to 10 cm. (Basler, 1914; Hill and McQueen, 1921b). Hooker and Danzer (1919), (1920) using a micro-tonometer found pressures between 18.0 and 26.5 mm. Hg.

These determinations have been frequently criticised since no correction is made for the pressure necessary to deform the superficial tissues, nor for the possible effect of total obstruction of flow. Liebesny (1923) believes blanching of the skin is due to the compression of the subpapillary venous plexuses, rather than to the emptying of capillaries. Apparently the values obtained by indirect methods are variable and difficult of interpretation as shown by the conflicting clinical observations on capillary pressure.

The first direct measurements of pressure in the microscopic vessels of the human skin were made by Carrier and Rehberg (1923), who introduced fine glass tubes, 10 to 20 micra in diameter at the tip, into superficial capillaries which had been made visible by an application of cedar oil, according to the technique of Lombard (1912). The pressure is determined by the level of the balancing water column at equilibrium. In the absence of any fixed mechanism to insert or hold the tubes, observations were limited to periods of a few seconds. Moreover, the size of
the tubes permitted measurements only in rather large dilated capillaries located in fairly resistant tissues, such as are found at the base of the finger-nail.

Apparently only indirect methods have so far been applied to the measurement of capillary pressure in the frog. Roy and Brown (1880) used a transparent, distensible membrane which was pushed against the tissue by a measured amount of pressure, while the vessels were observed under the microscope. This method has been criticised because flow was stopped and pressure thereby raised. L. Hill (1920) has used the same apparatus but regards the true value as that pressure which when suddenly applied will momentarily modify but not stop arteriolar or capillary flow. The pressures thus indirectly obtained were relatively low in the mesentery and web of the frog, 2 to 5 cm. of water, but somewhat higher in the kidney glomeruli and the tongue papillae, 5 to 10 mm. Hg.

The direct method here described has been used to measure the pressure in certain portions of the vessels in frog mesentery, in an effort to determine the average gradient of pressure fall through this particular portion of the peripheral circulation, and the possible relations of capillary pressure to certain factors such as flow, venous obstruction, and perhaps constriction. The pressures thus indirectly obtained were relatively low in the mesentery and web of the frog, 2 to 5 cm. of water, but somewhat higher in the kidney glomeruli and the tongue papillae, 5 to 10 mm. Hg.

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Material and methods. The mesentery of the frog (Rana pipiens and small R. catesbiana) has been used throughout since it seems best adapted for micro-injection by the greater accessibility and visibility of the capillaries and larger vessels. A lateral incision is made through the skin with a cautery to avoid loss of blood, and the remainder of the abdominal wall cut by scissors. A loop of intestine is delivered through the incision and laid loosely over a transparent glass stage. A constant, slow drip of saline from a small reservoir on the frog board keeps the preparation moist and clean.

The microscopic implements consist of a blunt rod and the micro-pipette (fig. 2). The rod is made by fusing a small ball, 20 to 50 micra in diameter, on the end of a glass micro-needle. It is convenient to stop flow in a capillary temporarily or permanently by pressure, or to hold the mesentery firm during the introduction of the pipette into the more resistant larger vessels. The micro-pipettes are made of Pyrex capillary tubing with the small gas flame or micro-burner described by Chambers (1918). The tip is broken off against the edge of a glass slide until the diameter of the orifice is from one-sixth to one-third the length of a frog's red corpuscle, i.e., from 4 to 8 micra.

The movements of each implement are governed by a Chambers
micro-manipulator. It has been necessary to make some changes in the arrangement of the separate parts. The pipette and rod both come to the microscopic field from the right side of the stage, and the pipette is directed at a gentle slope downward instead of sharply upward as in a hanging drop preparation.

The other end of the micro-pipette is connected with a device for the application and measurement of pressure (fig. 1). This consists essentially of a reservoir, \( R \), a syringe, \( S \), for exerting large positive or negative pressures, and a column of water, \( W \), by which measured amount of pressure up to 30 cm. of water may be applied to the contents of the micro-pipette. A dye solution may be drawn into the tip of the micro-pipette by a column of mercury, \( M \), or by carefully withdrawing the plunger of the syringe when the two stopcocks, \( C', C'' \), are properly arranged. For accuracy of control the plunger of the syringe is moved by a screw adjustment.

After the pipette has been introduced into the capillary the stopcocks are so turned that the pressure is applied to the dye in the pipette by the column of water, \( W \), the height of which can be changed rapidly by the movement of the syringe plunger. To correct for capillarity in the upright tube the instrument has been calibrated by balancing the water column against known pressures.
For measuring the higher pressures found in the arteries a mercury column is more convenient. This is connected through a U-tube with the water manometer. If the opening at the tip of the tube, W, is open the whole system functions as a column of water, but if it is closed by a ringlet of rubber tubing, T, the pressure applied is measured by the mercury manometer providing correction be made for the column of water between the surface of the mercury and the tip of the pipette. This arrangement allows a more rapid succession of pressure determinations in arterioles and arteries than would otherwise be possible.

Photographs with a magnification of 80X have been made by a photomicrographic ocular permitting simultaneous observation and exposure.

Methods of measuring capillary pressure. Objections have been raised against the indirect values because of the blockage of flow, and apparently this same source of error must be guarded against in single capillaries. If flow be stopped in a capillary at some point and the pipette is introduced on the arterial side of the obstruction the pressure there is some centimeters of water higher, depending upon the fall in pressure in in the region at the time of measurement, than on the venous side. Thus in figure 3 is shown a photomicrograph of such a capillary in which after occlusion the pressure on the arterial side was 15.0 cm. while on the venous side it was only 10.2 cm. of water. Hence in measuring pressure in any capillary, flow should not be stopped by the introduction of the pipette since the pressure then measured is not that of the capillary pierced but of the vessel with which it is connected. The error is apparently small if the vessel is a part of a network of short interlacing capillaries, but becomes greater in the long vessels connecting more directly arteriole and venule.

Two methods have been used in the determination of pressure in individual capillaries. The first is the more difficult but under proper conditions will measure both diastolic and systolic pressures in the vessel pierced. A very fine pipette, filled with a dye such as methylene blue, may be introduced into a dilated capillary or venous capillary and the flow not be hindered if the tip is brought as close as possible to the endothelial wall (fig. 4). When flow has continued for some minutes and is to all appearances normal the measurement may be made. As the pressure on the dye in the micro-pipette is gradually raised by increasing the height of the column of water in the upright tube, a point is reached where there is a barely visible spurt of dye during diastole. This would indicate the lowest pressure in the circulatory cycle,—diastolic pressure. As the height of the water column is further increased another point is reached at which the dye flows into the passing plasma continuously, though the amount is larger during diastole, and just perceptible during systole. This gives some indication of systolic pressure. The determination is
Fig. 2. Photomicrograph (× 80) showing the micro-pipette used to pierce the capillaries and the glass rod for holding the mesentery or stopping flow.

Fig. 3. Photomicrograph (× 80) of capillary in which after stoppage of flow the pressure on the arteriolar side was 15.0 cm. of water, while on the venous side of the obstruction it was only 10.2 cm.

Fig. 4. Photomicrograph (× 80) showing the determination of systolic and diastolic pressures in a venous capillary by the introduction of a fine pipette close to the endothelial wall, thus avoiding any interference with flow. For the sake of contrast the corpuscles were washed out by a stream of saline from the pipette during the period of exposure.

Fig. 5. Photomicrograph (× 80) showing the method of determining pressure with uninterrupted flow by the use of a collateral capillary as a side tube for the insertion of the micro-pipette. Saline is flowing from the pipette and is passing into the vein. The pressure is being measured in one of the larger connections which resemble venous capillaries in size but pass directly from the arteriole to the small venule. The vessel into which the pipette was inserted had all the characteristics of a true capillary.
not always feasible and must be made with care since a too rapid flow of blood may dilute the dye and give falsely high results. The patency of the tip of the pipette must be frequently tested since a fold of endothelium, practically invisible under low power, may cover it and partially or completely obstruct flow.

The second method has been the more generally useful and is entirely accurate for measurement of mean pressure but permits only a rough estimation of pulse pressure. It requires the insertion of the pipette into some collateral of the capillary, the pressure changes of which are to be measured (fig. 5). The pressure in this occluded collateral will be equal to the lateral pressure in the straight vessel at the point opposite the opening. Flow proceeds uninterruptedly throughout the entire period in the capillary observed. After the pipette is introduced into the collateral a few corpuscles may be drawn out of the main stream back into the vessel functioning as a side tube. Intermittent movement toward or away from the pipette occurs synchronously with the heartbeat when the pressure of the water column is below or above mean pressure respectively. When the proper height is reached the dye-plasma junction, or the corpuscles, whichever is used to determine the point of equilibrium, will oscillate back and forth about a single point, thus giving the mean pressure in the capillary at the moment. With lowering of pressure, the corpuscles will move toward the pipette only in systole, remaining stationary in diastole. At a higher pressure the converse will be true; the movement is away from the pipette in diastole but ceases in systole. The difference between these two levels gives a general idea of the magnitude of pulse pressure, but in all probability not so accurately as the first method.

Although both a water column and a mercury manometer have been used all figures used in this paper have been changed to centimeters of water pressure.

Observations. 1. The capillaries of the exposed mesentery. The term "capillary" has been here reserved for the vessels which are composed of a simple endothelial wall, and are in more or less direct relation with the finest arterioles. They have sometimes been referred to as arteriolar capillaries, in contrast to the venous capillaries. Frog mesentery contains in addition vessels whose diameter is little greater than that of the fully dilated capillary but whose walls are composed of endothelium enclosed by a thin coat of connective tissue. Frequently two or more true capillaries can be seen emptying into one of these vessels, which are apparently a part of the venous or collecting system, and for structural identification are referred to as venous capillaries, though physiologically their function and innervation are probably similar to those of the purely endothelial vessels, according to Krogh (1922) and Vimtrup (1922).
When the mesentery of a pithed frog is first exposed to the air usually all the vessels are constricted and flow is at a minimum. Within a few moments the arterioles begin to increase in size, and the capillaries, in the beginning invisible, appear at first to be constricted, but finally nearly all become more or less dilated.

If the frog has been anesthetized there is apparently a tendency for the capillaries to remain in the constricted state, either throughout their length or especially in the portions nearer the arterioles. In the pithed frog also after the preparation has been in use for some hours there is a tendency for constriction to recur. The reactions of inflammation develop very slowly indeed if the preparation is kept well moistened by a constant flow of saline, or Ringer's solution.

![Fig. 6. Curve showing the distribution of 111 successive determinations of capillary pressure in the mesenteries of 21 frogs. Average pressure is 14.5 cm. of water.](image)

The size of the capillaries in the exposed mesentery varies from 3 to 30 micra, with the majority about 20 to 25 micra in diameter when flow is active. The smaller ones by constriction may disappear entirely, the larger are merely reduced in diameter.

Most of the capillaries are undoubtedly relaxed, but the extent of dilatation found is usually not sufficient to be accompanied by that degree of permeability which allows the outflow of all the fluid portions of the blood, stated by Krogh and Harrop (1921) and Krogh (1922) to accompany maximum dilatation. The vessels are to all appearances functioning normally, since, though dilated, if properly moistened, they have been observed for hours without showing any tendency to stasis.

2. **Range of pressure in the mesenteric capillaries.** In a series of several hundred determinations of capillary pressures made in the mesenteries
of pithed frogs the values have ranged under different conditions from 5.0 to 29.0 cm. of water. Of these, 111 consecutive readings in the mesenteries of 21 pithed frogs have been charted in a frequency curve (fig. 6) in which over half lie between 10 and 15 cm. with 13 cm. as the most frequent.

The wide variation is probably due in part to the manner in which the measurements were made, purposely including all conditions of flow, from its complete absence, with or without plasma-skimming, to exceedingly rapid rates in some instances, in the hope of obtaining a fair average figure. Constricted and dilated capillaries alike have been tested and the values included.

In general the lower pressures, up to 10 cm., have accompanied very slow, or totally absent capillary flow, either with marked arteriolar constriction, or in constricted capillaries, irrespective of the condition of the arterioles. The very high values, from 22 to 29 cm., occur apparently with stoppage of venous outflow from any cause and if the condition is long continued it is accompanied by much reduced rate of flow, and a visible concentration of the blood ultimately resulting in stasis. In capillaries with the more average rates of flow, pressure lies between 10 and 22 cm. of water, the rapidity of flow increasing as the pressure rises, with a relation which seems quite close within certain limits, in dilated capillaries at least.

3. The fall of pressure in the mesenteric vessels. The gradient of pressure fall from artery to vein through the capillary bed has been of certain general interest. Since the work of Dale and Richards (1919) the intrinsic tone of the capillary wall has been regarded as playing a part in the consideration of peripheral resistance. Dale and Richards state (loc. cit., p. 63), "We find no evidence, however, to warrant the assumption that the steep fall of pressure in the arterioles suffers an abrupt flattening, becomes in fact horizontal, at a sharp line of demarcation between the smallest arterioles and the first capillaries. We find nothing to exclude the view that a general tone of the capillaries, if it exists, will play an important part in determining the peripheral resistance, . . . ."

To determine the average fall of pressure 189 determinations were made at various points in the peripheral vessels, 22 in the larger arteries near the attachment of the mesentery, 5 in the first bifurcations of these arteries near the intestine, 11 in the small arterioles, 111 in the capillaries, 17 in the venous capillaries, and 23 in venules and veins. Table 1 presents the averages of these figures along with the highest and lowest values observed in each group. Systolic and diastolic pressures, in centimeters of water, are given where pulse pressure was sufficiently definite to measure, otherwise only mean pressures are recorded.

These figures are all inclusive; no limitation was made except that the
mesentery be in normal condition at the time the pressures were taken and remain so during the entire period of measurement. Arterial pressures were determined by introducing a pipette with an orifice sufficiently large (30 to 40 micra in diameter) to allow red corpuscles to enter freely. Systolic pressure was measured with the mercury manometer at such a level that dye flowed out of the tube except at the height of systole, when two or three corpuscles would be pushed into the tip of the pipette. Then the pressure was lowered until an almost steady flow of corpuscles into the pipette was observed with a periodic stoppage or marked slowing of flow, thus giving a measure of diastolic pressure. Mean pressure, at which point there was no flow, but merely a fluctuation of the corpuscles back and forth through a single zone, was usually found to be about half way between the other two values, or slightly lower.

TABLE 1

Average pressures in the peripheral vessels of frog mesentery in centimeters of water

<table>
<thead>
<tr>
<th>Diameter</th>
<th>ARTERY</th>
<th>ARTERY-FIRST BIFURCATION</th>
<th>ARTERIOS-OLE</th>
<th>CAPILLARY</th>
<th>VENOUS CAPILLARY</th>
<th>VENULE AND VEIN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25-0.30 mm.</td>
<td>22</td>
<td>5</td>
<td>11</td>
<td>111</td>
<td>17</td>
<td>23</td>
</tr>
<tr>
<td>0.12-0.15 mm.</td>
<td>40-50μ</td>
<td>10-30μ</td>
<td>30-45μ</td>
<td>0.25-0.35 mm.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of observations</td>
<td>50</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>18.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Highest value:</td>
<td>50</td>
<td>49</td>
<td>29</td>
<td>24</td>
<td>18.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Systolic ......</td>
<td>24</td>
<td>25</td>
<td>17</td>
<td>20</td>
<td>17.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Diastolic ......</td>
<td>24</td>
<td>25</td>
<td>17</td>
<td>20</td>
<td>17.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Lowest value:</td>
<td>24</td>
<td>35</td>
<td>6.5</td>
<td>5.0</td>
<td>6.7</td>
<td>4.5</td>
</tr>
<tr>
<td>Systolic ......</td>
<td>3</td>
<td>22</td>
<td>6.5</td>
<td>5.0</td>
<td>6.7</td>
<td>4.5</td>
</tr>
<tr>
<td>Average:</td>
<td>3</td>
<td>35</td>
<td>6.5</td>
<td>5.0</td>
<td>6.7</td>
<td>4.5</td>
</tr>
<tr>
<td>Systolic ......</td>
<td>39</td>
<td>38</td>
<td>22</td>
<td>14.4</td>
<td>10.1</td>
<td>7.5</td>
</tr>
<tr>
<td>Diastolic ......</td>
<td>23</td>
<td>24</td>
<td>16</td>
<td>14.4</td>
<td>10.1</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Pipettes were also inserted at the first bifurcation of the artery near the intestinal wall and pressures compared with those of the artery at the proximal part of the mesentery. In every case there was practically no fall in pressure in that region of the peripheral circulation, though the entire breadth of the mesentery had been traversed, indicating the flatness of the pressure curve in the unbranching portion of the artery. Beyond this point the vessels are too close to the intestine to allow further measurement. The large venules and veins were pierced with the larger pipettes and their pressure measured by the same method used for arteries, except that the much reduced pulse pressure, amounting to only a few millimeters of water, made mean pressure the only significant figure.

It has not been so far possible to take these pressures simultaneously but only as rapidly as possible one after the other while flow and vessel
diameter were observed to remain practically the same. A protocol is given to show the times of the measurements and the variations in the readings for short periods.

**PROTOCOL 1**

Young bull frog, of medium size pithed at 7:30 p.m. Abdomen opened without blood loss. Mercury manometer used throughout for all readings which have, however, been changed to centimeters of water.

7:47 Capillary of 20 micra diameter pierced and pressure determined
7:49 16.8 cm.—flow too fast to measure by eye since individual corpuscles were not visible
14.8 cm.—flow slower, about average speed
7:53 Terminal arteriole of 30 micra diameter pierced. It appeared to be of the same size as a dilated capillary but with thicker walls. It split into two capillaries of almost the same diameter within the microscopic field
7:54 Systolic pressure 26.0 cm. water
7:56 Mean pressure 21.0
7:57 Mean pressure 21.0
7:58 Larger arteriole, diameter of 45 micra entered
7:59 Systolic pressure 29.0 cm. water
 Diastolic pressure 17.0
 Systolic pressure 31.5

8:02 Systolic pressure 33.0
8:04 Pipette introduced into venous capillary of 35 micra
8:05 Mean pressure 12.0 cm.
 Mean pressure 12.5
8:06 Mean pressure 10.4
 Pulse pressure was too slight to measure with a column of mercury

The fine tip of the pipette was now broken off till the orifice was about 35 micra in diameter.

8:10 Blunted pipette introduced into the vein which drained the region in which the previous pressures had been taken
8:17 6.5 cm. water
 6.0 cm.
8:23 Entered large artery at the base of the mesentery
 Systolic pressure 50.0 cm.
 Diastolic pressure 25.0
8:24 Systolic pressure 45.5
 Diastolic pressure 24.0
8:26 Systolic pressure 48.0
8:27 Mean pressure 36.0
8:29 Diastolic pressure 25.6
 Systolic pressure 45.5
8:30 Diastolic pressure 25.6
8:32 Pipette introduced into the bifurcation of the same artery at a distance of 8 mm. from the first point
Systolic pressure  45.0 cm.
Diastolic pressure  24.5
Mean pressure  35.0

Frog still in good condition, flow is rapid, vessels well dilated, and there is no evidence of stasis.

The data obtained by experiments like the above, or others less complete, have been averaged (table 1) and plotted in the form of a curve in figure 7 in which the average length of each type of vessel forms the abscissa and the average pressure the ordinates. Below the curve are shown the diameters of the vessels plotted to scale. This represents therefore an average gradient of pressure fall in the peripheral circulation of the mesentery.

Virtually the fall of pressure presents wide variations under different conditions of arteriolar resistance, capillary pressure and flow. Five individual experiments have therefore been charted to the same scale as the average curve in figure 8 to show the types of gradients which are included.

With very low capillary pressures, as shown in the lowest gradient of figure 8 there is frequently no pulse pressure in the capillary, and little or none in the arteriole, the major fall of pressure occurring proximal to these vessels. Under these conditions the larger arteries of the mesentery are frequently contracted to such an extent that only two or three corpuscles can pass through a lumen that before was 0.15 mm. in diameter. The resistance has apparently shifted centrally due to the constricted larger vessels and the smaller arterioles and the capillaries have less importance in the determination of peripheral resistance. This condition is seen after application of adrenalin to the mesentery when arteriolar pressure is very low, and approximately equal to that in the capillaries as shown by direct measurement and as indicated by plasma skimming. Both Krogh (1921) and Froelich and Lak (1924) state that adrenalin has little or no effect on capillaries but constricts the arteries or arterioles above 0.1 mm. diameter. Resistance is also shifted centrally in the same way after hemorrhage when the mesentery may be almost bloodless, though the capillaries are widely dilated.

When the capillaries and arterioles are dilated and present the more rapid rates of flow on the contrary the fall of pressure may in extreme cases occur to a very large part between the capillary and the vein. In such instances as shown in the two highest gradients of figure 8, capillary pressure is very high, from 17 to 22 cm. of water, pulse pressure is increased, and frequently venous pressure is raised with a more than usually definite, though small pulse pressure of barely measurable magnitude.

In the average curve (fig. 7) and in the more moderate gradients of figure 8 there is a rather sharp reduction of pulse pressure between the arterioles and capillaries, the usual finding with capillary pressures of
Fig. 7. Curve showing graphically the average fall in pressure through the vessels of the mesentery, using the data given in table 1. Pressure is charted in centimeters of water against the length of the respective vessels. Below the relative diameters of the vessels are shown in micra.

Fig. 8. Chart showing five pressure gradients determined in individual experiments in which pressures were determined one after the other as rapidly as possible, other things remaining constant. It shows the range of variation in the gradients forming the average curve in figure 7.
13 to 15 cm., and a moderate fall in pressure from the capillary to the venous capillary. If capillary pressure is less than 10 cm. a pulse pressure can only rarely be measured. At 15 cm. there is usually from 0.5 to 1.0 cm. difference in the systolic and diastolic levels, while at the very high pressures the difference may reach 4 cm. Except in the exceedingly rapid flow all over the mesentery there is little or no difference in the systolic and diastolic levels in the venule and vein. A slight difference may be made out in the venous capillaries, especially when flow is rapid and pressure high (fig. 8).

The rather sharp reduction in pulse pressure at the arteriole-capillary junction may be due partly to the changed conditions accompanying branching, partly to the narrow, at times almost slit-like openings frequently seen in the mesentery at the points where capillaries spring from the arterioles. Such narrowed orifices are believed by Richards and Schmidt (1924) to exist in the kidney at the junction of arteriole and capillary. With rapid rates of flow in the mesentery these openings can be observed to be widely patent and it is under such conditions that the higher pulse pressures are found.

There is noted both in the average gradient and also in two of the gradients of figure 8 a peculiar flattening of diastolic pressure in the region between the arteriole and the capillary. This has been noted very frequently, and in some cases the diastolic line may become almost horizontal in this zone. No explanation is offered at the present time. Speculation might include the possible effect of a velocity factor, or of a changed systolic pressure due to wave reflection in that region, or possibly also the presence of a special type of resistance, such as a slit-like opening between the arteriole and its capillaries, which would become more effective during diastole, and less so in systole since the force would then be sufficient to neutralize it to a certain extent by stretching.

Venous capillaries have given quite variable results, possibly because they are frequently hard to define, since a vessel which is obviously an arteriole at one extremity may within the same microscopic field receive capillaries and to all appearance become a venous capillary or even a small venule, with rapid flow, and a correspondingly high pressure. These have been mentioned by Hill (1921a) as short cuts forming a safety factor when blood stagnates in the capillaries.

4. Relation of pressure to velocity of flow in capillaries. The rate of flow in a capillary is apparently related to the gradient of pressure fall from capillary to vein, and a series of such gradients have been arranged in order of rate of flow in table 2. With reduction of the pressure difference between capillary and vein there is a reduction of the velocity of flow. At the average rate, somewhere about 0.5 mm. per second, the pressure difference between capillary and vein is usually between 7 and
8 cm. The fall in pressure through the same region as given in the average curve is 6.9 cm.

Capillary flow has long been known to be variable in character, differing not only in capillaries which lie side by side but also in the same capillary at different moments. It has been inferred that capillary pressure likewise changes from moment to moment, and such variations in the same capillary have been indicated by the work of Roy and Brown (1880) using the indirect method of measurement.

Direct measurement in a single capillary shows that pressure as well as flow is constantly varying and that the two apparently change in the same direction providing the pressure in the vein remains approximately the same. Within the limits mentioned above high capillary pressures are nearly always found to occur in regions showing more rapid flow, and low pressures where flow is very slow or absent.

The relation between pressure and velocity may be measured by inserting the pipette into the side capillary, taking pressure as usual, then immediately afterward determining the time it requires for a corpuscle or group of corpuscles to pass from a certain point to the opening of the side capillary. It is possible to measure the pressure in the venule or vein only at the beginning and the end of the experiment, but this pres-

**TABLE 2**

<table>
<thead>
<tr>
<th>Mode of Flow</th>
<th>Average Pressure (mm.)</th>
<th>Average Velocity (mm. sec.)</th>
<th>Average Time (sec.)</th>
<th>Pressure in Vein (mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid flow—moving so rapidly corpuscles cannot be seen individually</td>
<td>22.0</td>
<td>18.7</td>
<td>12.5</td>
<td>9.5</td>
</tr>
<tr>
<td>Moderate flow—about 0.5 mm. per second</td>
<td>22.0</td>
<td>18.5</td>
<td>12.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Slow flow—about 0.25 mm. per second</td>
<td>17.0</td>
<td>12.5</td>
<td>7.5</td>
<td>9.5</td>
</tr>
<tr>
<td>Very slow—barely perceptible with plasma skimming</td>
<td>18.0</td>
<td>7.5</td>
<td>7.5</td>
<td>9.5</td>
</tr>
<tr>
<td>No flow........................................</td>
<td>10.5</td>
<td>7.5</td>
<td>2.0</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**TABLE 2**

Relation of flow to the fall of pressure from capillary to vein.
sure has been found to remain quite constant if capillary flow is increased over a small area only, as is usually the case.

Figure 9 shows the results of such an experiment plotted with velocity of flow and pressure against time. The chart shows the fairly close parallelism between pressure in the capillary and the flow in the same vessel. It indicates also the relatively large variations of pressure that may occur in a single capillary in successive moments. Again if 0.5 mm. per second be regarded as average velocity it required in this case a pressure difference of 6.5 to 7.5 cm. of water between the capillary at the point measured, near the arteriole, and the venule into which it emptied, which had a pressure of 7.5 cm. This compares favorably with the average fall of 6.9 cm. obtained statistically.

Apparently average rate of flow usually accompanies a capillary pressure of 13 to 15 cm. when venous pressure is from 6 to 8 cm. As the capillary pressure decreases, venous pressure remaining constant, there is first noted a slower rate of flow, then in addition a reduction in the number of erythrocytes with increasing proportions of plasma, and finally plasma-skimming.

5. Plasma-skimming. Plasma skimming is most often seen in the presence of low capillary pressures but it seems to depend not so much
upon the level of pressure as upon the velocity of the stream of plasma passing from the arteriole to the capillary. If a widely dilated capillary be temporarily occluded and then gradually opened by slow elevation of the glass ball, plasma skimming occurs first, both leucocytes and erythrocytes passing by the capillary opening though a slow flow of fluid is passing through the partially occluded vessel as can be observed by following small fragments of cells which are nearly always present in the blood stream. As the obstruction is further lessened leucocytes can be skimmed off the axial stream and caused to flow through the partially occluded capillary. Finally, when entirely open, erythrocytes will be swept through as before the obstruction.

Frequently also when the heart rate is very slow a mass of corpuscles pass into a partially constricted capillary in systole, followed by a stream of plasma with relatively fewer corpuscles, or in extreme cases almost no cells, in diastole, apparently caused by the reduced stream when the arteriolar pressure falls during the latter period. Both of these are examples of plasma skimming through large orifices.

With normally high arteriolar pressure plasma skimming is also seen in constricted vessels, where frequently only a minute orifice at the junction of the capillary to the arteriole remains open. At intervals an erythrocyte can be seen to dart from the axial stream, plug the opening, and gradually squeeze through. On escaping this narrowed space it passes through the constricted vessel rapidly followed by plasma, until another erythrocyte is swept from the axial stream when again flow almost stops until the second cell has passed through the constriction. It is possible that some such occurrence may be the cause of the "stoszförmig" flow shown in the curves of Hurthle (1923), after the administration of adrenalin.

In the use of the collateral capillary as a side tube difficulty has frequently been experienced in causing corpuscles to leave the axial stream and enter the capillary, there to act as indicators of balance of pressure. The capillary in such a case is obstructed so that flow ceases, and pressure is equal to that of the arteriole. It apparently requires a lowering of the pressure in the micro-pipette of 1 to 2 cm. below that in the arteriole to cause corpuscles to enter the capillary. Such lowering of the water column causes plasma to flow into the pipette, and when this stream becomes sufficient a corpuscle or two will enter the capillary. Balance of pressure is then obtained by increasing the height of the column of water again until the corpuscle remains motionless. The more rapid the flow in the arteriole, and the smaller the entrance of the capillary, the greater the lowering of pressure must be.

Apparently plasma skimming though far more common with low pressures in the capillary or arteriole, may occur at any level, the sole require-
ment being a reduction in the stream of plasma passing into the entrance of the capillary. This may be accomplished by a small orifice which will reduce the stream of plasma though a fairly marked difference of pressure exists between the arteriole and its capillary. It may also occur with a large, dilated orifice if pressure is equalized by occluding flow in the capillary, as by the glass rod, or the micro-pipette which fills the lumen, in which case capillary pressure tends to approach the arteriolar level. It may also be observed with a large orifice after severe hemorrhage, or after the application of adrenalin to the mesentery, where constriction occurs in the larger arterioles, or the smaller arteries, above 0.1 mm. diameter as described by Krogh (1921) and others while the capillaries are dilated or unaffected, thus causing arteriolar pressure to approach capillary pressure, and reducing the flow of plasma and tending to emphasize the action of the larger, more direct channels leading from the arterial to the venous system.

6. Effect of venous occlusion on capillary pressure. The condition of the veins has been recognized as an important factor in determining the rate of peripheral flow and capillary pressure. Basch (1903) mentions resistance to outflow as one of the four factors determining capillary pressure. Hooker (1920), (1921) has emphasized the importance particularly of the small veins and Liebesny (1923) states that venous congestion constantly produces a marked rise of capillary pressure. According to Carrier and Rehberg (1923) capillary pressure follows venous pressure very closely with different levels of the hand.

If a single capillary is blocked by means of the microscopic rod so that its communications by collaterals are removed and it is connected only to its arteriole and venule, and flow is then stopped in the venous end, pressure rises rapidly to the arteriolar level, falling back to the lower value or in many cases slightly below, when the occlusion is removed.

Similar pressure changes, though smaller, are noted also with full collateral circulation. If flow in a capillary, venous capillary or even venule be shut off the pressure on the arterial side of the occlusion increases markedly for a few moments, then gradually lowers slightly to a level of 3 or more centimeters above the original. On removal of the obstruction it also falls below its first value, then climbs slowly back to the normal observed before circulation was stopped. An example is given under protocol II, in which a capillary was compressed 0.5 mm. from the arteriole.

**Protocol II**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure before occlusion</td>
<td>14.5 cm. water</td>
</tr>
<tr>
<td>Immediately after occlusion</td>
<td>20.0</td>
</tr>
<tr>
<td>After opening of adjacent capillary</td>
<td>17.0</td>
</tr>
<tr>
<td>Occlusion removed</td>
<td>13.5</td>
</tr>
<tr>
<td>Two minutes later</td>
<td>14.0</td>
</tr>
</tbody>
</table>
This may be repeated several times with similar results. The rise in pressure apparently depends on the rate of flow in the region, and the size and number of collaterals. The obstruction of a single fairly large venule results in a local rise of pressure of 3 to 4 cm., accompanied by a more rapid outflow of such a dye as methylene blue through the capillary wall, indicating an increased outflow of fluid from the vessel with the higher pressure. If pressure is above 20 cm. passage of fluid through the wall is rapid even in the venous capillary and a visible concentration of the corpuscles occurs. It appears from this and other experiments that fluid and certain dyes pass through walls of the venous capillary almost as easily as through simple endothelium. Krogh (1922) states that morphologically the venous capillary is very slightly different from the capillary itself and may well be a factor in the exchange of fluid.

7. Effect of hemorrhage on capillary pressure. After severe hemorrhage there is a marked vaso-constriction of the smaller and larger arteries in the mesentery. Capillary and arteriolar pressure is low, commonly 5.0 to 8.5 cm. and the number of corpuscles in the plasma of the capillary bed is much reduced, due to more or less marked plasma skimming. The activity of the channels which intervene between arteriole and venule is more evident, in spite of which venous pressure is usually lowered, the constriction more than compensating for this increased flow through the more direct communications.

8. Pressure in capillaries of varying diameter. Roy and Brown in their studies on pressure noted that an increase of external pressure caused a diminution of diameter of capillaries but never more than fifteen per cent, thus limiting the effect that internal pressure can have upon the size of the capillary lumen. Oinuma (1924) found that capillary size depends partly on blood pressure, partly on the condition of the capillaries themselves, but the relation of capillary dilatation to arteriolar dilatation while usually present was not constant.

Efforts have been made with this apparatus to measure the pressure in constricted capillaries in frog mesentery for comparison with those of dilated vessels. These have so far been consistently low, rarely above 10 cm. and never above 11, averaging for nine determinations 9.5 cm. The number obtained is small because of the difficulty of entering the vessels without clogging the very fine pointed pipette necessary. Certainly constricted vessels may have much lower pressures than dilated capillaries lying in the area adjacent and arising from the same arteriole. The proximal resistance in such a case could not determine the lowered pressure since the same arteriole supplies both nets of capillaries. If this is found to be consistently the case, that constricted capillaries have a lower pressure than dilated vessels, independent of arteriolar change it would seem to be an additional factor to be taken into consideration in
the explanation of the reduced rate of passage of substances through the walls of such vessels and possibly aid in explaining the reduced permeability of constricted capillaries as compared to dilated capillaries.

Spontaneous variations of pressure have been found to be accompanied by changes in diameter as in the following protocol:

**Protocol III**

Pipette introduced into capillary, and pressure found to be

<table>
<thead>
<tr>
<th>Pressure (cm. water)</th>
<th>Diameter (micra)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.0</td>
<td>21</td>
</tr>
<tr>
<td>12.5</td>
<td></td>
</tr>
</tbody>
</table>

Gradually the flow increased in velocity till the corpuscles passed so rapidly that no individual cells could be seen. At this time pressure was 17.5 cm. Capillary diameter increased to 29 micra. The dilatation was obvious to the eye, even without measurement by the ocular micrometer. Coincidently pulse pressure was much increased over that at the lower level.

<table>
<thead>
<tr>
<th>Pressure (cm. water)</th>
<th>Diameter (micra)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.5</td>
<td>29</td>
</tr>
<tr>
<td>17.4</td>
<td></td>
</tr>
<tr>
<td>17.0</td>
<td>27-28</td>
</tr>
<tr>
<td>16.0</td>
<td></td>
</tr>
<tr>
<td>15.0</td>
<td>24</td>
</tr>
<tr>
<td>14.5</td>
<td>20-21</td>
</tr>
<tr>
<td>13.2</td>
<td>22</td>
</tr>
<tr>
<td>12.7</td>
<td>21</td>
</tr>
<tr>
<td>12.7</td>
<td>20</td>
</tr>
<tr>
<td>14.0</td>
<td>21</td>
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<tr>
<td>15.0</td>
<td>25-26</td>
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<tr>
<td>13.7</td>
<td>21</td>
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<tr>
<td>13.2</td>
<td>22</td>
</tr>
<tr>
<td>12.5</td>
<td>25</td>
</tr>
</tbody>
</table>

Finally stasis with a diameter once more of 29 micra.

Whether dilatation in such a case is the cause or effect of the change of pressure is entirely uncertain. Efforts have therefore been made to measure the pressure in a dilated capillary, to introduce from the pipette some substance, such as acid, or pituitrin, which should constrict the capillary while the arteriole remains the same, then to repeat the pressure determination. In some cases there is a definite fall of pressure coinciding with the constriction but the results are not sufficiently uniform to draw conclusions, though the tendency is definitely downward.

Measurements have also been made of pressure in capillaries under conditions producing increased flow and raised pressure coincidently determining changes in diameter of the vessels observed, to detect possible increase in size with rising pressure. The dilatation and increased rate of flow observed on the application of urethane to the mesentery is accompanied also by a slightly raised pressure as shown in figure 10. This
is independent of arteriolar change since urethane has no effect on any vessels but capillaries (Krogh, 1922). The dilatation may be measured and shows some correlation with the rise in pressure. The application of saline at a higher temperature to the mesentery results in a more rapid flow, dilatation of capillaries and higher pressure. Arteriolar changes may complicate this rise, however.

The evidence given is not definite enough to draw conclusions of any sort but apparently the tendency is toward a more rapid flow, higher pressure, and increased pulse pressure in more dilated vessels. This point will necessarily be considered in more detail in a later paper in relation to permeability, especially of constricted and dilated capillaries.

Discussion. The capillary pressures measured in the frog by Roy and Brown are generally conceded to be too high because of obstructed flow at the time of determination. A recent series of papers by Hill and McQueen (1921), (1922) detail the results obtained by the use of the same apparatus, by which pressure is applied over a large area of membrane, as the web or mesentery, or even a mass of tissue, as in the tongue or kidney. Pressure is applied to veins, venules, capillaries and arterioles alike. They record the pressure which, when momentarily applied to

![Fig. 10. Record of the changes in venous capillary pressure and capillary diameter after application of urethane to the mesentery. The inset shows the arrangement of the vessels. A was a small arteriole from which two capillaries rose to fuse into one, 2, which emptied into the venous capillary, V, into which the pipette was introduced. The diameter of capillaries 2 and 3 were measured at intervals and the change in diameter is charted below in arbitrary units.](http://ajplegacy.physiology.org/Downloaded from http://ajplegacy.physiology.org/ by 10.220.33.6 on May 8, 2017)
all these vessels, is sufficient to modify but not to stop flow in the capillaries or arterioles. The pressures thus determined have been very low, 2 to 5 cm. of water in the mesentery and web, 5 to 10 mm. Hg in the tongue and kidney.

Venous pressure in the mesentery, according to the micro-injection method varies from 4.5 to 12.5 cm. of water with most values lying between 5 and 8 cm. A momentary stoppage or even reversal of flow in a few capillaries can be produced locally by but partially compressing one of the larger veins of the mesentery, with the glass ball. Since occlusion is only partial the pressure exerted is less than full venous pressure.

It would appear possible that momentary pressure producing a partial occlusion of all the veins in the area might slightly modify flow in a few capillaries without equalling even venous pressure, much less the true capillary pressure. It is also suggestive possibly that the lowest pressures have been found in the mesentery and web, both thin tissues in which the veins, by their greater diameter, project above the other vessels, and would be first compressed by the distended membrane of the apparatus, whereas in the masses of tissue, where less direct pressure is brought to bear on the veins, higher pressures are recorded,—5 to 6 cm. of water in the tongue papillae, and for the kidney glomeruli 5 to 10 mm. Hg.

The relationship between the pressure in the capillary field and the osmotic pressure of blood colloids for the frog is of interest with reference to the interchange of fluid. White (1924) has found the osmotic pressure of plasma proteins of three frogs ranging between 9.6 and 11.5 cm. of serum, or 10.0 to 11.9 cm. water. Thus the average pressure in the arterial end of the capillary is above, and that in the venous capillary is slightly below, the average osmotic pressure of the colloids as reported by White. Moreover capillary pressure changes continually, at some moments above, at others below, the reported colloid osmotic pressure, and flow is rapid or slow in the same relation. It is conceivable that in any capillary the flow of water through the wall might be outward at one moment, and inward at another, throughout the whole length of the capillary.

Apparently peripheral resistance, as suggested by Dale and Richards, is not limited to the arterioles alone. As the arterioles dilate the fall of pressure may be shifted more and more into the capillary area. On the other hand after hemorrhage, or after the application of adrenalin, the mesenteric circulation may be in large part cut out by the constriction of the larger arterioles and smaller arteries. Pressure is then at a low level in the capillary vessels irrespective of their condition, whether dilated or constricted, and the flow of blood is reduced, the mesentery becoming pale and bloodless. The dual resistance may have a local and general significance. The arteriolar resistance can apparently divert
much of the blood from the mesenteric circulation, thus increasing the
blood volume passing to the remainder of the body, and diminishing the
volume of the peripheral circulation. In the absence of such need for
additional blood volume this resistance may be lowered, and capillary
reactions would then be able to determine a local distribution to fulfill
needs in the different portions of the mesentery or intestine. Such
resistance would determine the high pressure and rapid flow in one capil-
lar net, while an adjacent one arising from the same arteriole is com-
posed of constricted vessels, with low pressure and reduced flow.

Further determinations of complete gradients under diverse condi-
tions, local and general, will in all probability do much in clarifying the
relationship between these two sets of resistance, the mere existence of
which is here indicated.

SUMMARY

A method for the determination of pressure in individual capillaries
by micro-injection is described. It permits the measurement of mean
pressure, its fluctuations in successive periods, without disturbance of
flow, and in certain cases it can be used to determine systolic and diastolic
pressures in the capillary vessels of frog mesentery.

Capillary pressure in frog mesentery varies normally from 5.0 to 22.0
cm. of water, the large majority of determinations lying between 10 and
15 cm. Average pressure in the arteriolar capillary is apparently about
14.5 cm. and in the venous capillary 10.0 cm.

At the average rate of flow the fall of pressure in the peripheral vessels
of the mesentery does not cease abruptly at the junction of the arterioles
and capillaries, but continues to the venous capillary before flattening,
though pulse pressure is sharply diminished at the junction of arteriole
and capillary. The gradient varies markedly under different conditions
of arteriolar resistance, capillary pressure and flow.

Pressure and velocity of flow in the capillary vary in the same direction
and with rather close relationship. With an average flow of 0.5 mm.
per second capillary pressure is usually about 13.0 to 15.0 cm. when venous
pressure is 6.0 to 8.0 cm.,—a fall of 6 to 8 cm. from capillary to vein.
The average fall through the same region determined statistically is
6.9 cm.

Pressure differs not only in different capillaries coming from the same
arteriole, but also in the same capillary from moment to moment. Pres-
sure may be above or below the reported osmotic pressure of plasma
colloids throughout the whole length of any capillary in successive
moments.

Occlusion of flow in a single capillary raises the pressure on the arterial
side of the obstruction 3 or more centimeters even with fully open col-
laterals. Hemorrhage was found to reduce capillary pressure and increase plasma skimming. It is suggested that constricted capillaries may possibly have lower pressures than dilated vessels, independently of changes in the arteriole.

I wish to express my indebtedness and gratitude to Dr. M. H. Jacobs who suggested the application of the technique of micro-injection to the study of capillary permeability, and to Dr. R. Chambers for his kindness in demonstrating the use of his micro-injection apparatus and his method of making micro-pipettes.

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