Determinants of Tumor Blood Flow: A Review

Rakesh K. Jain

Department of Chemical Engineering, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213-3890

Abstract

Blood flow rate in a vascular network is proportional to the pressure difference between the arterial and venous sides and inversely proportional to the viscous and geometric resistances. Despite rapid progress in recent years, there is a paucity of quantitative data on these three determinants of blood flow in tumors and several questions remain unanswered. This paper reviews our current knowledge of these three parameters for normal and neoplastic tissues, the methods of their measurements, and the implications of the results in the growth and metastasis formation as well as in the detection and treatment of tumors.

Microvascular pressures in the arterial side are nearly equal in tumor and nontumorous vessels. Pressures in vesicular vessels, which are numerically dominant in tumors, are significantly lower in a tumor than those in a nontumorous tissue. Decreased intravascular pressure and increased intrastitial pressure in tumors are partly responsible for the vessel collapse as well as the flow stasis and reversal in tumors.

The apparent viscosity (viscous resistance) of blood is governed by the viscosity of plasma and the number, size, and rigidity of blood cells. Plasma viscosity can be increased by adding certain solutes. The concentration of cells can be increased by adding cells to blood or by reducing plasma volume. The rigidity of RBC, which are numerically dominant in blood, can be increased by lowering pH, elevating temperature, increasing extracellular glucose concentration, or making the suspending medium hypo- or hypertonic. Effective size of blood cells can be increased by forming RBC aggregates (also referred to as rouleaux). RBC aggregation can be facilitated by lowering the shear rate (i.e., decreasing velocity gradients) or by adding macromolecules (e.g., fibrinogen, globulins, dextrans). Since cancer cells and WBC are significantly more rigid than RBC, their presence in a vessel may also increase blood viscosity and may even cause transient stasis. Finally, due to the relatively large diameters of tumor microvessels the Fahraeus effect (i.e., reduction in hematocrit in small vessels) and the Fahraeus-Lindqvist effect (i.e., reduction in blood viscosity in small vessels) may be less pronounced in tumors than in normal tissues.

Geometric resistance for a network of vessels is a complex function of the vascular morphology, i.e., the number of vessels of various types, their branching pattern, and their length and diameter. Geometric resistance to flow in a single vessel is proportional to the vessel length and inversely proportional to vessel diameter to the fourth power. Hence, vessel diameter is the dominant parameter in flow regulation. In normal tissues, changes in vessel diameter are mediated primarily via smooth muscle cells. A tumor has two types of vessels: those recruited from the preexisting network of the host vasculature; and those resulting from the angiogenic response to cancer cells. Since a tumor rarely invades the arteries and arterioles of the host vasculature; and those resulting from the angiogenic response to cancer cells. Since a tumor rarely invades the arteries and arterioles of the host vasculature, the smooth muscle cells, with their contractile and nervous apparatus, surrounding these vessels may respond to physical or chemical stimuli. Since the newly formed vessels lack smooth muscle cells, they may not respond to these stimuli. As a result, the overall response of tumors will depend on the ratio of host vessels to newly formed vessels. This ratio would vary from one location to another and from one day to the next in the same tumor and from one tumor to another.

Recent studies suggest that endothelial cells and pericytes also possess contractile elements capable of causing changes in vessel diameter. The extent to which these cells control blood flow is not known. Other factors which may contribute to changes in effective diameter of tumor vessels include: swelling or destruction of endothelial cells; adhesion of platelets, WBC, or cancer cells to the vascular endothelium; and partial or total collapse of a vessel due to increased interstitial and decreased microvascular pressures. Due to micro- and macroscopic heterogeneities in the tumor microcirculation, care must be exercised in extrapolating from one tumor to another.

I. Introduction

Microcirculation plays an important role in the growth, metastasis, detection, and treatment of tumors. For example, angiogenesis and the resulting microcirculation are essential for supplying nutrients and for removing waste products during tumor growth. Similarly, blood and lymph vessels provide the vehicle for cancer cells to metastasize to distant organs/tissues. In radiotherapy, the efficacy of treatment depends upon the local oxygen concentration which is governed by the local blood flow. In chemotherapy and immunotherapy, blood flow plays an important role in the delivery of appropriate pharmacological agents. Finally, in hyperthermia, the temperature distribution and interstitial microenvironment in tumors are influenced by the local perfusion rates.

Flow rate of blood, Q, in any tissue, whether normal or neoplastic, is given by

\[ Q = \frac{\Delta P}{FR} \]  

where the pressure drop, \( \Delta P \), is the pressure difference between the arterial and venous ends of the tissue circulation. The flow resistance, \( FR \), is a complex function of the vascular morphology (i.e., the number of blood vessels of various types; their branching pattern; and their diameter, length, and volume) and the blood rheology (i.e., their viscosity). For the most simple case of laminar flow through a circular rigid vessel of radius \( R \) and length \( L \), the flow resistance is given by the Hagen-Poiseuille equation (1–3):

\[ FR = \frac{8\eta L}{\pi R^4} \]  

Thus, flow resistance is proportional to the blood viscosity, \( \eta \), and vessel length and inversely proportional to the fourth power of the vessel radius. For a network of blood vessels, it is convenient to express flow resistance as a product of \( \eta \) and where \( Z \) is referred to as a geometrical resistance (hindrance). Therefore, blood flow through a tissue is given by

\[ Q = \frac{\Delta P}{\eta \cdot Z} \]  

There are several comprehensive reviews on the vascular-extravascular exchange in tumors (4, 5) and the tumor blood flow and its modifications (e.g., Refs. 6–9); however, there are no reviews in the literature on the parameters which govern
tumor blood flow. Therefore, in this article I will critically review the three determinants of blood flow \((Z, \eta, \Delta p)\), the methods of their measurement, and their values in tumors under defined conditions. I will also attempt to identify the key unanswered questions for each of these parameters and suggest possible approaches for resolving these issues.

II. Vascular Morphology of Tumors: Structure, Organization, and Volume of Tumor Vascular Compartment

The literature on the vascular morphology of human and animal tumors has been reviewed recently by Warren (10) and Shubik (11). The structure, geometry, and arrangement of tumor vessels have been studied by many investigators since the work of Virchow (12). The motivations of these works have ranged from understanding pathophysiology (e.g., angiogenesis, growth, invasion, metastasis) to improving diagnosis and treatment of tumors. In what follows, I will briefly discuss the methods used, and then present qualitative and quantitative results.

A. Methods

The methodologies used to obtain tumor vascular parameters have included: (a) standard histological examination of tumors using light or electron microscopy (e.g., Refs. 10 and 13); (b) examination of tumor tissue injected with agents (e.g., India ink, benzidine, macromolecules, labeled RBC, colloidal carbon) which are retained by blood vessels (e.g., Refs. 14 and 15); (c) examination of the cast of the tumor vasculature made using a polymer (e.g., silicone rubber, vinyl acetate) or other substances (e.g., gallium) (e.g., Refs. 16–19); (d) gross or microscopic radiography and angiography (i.e., radiography with radiopaque contrast media) with or without the use of vasoactive agents (e.g., Ref. 20); and (e) intravital observation of tumor microcirculation with or without contrast agents (e.g., Ref. 21). The results have been analyzed either qualitatively to discern macro- and microscopic organization of the vasculature or quantitatively using stereological principles to obtain morphological parameters (e.g., vessel length, diameter, surface area, and volume).

B. Macroscopic Organization

Unlike most normal tissues, the tumor vasculature is highly heterogeneous and does not conform to the standard "normal" morphology (i.e., artery to capillary bed to vein). The vascular morphology of one tumor differs from another and to some extent is determined by the growth pattern of cancer cells. Macroscopically, the tumor vasculature can be studied in terms of two idealized categories (22).

In tumors with peripheral vascularization, the vessels are localized primarily at the periphery. The centers of these tumors are usually poorly perfused; hence penetration of blood-borne substances is difficult. As the tumor grows and invades the surrounding tissue, the vessels proliferate at the periphery. In tumors with central vascularization, vessels proliferate from the center like branches from a tree. One would expect a high vascular volume in the center of these tumors and a low vascular volume in the periphery.

In reality, the tumors may have many modules, each module exhibiting one of these two types of idealized vascular patterns. The macroscopic architecture is different among various tumor types and even between a spontaneous tumor and its transplants (23–25). As expected, the response of a tumor to therapy depends on both the macroscopic and microscopic vascular morphology (see, e.g., Refs. 23 and 26).

C. Microscopic Organization

Before I discuss the organization of vessels in the tumor microcirculation, it is worthwhile to review the most commonly accepted terminology of blood vessels in an "ideal" normal tissue with a simple route between arterial and venous sides (27–29). In such a tissue, blood flows successively through large arteries, small arteries, arterioles, terminal arterioles, capillaries, postcapillary venules, venules, small veins, and large veins.

The large and small arteries and arterioles are invested in smooth muscle cells and are capable of vasomotor adjustments. The terminal arterioles, the next branching order of arterial vessels, are invested proximally in vascular smooth muscle that gradually decreases until a single smooth muscle cell, spirally wrapped, marks the end of the muscular investment. This final smooth muscle cell is referred to as the precapillary sphincter and serves as the final control site for blood flow into the capillary bed.

Capillaries are vessels made of endothelial cells surrounded by a basement membrane. These vessels are devoid of smooth muscle cells and hence are incapable of active vasoconstriction/vasodilation. (The capability of endothelial cells and pericytes to cause vasomotion cannot be excluded.) Their large surface areas and structure make them the ideal site for the exchange of material between the blood and tissues. Normal capillaries can be divided into three categories based on their wall structure: (a) nonfenestrated (continuous); (b) fenestrated; and (c) discontinuous (sinusoids). Nonfenestrated capillaries are the most common type and are located in skin, connective tissue, skeletal and cardiac muscle, alveolar capillaries of the lung, and the brain. In fenestrated capillaries there are fenestrae or transendothelial circular openings of about 400–800 Å between the lumen and interstitium across the endothelial cells. These fenestrae may be open (e.g., in glomerular capillaries) or covered by a thin ~60–80-Å layered diaphragm similar to the diaphragm of vesicles. These capillaries are located in the intestinal mucosa, pancreas, glomerulus, peritubular capillaries, endocrine glands, the choroid plexus of the brain, and the ciliary body of the eye. Discontinuous capillaries have large diameters and wide openings between endothelial cells. Basal lamina is either discontinuous or absent. Examples of these capillaries include sinusoids of the liver, the spleen, and the bone marrow. (See Ref. 5 for relationship of structure with transport properties.)

When vessels converge toward the exit of the capillary network, the resulting vessel is referred to as a postcapillary venule. These vessels are larger in diameter than capillaries, are composed of a single layer of endothelial cells and a basement membrane, and are usually devoid of smooth muscle cells. These vessels have the weakest interendothelial junctions. Their sensitivity to prostaglandins, histamine, serotonin, and bradykinin makes them preferential sites for plasma extravasation and diapedesis in inflammation (30). These vessels join to form venules where the smooth muscle cells reappear.

In regions where the blood flow varies significantly over time, arteries may be directly connected to venules by arteriovenous anastomoses. The arterial side of arteriovenous anastomoses is invested in smooth muscle cells. These vessels are found in fingertips, toes, skin, nailfolds, lips, intestinal mucosa, thyroid, erectile tissue, etc.

In a normal tissue, these idealized vascular beds may be
connected in parallel or series depending upon the organ. For example, in the kidney, the glomerular capillaries are in series with the tubular capillaries. In the enterohepatic circulation, the capillary beds of the spleen are in parallel with those of the mesentery and in series with the liver microcirculation. For details of the vascular morphology of various organs and tissues, the reader is referred to the Handbook of Physiology (31).

When a group of cancer cells is placed in a host tissue, tumor angiogenesis leads to the formation of capillary sprouts with eventual development of a tumor microcirculatory network. Therefore there are two sources for blood vessels in a tumor: (a) those recruited from the preexisting network of the host tissue; and (b) those resulting from the angiogenic response of tumors. (For details on tumor angiogenesis, see Ref. 32.) Although the tumor microcirculation originates from the host vasculature, its organization may be completely different depending upon the tumor type, its growth rate, and its location in the tumor mass. As a result, the classification of blood vessels developed for normal tissues based on structure (anatomy) and function (physiology) may not be applicable to tumors. Warren (10) classified the blood vessels found in tumors into nine categories based on their ultrastructure. I have modified his nomenclature so that it corresponds as closely as possible to that discussed earlier for a normal tissue: Class 1, arteries and arterioles; Class 2, nonfenestrated capillaries; Class 3, fenestrated capillaries; Class 4, discontinuous capillaries (sinusoids); Class 5, blood channels without endothelial lining; Class 6, capillary sprouts; Class 7, postcapillary venules (giant capillaries); Class 8, venules and veins; and Class 9, arteriovenous anastomoses. Note that except for the capillary sprouts (Class 5) and the blood channels (Class 6), the remaining vessel types are structurally analogous to those found in a normal tissue. A key difference is that the tumor vessels may contain tumor cells within the internal lining of endothelial cells (33). The vessels of Classes 5 and 6 are found in a healing (granulation) tissue (34, 35). In the following, I will discuss characteristics of these vessels with examples of tumors where they are found.

Arteries and Arterioles. Aligire and Chalkley (36) and Gullino and Grantham (16) using a transparent mouse chamber and an isolated rat tumor preparation, respectively, showed that various sarcomas and carcinomas increased the caliber of the arteries of the host tissue. Willis (37), on the basis of his own work and that in the literature, asserted that even in the most invasive tumors, the structure of arterial wall does not change, and malignant invasion of arterioles is extremely rare. Perhaps the incorporation of “intact” arterial vessels in a tumor is partly responsible for vasoemotion and blood flow fluctuations observed in sandwiched tumors (38, 39) and for blood flow modifications due to heat and vasoactive agents (for a review, see Refs. 8 and 9).

Nonfenestrated Capillaries. Warren (40), on the basis of his study of hemangiopericytoma (a tumor derived from blood vessels) grown in a hamster cheek pouch chamber, concluded that nonfenestrated capillaries are found in the milieu of a highly differentiated tumor. These vessels possess intact endothelial walls and a distinct basement membrane supported by fine strands of collagen fibers.

Fenestrated Capillaries. These capillaries with openings in the endothelium have been found in the W256 carcinoma (41), in human renal carcinoma transplanted in nude mouse (42), and in various brain tumors (43). These fenestrations may be partially responsible for the large permeability of tumor vessels (5).

Discontinuous Capillaries. These vessels, which resemble the sinusoids in the liver and the spleen, are present in poorly differentiated mammary (44) and human renal carcinomas (45) and may be responsible for fibrin deposit in the extravascular space. Plasma fibrinogen may extravasate through these interendothelial openings, clot, and become cross-linked in the tissue space (5, 46).

Blood Channels. In melanomas and some sarcomas, blood channels are present which are not lined with endothelial cells (10). In these tumors, blood percolates between and around tumor cells; hence RBC come in contact with tumor cells. These channels are connected to sinusoids or other types of vessels.

Capillary Sprouts. Similar to neovascularization in a granulation tissue, tumor angiogenesis leads to capillary sprouts. These sprouts may be of the tapering type or saccular depending upon the caliber of the lumen. Endothelial cells of these sprouts differ from those of normal mature vessels (35). These newly formed vessels are extremely fragile and are surrounded by RBC and fibrin deposits due to petechial (pinpoint) hemorrhage. The lumen of the sprouts contains a static column of RBC which begin to flow when a capillary network is formed.

Postcapillary Venules. These vessels are highly tortuous and large in diameter. Unlike the postcapillary venules in normal tissues, these vessels are mostly devoid of basement membrane, and partly surrounded by fibrous tissue. These vessels are also referred to as giant capillaries (10) or as venous capsules. These vessels have also been identified as a site for cancer cell intra-vasation (47, 48).

Venules and Veins. Similar to postcapillary venules these vessels are highly tortuous, saccular, and dilated. The blood flow and oxygen content in these vessels change with time.

Arteriovenous Anastomoses. A tumor may contain a large number of arteriovenous anastomoses. This direct shunting of blood from the arterial to the venous side creates problems for delivery of materials (e.g., anticancer agents) into a tumor.

Using the above vessel classification it should be possible, at least in principle, to describe the organization of vessels in a tumor. At a given location, blood may be routed from the arterial (Class 1) to the venous side (Class 8) via vessels of Classes 2 through 7, or directly via arteriovenous shunts. Due to the transient nature of tumor microcirculation, it is also possible that blood may flow from a venule (Class 8) via blood vessels of Classes 2 through 7 and return to a venule (Class 8), similar to the “portal” system. Therefore, combinations of these vessel types may lead to a nearly infinite number of vessel organizations. Furthermore, due to the inherent heterogeneity in a tumor, the organization of vessels may be different from one location to another and from one day to the next day. As a result, one would expect different routes for blood flow in the well-perfused advancing zone, in the intermediate seminecrotic zone, and in the necrotic zone. A detailed study of vascular branching patterns in a tumor has not been done to date.

D. Quantitative Studies on Vascular Morphology

The spatial and temporal distributions of vascular volume, length, diameter, and vascular surface are defined as the vascular morphology of tumors. While there have been several studies in the literature since 1893 (10) on the vascular morphology of tumors, most of these are qualitative. The first quantitative study on tumor vascular morphology was conducted by Aligire and Chalkley (36) in two-dimensional tumors using the method of Chalkley (49). They examined the vascular volume of several rapidly growing tumors (e.g., sarcomas, malignant melanomas) and slowly growing tumors (e.g., pig-
mented and amelanotic melanomas) in a mouse transparent chamber. The rapidly growing tumors elicited new capillary sprouts from the host vasculature in 2–3 days post-tumor implant, and the vascular space increased to 40–50% within 5–8 days. The slowly growing tumors took at least 8 days for the initial sprout formation and the vascular space never exceeded 25% during the entire observation period. The capillaries in rapidly growing tumors were about 5 times the diameter of those in normal tissue, appeared like sinusoids, and rarely differentiated into arterioles and venules. Whereas in slowly growing tumors, the capillaries had the same diameter as normal capillaries and showed considerable differentiation into arterioles and venules.

Following Algire’s pioneering work, several investigators have analyzed growth of vessels in two-dimensional tumors in transparent windows using intravital microscopy. Yamamura and Sato (50) have carried out perhaps the most extensive quantitative study of the vasculature developed in ascites hepatoma AH109A transplanted in the dorsal skin chamber in Donryu rats. These authors divided the growth period of 25 days into four stages according to changes in vascular morphology: Stage 1, slight capillary alterations; Stage 2, formation of fine capillary network; Stage 3, modifications in arteries; and Stage 4, necrosis. These authors used Chalkley’s method to measure vascular volume and Vogel’s approach (44) to measure length and surface area. The diameters of 200 vessels were measured at random and represented in a histogram, where vessels in each diameter class were recorded. The vessel length (cm/m³ tissue), surface area (mm²/mm³ tissue), and volume (%) increased from 36, 20, and 20, respectively, in the s.c. tissue (control) to 160, 70, and 50 in the second stage of growth and then decayed to nearly zero in Stage 4. In the second stage of tumor growth, the speed of neovascularization was 40–50 times higher than that in a granulation tissue (51). The number of small vessels (<10 μm) increased from 40% in Stage 1 to 70% in Stage 2. In Stages 3 and 4 the frequency of small diameter vessels decreased, and the frequency of larger diameter vessels increased. Vessels with diameters of up to 200 μm have been reported by Eddy and Casarett (52) in a malignant neurilemoma grown in a hamster cheek pouch and by Peters et al. (53) in a DMBA²-induced mammary adenocarcinoma grown in a dorsal chamber in rats.

Using photomicroscopy, Endrich et al. (39) and Asaishi et al. (54) have measured the frequency distribution of the capillary diameter and length as a function of days postimplant in BA1112 sarcoma in dorsal skin chambers in Wistar (WAG/Rij) rats and in A-Mel-3 amelanotic melanoma in dorsal skin chambers in hamsters, respectively. Both of these studies show an increase in capillary diameter and length with tumor growth. Recently, using photomicroscopy and the stereological methods of Underwood (53), we have measured the vessel diameter distribution, vessel length, surface area, and volume in granulation tissue (51) and in VX2 carcinoma grown in a rabbit ear chamber (56). The similarity in the growth characteristics of the granulation tissue and the tumor is noteworthy. Our tumor data show increases in length, volume, and surface area similar to those reported in other two-dimensional tumors.

While tumors grown in transparent chambers provide the luxury of intravital observation, the results obtained must be interpreted with caution. A major criticism of this approach is the elevation of tissue pressure due to the proliferation of tumor cells and blood vessels in a rigid enclosure. This increase in tissue pressure may lead to vascular stasis by crushing the blood vessels and ultimately to pressure necrosis. This was confirmed by Younger and Algire (57) who reported return of blood flow in several vessels after the chamber was loosened. Therefore, the development of necrosis, although an inherent property of tumors, may be partly due to the technique itself in sandwich tumors. A second related problem with the chamber technique is that once the vessel density increases beyond a certain value, it is hard to observe the microcirculation. Therefore, reliable data can be obtained only in the early stages of tumor growth. Finally, the tumors must be transplanted in s.c. tissues, and their growth is restricted in the lateral direction.

The injection of dyes (microangiography), polymers, macromolecules (dextrans), labeled erythrocytes, or other stain permits quantification of vascular morphology of three-dimensional tumors in a variety of sites. Due to unrestricted growth of tumors, the development of necrosis is less artificial. The main disadvantage of this approach is the inability to follow the growth of the same tumor. As a result, these techniques are invasive in that they require the sacrifice of animals and removal of tissue and require a large number of experimental techniques to relate structure with function.

Following the pioneering work of Gullino and Grantham (58), several investigators have used these injection techniques to measure vascular volume of tumors at various stages of growth (Table 1). Note that the vascular space in tumors varies from 1% in transplanted fibrosarcoma 4956 (58) to 27.5% in a spontaneous canine lymphosarcoma (69). The measured value of vascular space of a given tumor depended upon the marker used. For example, the vascular space of W256 carcinoma was found to be 10.6 ± 0.5% (SD) using dextran 500 (M, 375,000; Mw, 180,000) (58) and to be 0.79 ± 0.06% using 51Cr-labeled erythrocytes (65). The reasons for this profound discrepancy are not completely clear. Possible reasons for overestimation using dextran may be the extravasation and binding of dextrans (5, 70) and reasons for underestimation using erythrocytes may be the significantly lower microvessel hematocrit compared to systemic hematocrit (Refs. 3 and 68; also see Table 2 and Section III).

The results on the changes in vascular volume as a function of tumor growth also depend on the tumor type. For example, Gullino and Grantham (58) found that the vascular space increased linearly with tumor size for W256 when the tumors were small (4–12 g), but no correlation was possible for large tumors. On the contrary, Song and Levitt (65) reported a decrease in vascular space as W256 tumors grew from 1 to 25 g. Similarly, Vaupel (66) reported a decrease in vascular space and an increase in intercapillary distance in the growing DS carcinosarcoma in rats, especially in the initial phase of tumor growth. Song et al. (62) found no significant changes during growth in the vascular volume of a mouse neuroblastoma. Liotta et al. (77) measured the vessel diameter distribution in T241 fibrosarcoma grown in C57BL mouse and found that the fraction of vessels with diameters of >5 μm decreases exponentially. The size distribution and the mean diameter of vessels did not change significantly during tumor growth (7–15 days), except for an increase in the number of large vessels (>100 μm diameter) at later time periods. Recently, Karlsson et al. (67) measured the intratumor distribution of RBC volume and plasma volume in a rat sarcoma at 14 and 20 days. Similar to Gullino and Grantham (58), these authors found no significant difference in distribution of spaces in the peripheral and central (necrotic) regions of these tumors, whereas they found a wide heterogeneity of spaces in each region. Both RBC volume and

² The abbreviation used is: DMBA, 7,12-dimethyl-benz(α)anthracene.

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Table 1  Typical values of vascular space in solid tumors

<table>
<thead>
<tr>
<th>Host</th>
<th>Tumor</th>
<th>Wt range (g)</th>
<th>Method</th>
<th>Vascular space (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>72j mammary carcinoma</td>
<td>0.1-2.4</td>
<td>Morphometry</td>
<td>15-18</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Mammary adenocarcinoma</td>
<td>0.1-2.4</td>
<td>Morphometry</td>
<td>17</td>
<td>59</td>
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<tr>
<td></td>
<td>SCK mammary carcinoma</td>
<td></td>
<td>RBC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>SLC carcinoma</td>
<td>0-0.9</td>
<td>RBC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.7</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Neuroblastoma</td>
<td>0.05-13</td>
<td>RBC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.9</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Gardner lymphosarcoma</td>
<td>(14 days)</td>
<td>RBC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>~2</td>
<td>63</td>
</tr>
<tr>
<td>Nude mouse</td>
<td>Clouser human breast carcinoma</td>
<td>0-1</td>
<td>RBC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>~4</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>SL2 murine lymphoma</td>
<td>0-1</td>
<td>RBC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>~5</td>
<td>64</td>
</tr>
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<td>Human melanomas</td>
<td>0.2</td>
<td>Morphometry</td>
<td>0.9-2.2</td>
<td>15</td>
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<td>Rat</td>
<td>Walker 256 carcinoma</td>
<td>3.5-13.0</td>
<td>Dextran</td>
<td>10.6</td>
<td>58</td>
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<td></td>
<td>Walker 256 carcinoma</td>
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<td>0.8</td>
<td>65</td>
</tr>
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<td>Dextran</td>
<td>1.0</td>
<td>58</td>
</tr>
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<td></td>
<td>Hepatoma 5123</td>
<td>1.0-12.9</td>
<td>Dextran</td>
<td>4.5</td>
<td>58</td>
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<td>Hepatoma 3683</td>
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<td>Dextran</td>
<td>5.0</td>
<td>58</td>
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<td>Novikoff hepatoma</td>
<td>1.8-6.9</td>
<td>Dextran</td>
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<td>58</td>
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<td></td>
<td>Hepatoma LC&lt;sup&gt;+&lt;/sup&gt;</td>
<td>7.0-32.1</td>
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<td>Dextran</td>
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<td>Dextran</td>
<td>8.1</td>
<td>58</td>
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<td>Liver</td>
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<td>Dextran</td>
<td>18.6</td>
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<td>DS carcinosarcoma</td>
<td>3-11</td>
<td>Morphometry</td>
<td>0.4-4.0</td>
<td>66</td>
</tr>
<tr>
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<td>MCA sarcoma</td>
<td>0.5</td>
<td>RBC and albumin</td>
<td>7-14</td>
<td>67</td>
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<td>Fibrosarcomas</td>
<td>?</td>
<td>RBC and IgG</td>
<td>1.0</td>
<td>68</td>
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<td></td>
<td>A-MC</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>C-MC</td>
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<td>BP-11</td>
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<tr>
<td>Hamster</td>
<td>Hemangiopericytoma</td>
<td></td>
<td>Morphometry</td>
<td>21</td>
<td>40</td>
</tr>
<tr>
<td>Dog</td>
<td>Spontaneous lymphosarcoma</td>
<td></td>
<td>Antipyrine</td>
<td>14-27.5</td>
<td>69</td>
</tr>
</tbody>
</table>

<sup>LC</sup>, low catalase; <sup>HC</sup>, high catalase; <sup>a</sup>, ascites form; <sup>S</sup>, solid s.c. form.

* Assumed $H_{\text{sm}} = H_{\text{sys}}$.

Table 2  Average microvascular hematocrits of normal and neoplastic tissues obtained using labeled RBC and plasma<sup>4</sup>

<table>
<thead>
<tr>
<th>Species/tissue</th>
<th>$H_{\text{sm}}/H_{\text{sys}}$</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Cardiac muscle</td>
<td>0.65</td>
<td>71</td>
</tr>
<tr>
<td>Lung</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>1.22</td>
<td></td>
</tr>
<tr>
<td>Thyroid</td>
<td>0.65&lt;sup&gt;+&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>0.65&lt;sup&gt;+&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>0.43</td>
<td>68</td>
</tr>
<tr>
<td>A-MC fibrosarcoma</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>C-MC fibrosarcoma</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>BP-11 fibrosarcoma</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>Rabbit placenta</td>
<td>0.68</td>
<td>72</td>
</tr>
<tr>
<td>Cat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>0.18</td>
<td>73</td>
</tr>
<tr>
<td>Tongue</td>
<td>0.84</td>
<td>74</td>
</tr>
<tr>
<td>Dog lung</td>
<td>0.84-0.95</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cranium</td>
<td>0.84</td>
<td>75</td>
</tr>
<tr>
<td>Cerebrum</td>
<td>0.92</td>
<td>76</td>
</tr>
</tbody>
</table>

<sup>4</sup> Most of the normal tissue data were taken from Ref. 3.

<sup>a</sup> Ratio of average microvascular hematocrit to systemic hematocrit.

<sup>4</sup> Assuming $H_{\text{sys}} = 45\%$.

marked widening of diameter with growth, accompanied by a marked decrease in vessel length (134 to 19 cm/mm<sup>2</sup> tissue) and surface area (46 to 14 mm/mm<sup>2</sup> tissue). Vascular volume of 16% did not change with growth. He referred to vessels with diameter <12 μm as capillaries and those with diameter >12 μm as sinusoids. Vogel attributed the decrease in the surface area of vessels with growth to the increase in the ratio of sinusoids to capillaries.

Hilmas and Gillette (59) repeated similar studies in another mammary adenocarcinoma grown in the gastrocnemius muscle of the right rear leg of C3H/BL mouse. They reported their findings in terms of viable (nonnecrotic) tissue which decreased from >95% in 35 mm<sup>3</sup> to <60% in tumors over 1500 mm<sup>3</sup> (Fig. 1). The vascular volume remained constant around 17% during growth (Fig. 2). The vessel length decreased dramatically in the

![Figure 1](https://example.com/figure1.png)
beginning and then more gradually (Fig. 1). As seen in Fig. 2, the vessel surface area also decreased rapidly during the early phase of growth, and vessel diameter increased as more sinusoids appeared in a growing tumor. Interestingly, despite the differences in the methods of analysis, Hilmas and Gillette's results are in excellent agreement with those of Vogel (44). These authors also studied the effect of radiation therapy on the vascular morphology of tumors.

Recently, the vascular morphology of human tumor xenografts in nude mice has been characterized by Solesvik et al. (15) and Kraus et al. (79). Using stereological principles, Solesvik et al. studied five melanoma xenografts with volume-doubling times ranging from 4.2 to 21.6 days and found that each tumor had its own characteristic vascular structure. On a per mm$^3$ histologically intact tissue basis, the total vessel length ranged from 32 ± 2 to 80 ± 4 mm, the total vessel surface area ranged from 1.6 ± 0.1 to 3.8 ± 0.2 mm$^2$, and the total vessel volume ranged from 0.009 ± 0.001 to 0.022 ± 0.002 mm$^3$. The necrotic fractions ranged from 30 ± 1 to 49 ± 4%; they were inversely proportional to vessel volumes and directly proportional to doubling times. The vascular system of xenografts originates from the host animal, and hence the vascular structure may not be totally representative of human tumors in situ. Nevertheless these studies provide a bridge between experimental animal tumors and human tumors.

One aspect of vascular morphology where very little quantitative data are available is the distribution of intercapillary distance in tumors. Thomlinson and Gray (80) found histologically a band of viable tumor cells about 100 μm wide between the blood vessel and necrosis in human lung cancers. Tannock (81) found viable tumor cords with a radius of 60–120 μm surrounding capillaries in a highly necrotic mouse mammary carcinoma. These authors referred to this viable zone as the diffusion length of oxygen and nutrients in tumors. Tannock and Steel (82) showed that in this mouse mammary tumor, the intercapillary distance increased as the tumor grew. Similar results were reported by Vaupel (66) in rat tumors. The decrease in vascular surface area and capillary density in tumors with increasing weight was explained by Tannock (83) on the basis of difference in “turnover” times of endothelial and neoplastic cells. Tannock and Hayashi (84) found the average turnover time of endothelial cells to be about 50–60 h as compared to the turnover time of 22 h for neoplastic cells. Interestingly, Denekamp (85) and coworkers have found that endothelial cells of animal and human tumor multiply at least an order of magnitude faster than those of normal vessels. Hammersen et al. (33) propose that the increased turnover of tumor endothelial cells reported by Denekamp may be a result of integration of tumor cells into the lining of newly formed and growing vessels. Interestingly, Tannock et al. found several blood vessels within necrotic tissue with stagnant blood in them. These results point out the problems in relating structure with function.

E. Summary

The tumor vasculature is highly heterogeneous and does not conform to the “standard” normal vascular organization. The vascular morphology of one tumor differs from another and is determined to some extent by the growth pattern of cancer cells. Macroscopically, the gross architecture of a tumor vasculature falls into two categories, peripheral and central. Microscopically, the tumor vessels can be classified into nine categories: arteries and arterioles; nonfenestrated, fenestrated, and discontinuous capillaries; blood channels; capillary sprouts; postcapillary venules; venules and veins; and arteriovenous anastomoses. There are no quantitative data on the detailed branching pattern of these vessels in a tumor.

Quantitative morphometric studies in two-dimensional tumors show that vascular volume, length, and surface area increase during the early stages of tumor growth, and then decrease after the onset of necrosis. Frequency of large diameter vessels increases in the later stages of growth. Most quantitative studies in three-dimensional tumors miss the early growth period of increase in vascular volume, length, and surface area. While studies of later stages of growth show a decrease in vessel surface area and length, the results on vascular volume are mixed. Some studies show that the fractional vascular volume of tumors remains fairly constant during growth, suggesting an increase in the number of blood vessels with low blood flow, while others show that the fractional vascular volume decreases as a tumor grows, in agreement with the observation that tumor blood flow rate per unit tumor weight decreases as a tumor grows.

There are no quantitative data on the vascular morphology of human tumors. These data should be obtained by studying human tumors in situ using a variety of noninvasive techniques or by perfusing human tumors ex vivo (17, 86).

III. Rheological Properties of Blood and Cells in Normal and Tumor Circulation

A major resistance to fluid flow in a tube or vessel is the fluid viscosity. For simple shear flows, the viscosity $\eta$ is defined as the ratio of the shear stress $\gamma$ to the shear rate $\dot{\gamma}$

$$\eta = \gamma / \dot{\gamma}$$  \hspace{1cm} (D)

where shear stress on a surface is the force per unit area acting on the surface in a direction tangential to the surface and shear rate is the velocity gradient in the fluid. When $\dot{\gamma}$ varies linearly with $\gamma$ and $\eta$ is constant, the fluid is referred to as Newtonian. Examples of such fluid are water, saline, plasma, and honey. When $\dot{\gamma}$ is a nonlinear function of $\gamma$, the fluid is referred to as non-Newtonian, e.g., blood. For non-Newtonian fluids, the ratio of $\gamma / \dot{\gamma}$ is referred to as the apparent viscosity, $\eta_a$, and is a function of $\dot{\gamma}$. For example, the rheological behavior of blood can be described by the nonlinear equation developed by Casson.

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2 Units: Viscosity: poise (P) = 100. P = 1 dyn. s/cm² = dPa.s. Shear stress = Pa = N/m² = 10 dyn/cm². Shear rate = s⁻¹.
(E) \[ \sqrt{\tau} = \sqrt{\tau_r} + \sqrt{n_\eta \sqrt{\gamma}} \]
where \( \eta_r \) is the Casson viscosity and \( \tau_r \) is the yield stress (i.e., such a fluid will not flow if \( \tau < \tau_r \)). In this equation, \( \tau_r \) and \( \eta_r \) are functions of the hematocrit, \( H \), and the plasma protein concentrations. (For details, see Ref. 88.)

In Poiseuille flow through a tube of radius, \( R \), the velocity profile is parabolic with maximum velocity in the center and zero at the walls, and the shear rate (i.e., velocity gradient) increases linearly with radial distance from the center (where it is zero) and reaches it maximum value at the wall, \( \gamma_w \). For such profiles, the maximum velocity, \( V_{max} \), is equal to twice the mean velocity, \( \bar{V} \). It can be also shown that for Newtonian fluid, the wall (maximum) shear rate, \( \gamma_w \), is equal to \( 4\bar{V}/R \), and the mean shear rate (\( \gamma \)) is equal to two-thirds of the maximum shear rate:

- \( V_{max} = 2\bar{V} \)  
- \( \gamma_w = 4\bar{V}/R \)  
- \( \gamma = \frac{2}{3} \gamma_w = \frac{8\bar{V}}{3R} \)

For non-Newtonian fluids, the velocity profile resembles a blunted parabola, and the wall shear rate is usually higher than for a Newtonian fluid. For such fluids, \( 4\bar{V}/R \) may be used as an apparent wall shear rate (\( \gamma_w \)).

Under normal conditions, blood can be divided by volume into about 55–60% plasma and about 45–40% cells, or “formed elements.” The plasma is essentially a dilute electrolyte solution containing total solutes equal to 9.55 weight %, most of which are proteins (~8 weight %). Plasma behaves in a Newtonian fashion with a viscosity, \( \eta_p \), of 1.2–1.3 cP at 37°C. Plasma viscosity is a function of protein concentration, primarily fibrinogen and some of the globular proteins (2). The cells consist of ~95% RBC, ~0.13% WBC, and ~4.9% platelets, by number. In cancer patients, cancer cells are also present in blood. The deformability and interaction of these various types of cells with one another and the vessel wall affect the flow behavior of blood.

The rheological behavior of blood, therefore, depends not only on shear rate but also on cell concentration, cell deformability, cell aggregation, plasma viscosity, protein concentration, and temperature. In what follows, I will first outline the methods of measurement of blood viscosity and its key determinants, cell concentration and cell deformability, both in vitro and in vivo; then I will present pertinent in vitro and in vivo rheological data for normal blood and finally discuss the rheology of blood in cancer patients and in tumor microcirculation.

**A. Methods**

1. Apparent Viscosity. Apparent viscosity measurements of blood are made in vitro in either rotational viscometers (e.g., two concentric cylinders or a cone and plate geometry) or capillary tube viscometers. The former type provide well-defined shear rates; the latter types are cheaper, are easier to use, and require much smaller fluid volume. The problem with tube viscometers, however, is that \( \eta_r \) is a function of tube diameter for blood (see the Fahraeus-Lindqvist effect in Section IIIB).

Information on in vivo blood viscosity in the microcirculation has come from pressure-flow (\( \Delta P-Q \)) relationship obtained using macroscopic or microscopic approaches. In the macroscopic approach, an isolated organ or tissue is perfused alternately with Ringer’s solution and whole blood, and the flow resistances are computed using Equation C. If the geometric resistance is assumed to remain unchanged during alternate perfusions, and if the viscosity of Ringer’s solution is known, the in vivo apparent viscosity of blood, \( \eta_{app} \), is

\[ \eta_{app} = \frac{(FR)_{blood}}{(FR)_{Ringer}} \cdot \eta_{Ringer} \]

By varying the hematocrit and other constituents of blood, the effect of various parameters on \( \eta_r \) can be obtained.

In the microscopic approach, \( \Delta P, Q, L, \) and \( R \) are measured in a microcirculatory preparation in vivo using intravital microscopy and modern optoelectronic instrumentation. The apparent viscosity of blood is then calculated assuming that Hagen-Poiseuille equation (A and B) holds for all segments of the vasculature. (The methods of measurement of \( L \) and \( R \) were discussed in Section II, and those of \( \Delta P \) and \( Q \) are discussed in Section IV and Ref. 48, respectively.) Since it is possible to optically measure the hematocrit, \( H \), this approach also permits evaluation of the relationship between \( \eta_r \) and \( H \). The major problem with both macro- and microscopic methods is the inability to determine the shear rates precisely.

2. Cell Concentration and Hematocrit. Concentration of RBC (hematocrit) can be easily measured in vitro by centrifuging whole blood and evaluating fractional volume occupied by packed RBC. Measuring concentrations of WBC, platelets, and cancer cells requires separating or staining these cells and counting their number in a known volume of sample either manually or optoelectronically.

Since microvascular hematocrit is an important determinant of blood viscosity and of oxygen transport, several measurements have been made of in vivo hematocrit using macroscopic and microscopic approaches. In the macroscopic (or whole organ/tissue) approach, an average (lumped) hematocrit of all vessels in a tissue is made by indirectly measuring the fractional volume of RBC by radiolabeling RBC and plasma. The key problem with this method is the extravasation of plasma markers (e.g., albumin), which must be accounted for in estimating the volume (5).

In the microscopic approach, hematocrit in individual blood vessels is measured in various microcirculatory preparations using various optical and electronic techniques (3). These techniques include: (a) direct cell counting using still photography in a blood vessel after flow has been occluded by a blunt instrument, referred to as flow occlusion method; (b) direct cell counting using high speed cinephotomicrography or stroboscopic lighting; (c) electronic cell counting by monitoring the changes in light intensity caused by the single-file passage of RBC through a capillary; (d) measurement of the time-averaged intensity of light transmitted through a vessel, referred to as opacity; and (e) correlation between hematocrit and absorbance \( = \log \left(I_0/I\right) \) where \( I_0 \) and \( I \) are intensities of incident and transmitted light, respectively.) According to Zweifach and Lipowsky (3), the first four methods are generally limited to small capillaries and/or vessels with low hematocrits, while the fifth method is useful for larger vessels (>20 μm diameter).

While any of the above methods can be used to measure concentrations of other blood cells or cancer cells in microvessels, recent developments in intravital fluorescence microscopy facilitate their visualization and, hence, quantitative measurements.

3. Cell Deformability. Deformability of various types of cells can be studied qualitatively by observing/photographing cells...
as they pass through narrow glass tubes in vitro or capillaries in vivo. Quantitative measurements of cell deformability have come from two methods: (a) filtration experiments, in which pressure required to filter a cell suspension through a membrane of known pore size is used as a measure of cell rigidity; and (b) micropipet aspiration experiments, in which the rate and extent of deformation of a cell of radius \( R_c \) aspirated by a micropipet of radius \( R_p < R_c \) are used to calculate the rheological properties of the cell. The latter method has been used to quantify rigidity of RBC, WBC, and cancer cells. A less commonly used method to measure deformability is based on the analysis of cell deformation in a well-defined flow field in a rheoscope (89).

4. Cell Aggregation and Adhesion. At low shear rates, the bridging of adjacent RBC by fibrinogen, globulins, or other macromolecules (e.g., dextran) causes these cells to aggregate and form rouleaux. Stresses greater than \( >0.1 \text{ dyne/cm}^2 \) cause rouleaux disaggregation. The net aggregation energy can be estimated from the shear stress required to disaggregate the rouleaux or from changes in RBC membrane strain energy due to elastic deformation of cells in rouleaux (2, 90). Similarly, the shear stress required to detach WBC, platelets, or cancer cells from a surface (e.g., glass coverslip, vascular endothelium, another cell) can provide a measure of cellular adhesion.

B. Rheological Behavior of Normal Blood in Vitro and in Vivo

The importance of blood rheology in blood flow in normal microcirculation has prompted numerous studies on this subject. (For reviews, see, e.g. Refs. 2 and 3.) In the following, I will present and discuss typical data to provide a background for understanding alterations in blood rheology during the neoplastic disease.

1. Effect of Shear Rate. Shown in Fig. 3 is the in vitro relationship between apparent viscosity, \( \eta_a \), and shear rate, \( \dot{\gamma} \), in normal human blood with a hematocrit of 45%. Note that at low shear rates RBC aggregate and form rouleaux leading to an increase in \( \eta_a \). As shear rate (or stress) is increased, these rouleaux break up and \( \eta_a \) reaches an asymptotic value at shear rates \( >100 \text{ s}^{-1} \). An increase in shear stress also causes deformation of the dispersed RBC and the alignment of their major axes with the direction of flow. Thus, disaggregation and deformation both contribute to the reduction in \( \eta_a \).

Whitmore (91) estimated the apparent shear rates in the circulation of humans and found \( \dot{\gamma}_m \) to vary between 60 and 80 \( \text{s}^{-1} \). Lipowsky et al. (92) recently measured the mean blood velocity, \( P \), in the microcirculation of cat mesentery and found \( \dot{\gamma}_m \) for 10- to 60- \( \mu \text{m} \)-diameter vessels to be 1200 \( \text{s}^{-1} \) in the arterioles and 850 \( \text{s}^{-1} \) in the venules, with an overall average of 1330 \( \text{s}^{-1} \). More specifically, they found that \( \dot{\gamma}_m \) rose from \( \sim 1400 \text{ s}^{-1} \) in 50- \( \mu \text{m} \) arterioles to a maximum of \( \sim 1800 \text{ s}^{-1} \) in 20- \( \mu \text{m} \) terminal arterioles, and then it steadily declined to a minimum of 500 \( \text{s}^{-1} \) in the 20- \( \mu \text{m} \) postcapillary venules but rose to 800 \( \text{s}^{-1} \) in 50- \( \mu \text{m} \) venules. Based on the in vitro data in Fig. 3, \( \eta_a \) in the microcirculation should be constant. However, the in vivo data of Lipowsky and Zweifach (93) for the cat mesentery does not support this conclusion. In these experiments, the apparent viscosity was found to increase from \( \sim 3 \text{ cp} \) during normal flow velocity, \( \sim 1 \text{ cm/s} \), \( \sim 2000 \text{ s}^{-1} \), to \( \sim 20 \text{ cp} \) as flow was reduced to \( \sim 0.03 \text{ cm/s} \). Zweifach and Lipowsky (3) attribute this increase in \( \eta_a \) during reduction in \( \dot{\gamma}_m \) to both RBC aggregation, as suggested by in vitro studies, and leukocyte-endothelial adhesion, as evidenced by in vivo observation. According to these authors, the role of RBC aggregation in increasing \( \eta_a \) in vivo is still unresolved.

2. Effect of Hematocrit. The apparent viscosity of RBC suspension in vitro rises nearly exponentially with increasing hematocrit. The role RBC deformability plays in reducing \( \eta_a \) becomes apparent when one compares these viscosity results with those for suspension of RBC hardened with aldehyde treatment or rigid spheres.

Fig. 4 shows the in vivo apparent viscosity results as a function of hematocrit in the blood perfusing the isolated dog hind limb (94). The in vivo results are compared with in vitro results obtained in a large diameter tube viscometer. Although the in vivo and in vitro results show the same qualitative behavior, the in vivo \( \eta_a \) is about one-half of in vitro \( \eta_a \). (Similar results have been obtained for the rabbit ear (95); rat tail artery (96); cat calf muscle (97, 98); and dog lung (99)). The lower value of \( \eta_a \) in vivo was attributed to lower hematocrit in the microvasculature of the tissue (\( H_m \)) compared to the perfusate hematocrit, as a consequence of the Fahraeus effect (see next section). As a matter of fact, the average hematocrit of blood in the microcirculation of several normal and neoplastic tissues (measured using radiolabeled RBC and plasma) has been found to be considerably lower than the systemic hematocrit (Table 2). The lower microvesSEL hematocrit results in a lower apparent viscosity similar to the Fahraeus-Lindqvist effect (see Section IIIB3).

3. Effect of Vessel (Tube) Diameter: The Fahraeus Effect and the Fahraeus-Lindqvist Effect. Since the pioneering work of Fahraeus (1929) and Fahraeus and Lindqvist (1931) which showed that the hematocrit and apparent viscosity in a tube of diameter \( \leq 350 \mu \text{m} \) are less than those in a larger tube, many in vitro and in vivo studies have been performed on the effect of tube (vessel) diameter on these parameters. To understand
blood. Since $P_{cell}$ is greater than $P$, $H_T$ is less than $H_D$ in small tubes (Fig. 6A). In large tubes, the ratio of cell-free layer thickness to the tube radius is small. As tube radius grows smaller ($R < 250 \mu m$), this effect becomes more pronounced (Fig. 6A).

As tube diameter decreases below 15–20 \( \mu m \), the RBC occupy a larger fraction of the lumen, and as a result, the difference in the cell velocity and the plasma velocity decreases causing $H_T/HD$ to rise. This ratio reaches a value of 1.0 for a tube diameter of $\approx 2.7 \mu m$ (minimum tube diameter through which a normal human RBC can pass) (Fig. 6A).

As shown in Fig. 6B, the decrease in $H_T$ in tubes with diameters between 15 and 20 \( \mu m \) and 300 \( \mu m \) is accompanied by a similar decrease in $\eta_m$. However, unlike the Fahraeus effect, the trend reversal in the Fahraeus-Lindqvist effect does not begin until the diameter is lowered in the 5–7-\( \mu m \) range. Below 5–7 \( \mu m \) diameter, $\eta_m$ begins to increase rapidly to infinity as $d$ tends to $\approx 2.7 \mu m$ for human cells. The fact that $\eta_m$ is not determined by $H_T$ in the 5–20-\( \mu m \)-diameter range needs further research.

Shown in Fig. 7A are the in vivo $H_m$ data as a function of vessel diameter in the cat mesentery. These results are in qualitative agreement with the in vitro data shown in Fig. 6A. Note that the value of $H_m/H_m$ is about 0.23 for $\approx 10-\mu m$ capillaries (105); whereas the lowest value of $H_T/H_D$ in vitro is

*Fig. 5. Definitions of systemic (feed), microvessel (tube), and efferent (discharge) hematocrit. Adapted from Ref. 2.*

*Fig. 6. Effects of tube diameter on (A) tube hematocrit, $H_T$ (Fahraeus effect) and (B) apparent blood viscosity (Fahraeus-Lindqvist effect). Note that $H_T$ is normalized with respect to discharge hematocrit ($H_D$) and relative blood viscosity ($\eta_r$) is normalized with respect to suspending fluid viscosity. Note also that the minimum in $H_T/H_D$ occurs when tube diameter is $\approx 15–20 \mu m$, and in $\eta_r$ when tube diameter is $\approx 5–7 \mu m$. From Refs. 103 and 104 as adapted in Ref. 2, with permission.*

these results it is useful to define hematomics measured in vitro and in vivo, respectively, at three different locations: (a) tube ($H_T$) or microvessel ($H_m$) hematocrit; (b) feed ($H_F$) or systemic ($H_{sys}$); and (c) discharge ($H_D$) or efferent ($H_E$) hematocrit (Fig. 5). The Fahraeus effect refers to decrease in $H_T/H_D$ (or $H_m/H_D$) in narrow tubes, and the Fahraeus-Lindqvist effect refers to the concomitant decrease in the apparent viscosity in the tube or vessel.

The Fahraeus effect results from the migration of RBC toward the center of the tube, leaving the fluid near the wall relatively cell free. Since the velocity of plasma is highest in the center and lowest near the wall, the cells travel faster than the plasma (suspending media). From simple mass balance on cells, one can write

$$H_T P_{cell} = H_D P$$  (J)

where $P_{cell}$ and $P$ are the average velocities of cells and whole

*The Fahraeus effect ($H_T < H_D$) should not be confused with the possibility of (a) $H_{sys} < H_F$ or (b) $H_D > H_E$. The former occurs due to (a) cell screening, i.e., the cells are blocked at the tube entrance due to steric hindrance; and/ or (b) "plasma skimming", i.e., a disproportionate amount of fluid enters the tube (vessel) from the cell-free layer at the wall of the feeding reservoir (vessel) (2, 100). The latter ($H_D > H_E$) occurs when there is considerable fluid loss from the vessels, e.g., in shock (101) or in tumors (Refs. 4, 5, and 102; also see Section III).*
only ~0.5. This discrepancy between in vivo and in vitro results is not totally understood. A possible explanation is the preferential shunting of RBC through some part of the vasculature (105). It is interesting to note that $H_m/H_m$ was higher in venules (0.53 ± 0.25) than in arterioles (0.46 ± 0.25) of comparable size range (10–60 μm diameter).

Shown in Fig. 7B are the in vivo estimates of $\eta_r$ as a function of vessel diameter in the cat mesentery. Two conclusions can be drawn from this work: (a) $\eta_r$ values are lower for arterioles (3.6 ± 1.9 cP) than for venules (5.2 ± 2.7 cP) (92). Zweifach and Lipowsky (3) attribute the elevated viscosities in the venules to higher hematocrit (Fig. 7A) and lower shear rates in venules as compared to arterioles of the same diameter. Note that low shear rates can facilitate RBC aggregation and leukocyte-endothelium adhesion. In addition, in exteriorized preparations a mild inflammatory response may increase WBC flux and adhesion leading to reduced lumen (functional) diameter. If the unobstructed lumen diameter is used in Equation B to calculate $\eta_r$, the estimate of $\eta_r$ may be higher by a factor of 2 or more (105). Since most vessels in tumors are venular, these results suggest a higher viscosity in the tumor microcirculation. (b) The average $\eta_r$ value obtained from the macroscopic approach is higher than that from the macroscopic approach (~2 cP; Fig. 4). Zweifach and Lipowsky (3) attribute the relatively high $\eta_r$ value to the inability to measure hemodynamic parameters in vessels of zero $H_m$ (i.e., plasma only) or very low $H_m$. This bias should be kept in mind when measuring $\eta_r$ in tumor microcirculation which may contain a large number of plasma channels.

4. Effect of Cell Deformability and Aggregation. Since the deformability of the cellular elements of the blood contributes significantly to the apparent viscosity of blood in large tubes (see, e.g., Fig. 4) and in small tubes (see, e.g., Figs. 6 and 7), considerable information has been gathered in the last decade on the rheological properties of RBC, WBC, and cancer cells. Most of the quantitative data has come from micropipet aspiration experiments (90).

In these experiments a step negative pressure is applied to a cell via glass micropipet. The projection length of the cell in the pipet is measured as a function of time and applied pressure. The data for RBC are analyzed using a Kelvin model to obtain the elastic modulus, $E$, and the membrane viscosity, $\eta_m$ (90). The WBC and cancer cell data are analyzed using a standard viscoelastic model to obtain the elastic coefficients, $K_1$ and $K_2$, and the cell viscosity, $\eta_{cell}$. The reason for using different models is that for RBC the membrane and its associated cytoskeleton provide the principal resistance to cell deformation; whereas due to the presence of granules and nucleus, the cytoplasm provides the resistance to deformation of WBC and cancer cells. (For more details on this subject, see Refs. 48, 90, and 106.)

Under normal conditions, WBC are less deformable than RBC (106). Cancer cells are even less deformable than WBC (48). Activation of T-cells and large granular lymphocytes may also increase their rigidity (107). Low pH, elevated temperature, high extracellular glucose concentration, or changes in the tonicity of the suspending medium in either direction (hypo- or hypertonic) make RBC less deformable (2, 108). Reduced deformability of cells reduces their migration toward the tube center and hence causes a reduction in the Fahraeus effect. Seshadri et al. (109) found that heating of RBC led to an increase in $\eta_r$ in large tubes (~30 μm diameter). These results have interesting implications in the hyperthermic and hyperglycemic treatment of cancer (9, 110–112).

Aggregation of RBC caused by low shear stress and/or by macromolecules increases the effective size of cells. This, in turn, facilitates their migration toward center (hence accentuates the Fahraeus effect) and increases cell screening at the tube entrance. Both lower the tube hematocrit. On the other hand in large tubes RBC aggregates increase $\eta_r$ and hence resistance to flow.

In the absence of RBC aggregation, WBC migrate to the center of the vessel due to their effectively larger size, whereas in the presence of RBC aggregates (due to slow flow) the larger rouleaux occupy the central position and push WBC to the wall which raises their concentration in the vessel. Such lateral displacement of WBC by RBC also occurs in a tube with increasing diameter. In addition, in convergent flows, WBC entering from one capillary tend to be pushed to the wall by RBC entering from the second capillary. It is of interest to note here that postcapillary venules have nearly the lowest shear rate in the circulation, are formed by converging capillaries and have a gradually increasing diameter. All these conditions favor WBC migration to the wall, so that ~95% of WBC roll along the wall in the postcapillary venules. The location near the wall and slow rolling speed facilitate extravasation of WBC when needed to fight inflammation and/or help them respond to extravascular signal. At the same time, WBC margination increases resistance to blood flow during inflammation, shock, and other low-flow states (105, 113). The implications of these findings to the movement of cancer cells, single or in clumps, have not been explored completely.

C. Blood Rheology in Cancer Patients

The presence of cancer may alter the factors governing the rheological properties of blood. These factors include blood and plasma viscosity; concentration, deformability, and aggregation of RBC; and concentration of plasma proteins. These changes affect not only the flow of blood through normal and tumor microcirculation but also the onset of metastasis and the effectiveness of cancer treatment.

The effect of the long-term growth of a tumor, whether transplanted or spontaneous, in producing anemia in the host has been known for a long time (114). The effect of anemia on the fraction of hypoxic tumor cells was studied by Hill et al. (115). The systemic hematocrit drops as tumors grow larger. Interestingly enough, the average hematocrit in the tumor microcirculation ($H_m$) is lowered proportionately so that the $H_m/H_m$ ratio remains fairly constant. Using a tissue isolated tumor preparation, Butler et al. (102) and Sevick and Jain (112) found that due to fluid loss from the vasculature, the hematocrit in the efferent blood of W256 mammary carcinoma was about 5% higher than in the systemic blood (Table 3). Similar results were obtained by Vaupel et al. (116) for human breast cancer

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>$W_t$ (g)</th>
<th>$H_m/H_m$</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor 12</td>
<td>2.3 ± 0.4</td>
<td>1.051 ± 0.013</td>
<td>102</td>
</tr>
<tr>
<td>N-Nitroso-methylurea</td>
<td>3.9 ± 0.6</td>
<td>1.029 ± 0.007</td>
<td>102</td>
</tr>
<tr>
<td>W256</td>
<td>3.8 ± 1.0</td>
<td>1.068 ± 0.011</td>
<td>102</td>
</tr>
<tr>
<td>MTW9</td>
<td>4.3 ± 0.9</td>
<td>1.042 ± 0.006</td>
<td>102</td>
</tr>
<tr>
<td>Growing</td>
<td>2.0 ± 0.8</td>
<td>1.038 ± 0.007</td>
<td>102</td>
</tr>
<tr>
<td>Regressing</td>
<td>1.048 ± 0.011</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>W256</td>
<td>0.5–1.8</td>
<td>1.05 ± 0.05</td>
<td>116</td>
</tr>
<tr>
<td>Human medullary cell carcinoma</td>
<td>2.35 ± 0.28</td>
<td>1.08</td>
<td>116</td>
</tr>
<tr>
<td>Human squamous cell carcinoma</td>
<td>2.23 ± 0.27</td>
<td>1.09</td>
<td>116</td>
</tr>
</tbody>
</table>

* Mean ± SE; P < 0.01.

Xenographs in nude rats.
xenografts in nude rats. The effect of this hemoconcentration on tumor blood flow is not understood.

In one of the most extensive studies of the blood rheology of cancer patients, Dintenfass (117) found a decrease in hematocrit which was statistically significant (Table 4). Note that the hematocrit drop in malignant melanoma patients, both survivors and deceased, over 10 years was not as pronounced as in mice bearing a transplanted tumor.

Dintenfass (117) also reported a significant increase in the aggregation and rigidity of RBC (P < 0.001), fibrinogen and globulin concentrations, and plasma and blood viscosity (after adjusting to normal hematocrit) in melanoma patients (Table 4). The increase in RBC aggregation has been reported in lung and bowel cancers (118, 119), in breast cancer (120), and in various carcinomas (121). Associated with the aggregation of RBC are increased fibrinogen and globulin concentrations and decreased albumin/fibrinogen ratio. The increased fibrinogen and albumin concentrations may also explain the increased plasma viscosity. Similarly, the increased RBC aggregation may explain increased blood viscosity (corrected to standard hematocrit) (117, 121). The increase in RBC rigidity is difficult to explain, especially in light of the relationship between rigidity and aggregation (117).

On the basis of a 10-year follow-up of ~130 patients, Dintenfass (117) proposed the use of viscosity tests as diagnostic and prognostic tools. He correlated the sum of standard deviations from the mean of plasma viscosity and RBC aggregation with survival time. The greater the departure from the norm, as expressed by the sum of SD, the shorter was the survival time. He also tried to predict survivors (i.e., metastasis-free patients) from the patients who died of metastases, on the basis of his long-term data (~1971, 1973, 1975, 1977, 1980), and found that the discriminating power of viscosity tests (i.e., increased plasma viscosity and RBC aggregation in decreased subjects as compared to survivors) holds for a period of less than 2 years.

In the absence of RBC aggregation, the cancer cells similar to WBC may occupy the more central position in the post-capillary venules due to their larger size. However, when RBC aggregation occurs due to low shear rates and/or high plasma protein concentration, the cancer cells are pushed to the wall. Near vessel walls, cancer cells may be coated by platelets and fibrin, making them inaccessible to immunological attack (117), as well as extravasate to form metastatic foci. (For more details on metastasis, see Ref. 48.)

D. Summary

The apparent viscosity of blood in large blood vessels (>500 μm) is governed primarily by hematocrit and shear rate. Blood viscosity increases with increasing hematocrit. At low shear rates, RBC aggregate to form rouleaux which cause an increase in viscosity. RBC aggregation is also facilitated by macromolecules (e.g., fibrinogen, some fraction of globulins, dextrans).

Due to their deformability, cells migrate toward the center leaving a cell-free marginal layer at the vessel periphery. The migration toward center is increased with increasing effective size and deformability of cells or cell aggregates. Since the cells in the center travel faster than the plasma, hematocrit in small tubes (<500 μm diameter) is lowered (the Fahraeus effect). As tube diameter becomes comparable to cell diameter, both vessel hematocrit and apparent viscosity begin to increase (trend reversal in the Fahraeus and the Fahraeus-Lindqvist effects).

In vivo microvessel hematocrit also exhibits the Fahraeus effect; however, the drop in Hm in vivo is more than in vitro values for vessels of comparable diameter. On the contrary, the drop in apparent viscosity in vivo is not as pronounced as the in vitro hematocrit would suggest. These discrepancies between in vitro and in vivo results could be partly due to the limitations of measurement techniques.

The presence of neoplastic disease may alter factors governing apparent viscosity of blood, which may affect blood circulation through normal and tumor tissues, and the onset of metastases. Decrease in the systemic hematocrit with tumor growth has been reported for both humans and animals. The

Table 4 Rheological parameters of systemic blood in normal subjects and in patients who survived and who died of malignant melanoma

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal values (mean ± SD)</th>
<th>Survivors</th>
<th>Deceased</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1977 evaluation n</td>
<td>1980 evaluation n</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Blood viscosity (cP) at 180 s⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>5.44 ± 0.75</td>
<td>26</td>
<td>5.21 ± 1.06</td>
</tr>
<tr>
<td>Women</td>
<td>4.70 ± 0.30</td>
<td>12</td>
<td>4.57 ± 0.95</td>
</tr>
<tr>
<td>All</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>46.8 ± 3.7</td>
<td>26</td>
<td>42.6 ± 7.0</td>
</tr>
<tr>
<td>Women</td>
<td>41.2 ± 1.5</td>
<td>12</td>
<td>37.0 ± 5.4</td>
</tr>
<tr>
<td>All</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rrigidity of RBC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>0.956 ± 0.059</td>
<td>66</td>
<td>1.02 ± 0.10</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td>34</td>
<td>1.043 ± 0.156</td>
</tr>
<tr>
<td>All</td>
<td></td>
<td>66</td>
<td>1.02 ± 0.11</td>
</tr>
<tr>
<td>Aggregation of RBC; erythrocyte sedimentation rate (mm/h) (37°C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin/fibrinogen ratio</td>
<td>16.3 ± 3.7</td>
<td>58</td>
<td>11.2 ± 3.5</td>
</tr>
<tr>
<td>Albumin/globulin ratio</td>
<td>1.65 ± 0.28</td>
<td>63</td>
<td>1.28 ± 0.25</td>
</tr>
<tr>
<td>Plasma viscosity</td>
<td>1.23 ± 0.09</td>
<td>65</td>
<td>1.24 ± 0.10</td>
</tr>
</tbody>
</table>

a Data are from Ref. 117.

b Note that differences between parameters in patients and in normal subjects are significant at P < 0.001 or better, except for values marked d and e.

c Not significant.

d P < 0.05.
$R_{\text{m}}/H_{\text{m}}$ ratio remains unchanged despite the drop in $H_{\text{m}}$. Simultaneous rise in fibrinogen and/or globulin level increases the plasma viscosity and RBC aggregation. As a result, apparent blood viscosity goes up after adjusting to normal hematocrit. RBC aggregates, due to their effectively large size, push WBC and cancer cells toward vessel walls, facilitating their extravasation and ultimately metastasis formation.

Although one can speculate about the rheological behavior of blood in the tumor microcirculation based on the above information, distributions of $H_{\text{m}}$ and $n$, in individual vessels of tumors need to be measured directly. More specifically the questions that should be answered include: (a) How do abnormalities in the vascular morphology of tumors (e.g., presence of dilated and tortuous vessels, plasma channels, sinoids) and low shear rates affect $H_{\text{m}}$ and $n$? (b) What is the role of shape, size, and rigidity of cancer cells with respect to RBC and WBC on their radial distribution in vessels? (c) What is the effect of lowered hematocrit on flow resistance versus oxygen transport from the point of view of tumor growth and treatment (e.g., radiotherapy)? Recent developments in various optoelectronic methods to measure microcirculatory parameters as well as the possibility of perfusing isolated tumor tissue, ex vivo, should make it possible to answer these questions. (122–124).

IV. Microvascular Pressures in Normal and Tumor Tissues

Pressure, $P$, in a fluid is defined as the mean compressive force per unit area acting on fluid surfaces in directions normal to the surface at any point. Stephen Hales (125) was probably the first person to measure mean arterial pressure (by inserting a tube in the artery of the neck of an unanesthetized horse). Since that time, many direct and indirect techniques have been developed to measure pressure in macro- and microcirculation (for historical perspective, see, e.g., Refs. 3, 27, 126, and 127). The motivation for these studies has been 2-fold: (a) the blood flow in a vessel is governed by the pressure difference between its arterial and venous end (see Equation A); and (b) the transcapillary exchange of various solutes is associated with the exchange of fluid between the vascular and the interstitial spaces which, in turn, is governed by the hydraulic and osmotic (oncotic) pressure differences between these two spaces (5).

In what follows I will first outline various methods of blood pressure measurements in vessels, then present a brief summary of results on normal tissues, and discuss in detail the microvascular pressure measurements in tumor tissues, and finally point out the issues that need to be explored further.

A. Methods

The methods to measure blood pressure can be divided into two categories: (a) macroscopic, limited to larger blood vessels (diameter > 1 mm); and (b) microscopic, developed for smaller vessels (127). In each category, when the pressure sensor is in contact with the blood directly or hydraulically via a fluid, the method of pressure determination is referred to as a direct method; otherwise it is referred to as an indirect method. While we are interested here in the microscopic pressure measurements, a brief discussion of macroscopic methods is in order to understand the constraints of the former methods.

Macroscopic Methods. Sphygomanometry is the most widely used indirect method to measure systolic (maximum) and diastolic (minimum) arterial pressure in humans. This method, first developed in 1905 by Korotkoff, is based on balancing the pressure in an air-filled cuff against the vascular pressure. In this method the arterial blood flow is stopped by increasing the cuff pressure. The pressure is then slowly lowered until arterial pressure exceeds the cuff pressure and a sound is heard (Korotkoff sound), which indicates the onset of blood flow. This is assumed to be the systolic pressure, and the pressure when the blood flow becomes continuous is diastolic pressure. Korotkoff sounds can be detected by a stethoscope or by acoustic, optical, or electronic means (for details, see Refs. 126 and 127).

Direct pressure measurements are made using pressure transducers which convert hydraulic pressure into mechanical displacement of a thin elastic body. This displacement is converted into an electrical signal using electrical (e.g., strain gauge) or optical (e.g., fiber optics) means. Due to recent developments in solid state electronics (i.e., semiconductors) the miniaturized transducers can be placed on the catheter tip, which can then be directly placed in a blood vessel. However, due to cost, sterilization, and some technical problems (e.g., temperature, sensitivity, fragility) the pressure transducer is connected to the blood stream hydraulically, e.g., via a fluid-filled catheter. The dynamic response of the catheter-transducer system is governed by the overall compliance C, the change in volume per unit change in pressure. Units: cm$^3$/dyn = 1333 cm$^3$/mm Hg) of the system (i.e., the sum of catheter, fluid and transducer compliances) and the flow resistance (defined by Equation A. Units: dyn*s/cm$^2$) through the catheter (126, 127). In addition to the inherent catheter compliance, the presence of an air bubble may increase the overall compliance in an unpredictable way. For details of ways of handling these problems, the reader is referred to the review by Intaglia (127).

Microscopic Methods. Indirect estimates of microvascular pressure have come from two approaches: (a) monitoring pressure during mechanical obstruction until flow stasis and subsequent release of a vessel until flow reappears; and (b) monitoring exchange of fluid following vessel occlusion. The first method was probably used by Kreis (128) to estimate the capillary pressure in the human finger (see Ref. 27 for details). Roy and Brown (129), Hill (130), and Algire (131, 132) used a much improved system for mechanical occlusion and release of microvessels to measure microvascular pressures in the web of the frog, the bat wing, and the transparent dorsal skin chamber in the mouse, respectively. This technique was also applied to measure capillary pressures in the digits of the upper and lower extremities in humans (133–135). While these data generally agree with the direct pressure measurements, the error introduced due to mechanical properties of the vessel wall and the perivascular tissue cannot be ascertained.

The second indirect method is based on the technique developed by Landis (136) for measuring fluid exchange and was used by Intaglia and Zweifach (cited in Ref. 127) to measure pressure in capillaries. According to the Starling hypothesis, the net exchange of fluid across a vessel is zero if the difference between the intra- and extravascular hydrostatic pressure is counterbalanced by the difference in the oncotic pressures. If the oncotic pressures and the interstitial pressures can be measured or estimated independently, this method permits estimation of the microvascular pressure from the microocclusion experiments.

All methods for direct pressure measurement in microvessels require penetration of the vessel itself or its side branch by some type of microannulae (e.g., glass micropipet, polyethylene catheter). The microannulae may be connected to a low compliance pressure transducer either directly (passively) or via an active interface-nulling system. The passive systems are limited
by both the small amount of fluid that can be transferred from the vessel to overcome the compliance (C) of the system and the mechanical energy available to overcome to flow resistance (FR). Since the time constant of response of these systems is approximately \((C \times FR)\) and FR is proportional to \((1/R^2)\) (Equation B), the minimum tip diameter for the passive system is \(~15 \mu m\) (for details of analysis see Refs. 137 and 138).

Rappaport et al. (139) and Levasseur et al. (140) developed these systems with tip diameters \(~20 \mu m\) and obtained a frequency response of \(~20–45\) Hz.

Landis (136, 141, 142) developed the first active system in which he inserted a dye-filled micropipet into microvessels and manually adjusted the pressure in the pipette to obtain a stationary dye-plasma interface. Eichna and Bordley (135) and Levick and Michel (143) improved this method for use in humans. Wiederhielm et al. (144) improved this technique in two ways. (a) Instead of using a dye to visually track dye-plasma interface, the micropipet was filled with 2 M NaCl solution, the electrical resistance of which is much less than that of plasma. Movement of fluid into and out of the pipet would change the electrical resistance which can be monitored using a Wheatstone bridge. (b) Instead of adjusting counter pressure in the pipet manually, a servocontrol system was used. This system gave a frequency response of \(~20\) Hz for pipets with tip diameters of 0.5–5.4 \(\mu m\). Intaglia et al. (145, 146) further refined this system to the point that it is now available commercially (e.g., IPM, Inc., San Diego, CA) and is being used routinely in laboratories all over the world for pressure measurements in animals and humans (see Sections IVB and IVC).

A major problem in the use of these techniques is the fabrication of the micropipet itself. For details, see the work of Muheim (147) and Misiewicz (148).

**B. Microvascular Pressures in Normal Tissues**

Although the first microvascular pressure measurements in normal tissues were made more than 100 years ago (128, 129), most investigations have been made in the past 20 years due largely to the availability of the servo-null pressure system. This method has been used to map pressures in the microvasculature of the mesentery, interstitial muscle, various skeletal muscles, pial surface of the brain, lung, and heart of various animals; bat wing; and the nailfold of human fingers. (For detailed discussion of these results, see Refs. 3 and 148.)

Shown in Table 5 are typical pressure profiles in the microvascular networks of various two-dimensional tissues and skeletal muscles. The cat mesentery network data of Zweifach and Lipowsky (156) are shown in more detail in Fig. 8A. Note that most pressure drop occurs in the arterial side, with the sharpest fall occurring in the narrowest arterioles (\(~15–35 \mu m\) diameter).

This pressure drop must be differentiated from the pressure drop per unit length occurring in vessels of various types as shown in Fig. 8B. The pressure drops in the latter case were measured by using a dual servo-null system in each vessel. Note that unlike the network pressure gradients (Fig. 8A) \((\Delta P/R)\) along the length of single unbranched microvessels in the network reaches its maximum at the true capillary level (92). Since the capillary length is very small, the pressure drop across a given capillary is small (\(~1 \text{ mm Hg}\)). Due to these small pressure differences, even small changes in blood rheological factors may alter blood flow distribution. For instance, if bifurcations the entrance of one RBC into one branch raises the apparent viscosity and reduces the flow into that branch, favoring the entrance of the next cell into the next branch. Such effects are even stronger when the less deformable WBC enters a branch, and the resulting transient plugging (\(~5–10\) s to several minutes) leads to preferential redistribution of blood to other vessel(s) (157).

Micropressures undergo periodic fluctuations synchronous with the action of the heart, spontaneous vasomotion at the level of arterioles, and the respiratory cycle. Shown in Fig. 9 are pressure recordings of various vessels in the cat mesentery (149). Note that frictional resistance encountered in the successive branchings of the arterial tree dampens the pulsatile waveform but does not completely abolish it even in the capillaries. The amplitude of pulse is \(~1–2\) cm H$_2$O in capillaries and \(~4–5\) cm H$_2$O in venues where the mean pressure is as low as \(~10–15\) cm H$_2$O. These periodic as well as irregular pressure fluctuations in capillaries and postcapillary venues may affect the fluid exchange between the vascular and extravascular spaces (3–5).

A limited number of studies have been reported in the literature on changes in microvascular pressure due to various physical and physiological changes in the host. Zweifach (149, 158) found a positive correlation between systemic pressure and pressure in microvessels >40–50 \(\mu m\), whereas micropressure in smaller vessels (<20 \(\mu m\)) became relatively independent of systemic pressures. Bohlen et al. (153) on the other hand reported a linear relationship between systemic pressure and microvascular pressure even for capillaries. Further work is required in this area to resolve this controversy.

Several investigators have shown an increase in capillary

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**Table 5 Typical microvessel pressure* distribution in various normal tissues**

<table>
<thead>
<tr>
<th>Arterial diameter</th>
<th>Venous diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>40–50 (\mu m)</td>
<td>15 (\mu m)</td>
</tr>
<tr>
<td>Rabbit omentum</td>
<td>51</td>
</tr>
<tr>
<td>Cat mesentery</td>
<td>67</td>
</tr>
<tr>
<td>Cat brain (pia mater)</td>
<td>72</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Skeletal muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1*</td>
</tr>
<tr>
<td>Rat spinotrapezius</td>
</tr>
<tr>
<td>Rat cremaster</td>
</tr>
<tr>
<td>Rat anterior gracilis</td>
</tr>
<tr>
<td>Cat tenuissimus</td>
</tr>
</tbody>
</table>

* Values are mean pressures in mm Hg. From Ref. 3, with permission.

* Region of active venous vasomotion.

* Arterial (A) and venous (V) dichotomous branchings numbered beginning with sector that feeds the arteries.

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TUMOR BLOOD FLOW

Fig. 8. A, arteriovenous distribution of microvascular pressures as a function of vessel diameter in the cat mesentery. From Ref. 156, with permission. B, arteriovenous distribution of microvascular pressure gradients along the length of single unbranched microvessels in the network. From Ref. 92, with permission.

Fig. 9. Pulsatile nature of the pressure waveform is attenuated as the bloodstream moves through arteriolar and precapillary branchings but is not completely abolished in any of the microvessels, including the narrow true capillaries in the cat mesentery. From Ref. 149, with permission.

pressure due to hydrostatic load (134, 142, 143). Levick and Michel (1978) found that the capillary pressure on the toes increased only to about two-thirds of that based on hydrostatic load as the subject moved from the horizontal to the upright position. Zweifach and Lipowsky (3) attribute this discrepancy to modifications in pre- and postcapillary resistance.

Capillary pressure rises as the temperature of the skin and/or the room is raised, or the venous pressure is raised (143, 159). Finally, the use of anesthetics may affect blood pressure by suppressing spontaneous vasomotion. Spontaneous vasomotion is, presumably, responsible for the pressure fluctuations in capillaries (159).

C. Microvascular Pressure in Tumors

Unlike normal tissues, the studies on the microvascular pressure in tumors are limited. Algire and Legallais (160) were probably the first to measure the microvascular pressure in two sarcomas and a mammary adenocarcinoma grown in the dorsal chambers in mice. Using an indirect vascular occlusion method, Algire (131, 132) measured the blood pressure in the skin. Without giving any numerical results, Algire and Legallais (160) state that "the blood pressure of tumor vessels approaches that of venous pressure." They also reported slower blood flow in tumor vessels than in capillaries of striated muscle. These investigators decreased the peripheral vascular pressure of mice by histamine injections and found reductions in blood circulation in tumor and surrounding host tissues.

Decreased intravascular pressure and/or increased extravascular pressure in tumors has been demonstrated by several investigators working with tumors grown in transparent chambers (e.g. Refs. 4, 41, 50, 52, 53, 160, and 161). By raising venous pressure in the chamber (161) or by loosening the chamber (52, 53) blood flow could be restored in previously ischemic/necrotic tumor areas. Yamaura and Sato (50) attributed the flow instability (i.e., stasis and reversal) with the animal's movement to lowered microvascular pressure in tumors. While studying the dissemination of malignant tumors, Young and Griffith (162) proposed that the pressure in tumor vessels is "disproportionately high in relation to the tonicity of the vascular wall."

Peters et al. (53) reported the first direct measurements of microvascular pressures in a DMBA-induced mammary adenocarcinoma grown in the rat dorsal skin chamber. Their normal s.c. tissue and tumor data are shown in Table 6 and Fig. 10. Note that on the arterial side the values did not differ significantly between nontumorous and tumor vessels, whereas venous pressures in the tumor were significantly lower than those in nontumorous tissue. Furthermore, although the arterial pressures were identical in both tissue types, the pressure gradient is significantly higher along the tumor capillaries (sinusoids) and relatively small along the tumor venules. (These results suggest a higher flow resistance in tumor vasculature.) These investigators found the pulsatile nature of pressure in the arterioles; however, no pulsatility was detected in the venous side.

Wigl (164) measured microvascular pressures in the superficial layers (<100 μm) of DMBA-induced mammary tumors in rats. By making silicone rubber castings of the tumor vasculature, Wigl showed that the surface arterioles seemed to supply vascular branches going into the central tumor regions. However, pressure drop during passage of blood from surface to deeper areas makes it difficult to estimate microvascular pressure in deeper vessels. Nevertheless, his key finding is that the mean interstitial fluid pressure in the center of these tumors (5.5 g) is ~16 mm Hg which exceeds the surface vascular pressure (Table 6) and hence may be responsible for reduced blood flow and O2 in the center of these tumors.

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TUMOR BLOOD FLOW

Table 6 Microvascular pressures in tumors (mm Hg)

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Arterial</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Venous</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;60 μm</td>
<td>41–60 μm</td>
<td>21–40 μm</td>
<td>11–20 μm</td>
<td>≤10 μm</td>
<td>11–20 μm</td>
<td>21–30 μm</td>
<td>31–50 μm</td>
<td>51–100 μm</td>
<td>&gt;100 μm</td>
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<td>≤10 μm</td>
<td>11–20 μm</td>
<td>21–30 μm</td>
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<td>51–100 μm</td>
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<td>Mammary adenocarcinoma</td>
<td>23.0 ± 6.3</td>
<td>17.3 ± 2.5</td>
<td>9.1 ± 2.5*</td>
<td>8.7 ± 1.9*</td>
<td>8.2 ± 2.6*</td>
<td>7.9 ± 3.1*</td>
<td>8.8 ± 2.3</td>
<td>8.1 ± 1.8</td>
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<td>AH109 A hepatoma*</td>
<td>36–53</td>
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<td>163</td>
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<td>DMBA-induced mammary</td>
<td>44.5 ± 9.7</td>
<td>38.9 ± 5.8</td>
<td>23.8 ± 4.4</td>
<td>13.3 ± 3.0</td>
<td>9.7 ± 2.4</td>
<td>8.9 ± 2.6</td>
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<td>carcinoma*</td>
<td>(21)</td>
<td>(18)</td>
<td>(4)</td>
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<td>A-Mel-3 amelanotic melanoma</td>
<td>37.9 ± 1.5</td>
<td>36.8 ± 1.5</td>
<td>24.7 ± 1.2</td>
<td>12.8 ± 1.2</td>
<td>11.0 ± 1.1</td>
<td>165</td>
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* Significantly lower (P < 0.01) than nontumorous s.c. tissue (Fig. 10).
† Indirect method of pressure measurement.
‡ Measured in superficial layer (<100 μm) of tumor weighing 1–17 g. Mean interstitial fluid pressure in the center of tumor ~16 mm Hg.
§ 30°C.

Hori et al. (163), using an indirect method similar to that of Algire (132), measured microvascular pressure in normal s.c. tissue and in a hepatoma (AH109A) grown in a dorsal skin chamber in rats. Similar to Peters et al. (53) they found that the microvascular pressure in the tumor was lower than in the normal subcutis. There were many tumor vessels with pressure less than 4 mm Hg. When the mean arterial pressure was elevated from 100 to 150 mm Hg with a continuous infusion of angiotensin II, RBC velocity and diameter of tumor vessels increased without an increase in normal tissue. These results may explain increased efficacy of cancer chemotherapy due to angiotensin II.

Endrich and Hammersen (165) have measured microvascular pressure in A-Mel-3 amelanotic melanoma grown in the dorsal skin chamber in hamsters at 30°C, 35°C, and 42.5°C. At 30°C their microvascular pressures were significantly higher than those measured by Peters et al. (53) (Table 6). As temperature was increased precapillary pressures decreased while venular pressure increased. These reductions in arteriolar-venular pressure gradients are in agreement with simultaneous flow reduction in this tumor during hyperthermia.

D. Summary

Microvascular pressures in the arterial side are nearly equal in tumor and nontumorous vessels. Venous pressures on the other hand in tumors are significantly lower than those in nontumorous tissue. Decreased pressure in venular vessels, which are numerically dominant in a tumor, and increased interstitial fluid pressure may (a) reduce extravasation of fluid and solute molecules in tumors (see Ref. 5 for details); and (b) cause flow stasis and hypoxia in tumors. The reason(s) for low tumor microvascular pressure is not completely understood. Possible causes include: (a) increased number of vessels in tumors; (b) tortuous nature of tumor vessels; and (c) increased viscous resistance in tumors. Understanding the precise role of each of these geometric and rheological factors would help in modifying blood flow and transport of molecules in tumors.

V. Conclusions and Future Perspective

The tumor microcirculation is heterogeneous both temporally and spatially. The objective of this review article was to summarize critically current understanding of the parameters which are responsible for the heterogeneity in blood flow through this circulatory network. Various experimental and theoretical approaches to quantify these parameters were discussed. The unresolved problems were pointed out throughout the text in hopes of stimulating multidisciplinary research in this area. The data available in the literature suggest that there are significant differences in the perfusion pressures and viscous and geometric resistances between normal and neoplastic tissues, between two locations within the same tumor, and at the same location at different times. Evidence that microvascular pressure is low in transplanted tumors is direct and convincing. Evidence that viscous and geometric resistances in tumors are increased is indirect and must be proven quantitatively. Recent developments in the microvascular technology as well as non-invasive imaging have the potential of allowing the assessment of these parameters.

In the author’s opinion, the key unanswered questions in this area of research are: (a) What is the intratumor viscosity? How does it change with tumor size, type, and location within the tumor. How does it differ from that in the host tissue? Can the difference be explained on the basis of number, size, and rigidity of cells in blood; plasma viscosity; and vascular geometry? (b) What is the geometric resistance of a tumor? How does it change with tumor size, type, and location? How is it related to the geometry of the vascular network? (c) How do the three parameters (Δp, Z, and η) respond to various physical (e.g., radiation, heat, photodynamic therapy, hemodilution) and chemical (e.g., vasoactive drugs) stimuli? Are the mechanisms of tumor blood flow modification different from those of normal tissues? (d) How different are the three determinants of blood flow in human tumors from those for transplanted tu-
Acknowledgments

I wish to express my sincere gratitude to Dr. P. M. Gullino for his pioneering work in the pathophysiology of tumors and to my former and current students who have contributed in many ways to the research on blood flow in tumors. I also appreciate the helpful comments of Drs. S. Chien, W. Dewey, H. Eddy, G. Hahn, M. Intaglietta, H. Lipowsky, H. Reinhold, and P. Vaupel. I would like to acknowledge the skillful assistance of D. Bigos and D. Dlugoskecki in typing this manuscript.

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