

Review

Transport of Molecules in the Tumor Interstitium: A Review¹

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Abstract

The transport of fluid and solute molecules in the interstitium is governed by the biological and physicochemical properties of the interstitial compartment as well as the physicochemical properties of the test molecule. The composition of the interstitial compartment of neoplastic tissues is significantly different from that of most normal tissues. In general the tumor interstitial compartment is characterized by large interstitial space, high collagen concentration, low proteoglycan and hyaluronate concentrations, high interstitial fluid pressure and flow, absence of anatomically well-defined functioning lymphatic network, high effective interstitial diffusion coefficient of macromolecules, as well as large hydraulic conductivity and interstitial convection compared to most normal tissues. While these factors favor movement of macromolecules in the tumor interstitium, high interstitial pressure and low microvascular pressure may retard extravasation of molecules and cells, especially in large tumors. These differences in transport parameters have major implications in tumor growth and metastases, as well as in tumor detection and treatment.

I. Introduction

Most normal and neoplastic tissues can be divided into three subcompartments: vascular, interstitial, and cellular. In addition, most normal tissues also contain lymphatic channels in the interstitial compartment. Once a molecule used for cancer detection or treatment is injected into the blood stream, it encounters the following "resistances" before reaching the intracellular space: (a) distribution through vascular space; (b) transport across microvascular wall; (c) transport through interstitial space; and (d) transport across cell membrane. Each of these transport processes may involve both convection and diffusion. In addition, in each of these subcompartments the molecule may (a) be metabolized and undergo degradation, (b) bind nonspecifically to proteins or other components, or (c) bind specifically to the target element(s) (e.g., an enzyme, an antigen) involved in growth, detection, or treatment (1-4).

Most investigators to date have focused their research on understanding the biochemistry, biophysics, and molecular biology of cancer cells with limited attention paid to the *in vivo* interstitial environment they exist in. The advent of hybridoma technology and genetic engineering has led to large scale production of monoclonal antibodies and other biologically useful molecules. If these molecules are to be used clinically, methods must be developed to deliver them selectively to the target cells *in vivo* (5-7). Since no molecule can reach the tumor cells from blood without passing through the vascular and interstitial subcompartments, it seems reasonable to find out more about the structure and function of these two subcompartments. We have focused our research in the past few years on the experimental and mathematical characterization of transport through

these two spaces. We have recently summarized our work on blood flow and exchange in the tumor vascular subcompartment elsewhere (8, 9). In what follows we will discuss our own findings on the interstitial transport in tumors as well as those of others.

The transport of a solute or a fluid molecule in the interstitium is governed by the physiological (e.g., pressure) and physicochemical properties (e.g., size, charge, structure, composition) of the interstitial subcompartment as well as physicochemical properties of the test molecule. These properties have been recently reviewed for normal tissues by several authors (10-15). In this article we will discuss the tumor interstitial properties in the following order: volume, structure, and composition of the interstitial space (Section II); pressure-flow relationship in the interstitium (Section III); and interstitial transport parameters (Section IV). For each parameter, we will outline the methods of measurement, discuss the key results, and finally point out the implications for tumor growth, detection, and treatment.

II. Volume, Structure, and Composition of the Tumor Interstitial Space

The interstitial subcompartment of a tumor is bounded by the walls of blood vessels on one side and by the membranes of cells on the other. In normal tissues, the blood vessels are surrounded by a basement membrane, which may be damaged or missing in tumors (for review see, e.g., Ref. 8). In addition, the anatomically well-defined functioning lymphatic vessels present in normal tissues may be absent in solid tumors (16). [Note that a tumor may invade and hence incorporate lymphatic vessels of the host tissue.] Similar to normal tissues, the interstitial space of tumors is composed predominantly of a collagen and elastic fiber network. Interdispersed within this cross-linked structure are the interstitial fluid and macromolecular constituents (hyaluronate and proteoglycans) which form a hydrophilic gel. It is sometimes convenient to divide the interstitial space into two compartments: the colloid-rich gel space containing the hydrophilic hyaluronate and proteoglycans; and the colloid-poor free-fluid space. In this paper, we will discuss quantitative results on the volume and composition of each of these spaces.

A. Volume of Interstitial Space

The volume of the interstitial space is usually obtained by subtracting vascular space from the extracellular space. Vascular space is measured by a marker confined to blood vessels, and the extracellular space is measured by a marker excluded by cells. In a limited number of studies, these spaces have been measured morphometrically.

Shown in Table 1 are the data of Gullino *et al.* (17) for various carcinomas and a sarcoma. These investigators used sodium, chlorine, or D-mannitol as an extracellular marker and dextran 500 ($M_r \sim 375,000$) as a vascular marker. [Note that dextran 500 may overestimate the vascular space due to some

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Table 1 *Interstitial spaces of tumors*

Host	Tumor	Interstitial space (%)	Ref.
Rat	Fibrosarcoma 4956	52.6 ± 4.3 ^a	17
	W256 carcinoma	36.3 ± 2.8 ^a	
	H5123 carcinoma	43.3 ± 1.1 ^a	18
	H3683 carcinoma	50.6 ± 3.5 ^a	
	Novikoff hepatoma	54.6 ± 4.4 ^a	
	Normal liver	20.5 ± 0.6 ^a	
	Gastrocnemius muscle	15.6 ± 0.7 ^a	
	DS-carcinosarcoma	38 ^b	
	Sarcoma-M	40 ^c	
	Sarcoma-B	50 ^c	
	Skeletal muscle	13 ^c	
	Fibrosarcoma A-MC	60 ± 5	
	Fibrosarcoma C-MC	55 ± 1	
	Fibrosarcoma BP-II	33	
	Muscle	14 ± 2	
Skin	34 ± 3		
Lung	29 ± 8		
Kidney	34 ± 6		
Human	Gliomas	20-40 ^b	21
	Meningiomas	13-15 ^b	
	Normal brain	6-7 ^b	

^a Interstitial space: mean ± SD; tumor weight, ~2-15 g; sodium, chlorine, or D-mannitol as extracellular marker and dextran 500 as vascular marker.

^b Extracellular space; morphometric analysis.

^c Extracellular space; tracer, ⁵¹Cr-EDTA; measurement made ~50 min postinjection.

extravasation (8).] The interstitial space of tumors in general is very large, and that of hepatomas is more than twice that of the host liver. Similar results were obtained by Rauen *et al.* (18), Appelgren *et al.* (19), and O'Connor and Bale (20) in various sarcomas and by Bakay (21) in human brain tumors (Table 1).

Although implications of the large interstitial space are not completely understood, it seems reasonable to assume that the large "free-fluid" space would offer less resistance to interstitial transport (22). In addition, this large space would serve as a "sink" or a "reservoir" for substances injected into the body (2). The large amount of blood-borne substance accumulated in this space may also give an erroneous impression of increased

vascular permeability in tumors and/or selective affinity of injected substances for tumor cells.

B. Collagen and Elastic Fiber Content

Histological examination of tissues using appropriate stains demonstrates the presence of collagenous and elastic fibers. The basic structural unit of the collagenous fibers is the collagen molecule. The main body of this protein molecule has a cylindrical structure (diameter, ~1.5 nm; length, ~300 nm; molecular weight, ~285,000) and is composed of 3 peptidic α chains (molecular weight, ~95,000) coiled in a rope-like fashion to form a triple helix. Depending upon the composition of the α chains, the collagen molecules can be divided into at least 10 types; each type has similar structure and size (23). While the collagen fibers offer considerable tensile strength along their length, elastic fibers provide the rubber-like elasticity to a tissue. The structural units of elastic fibers are the microfibrillar protein and elastin. The physical and mechanical properties of elastic fibers depend on their amino acid composition (14, 15, 23).

Collagen is usually measured by tissue content of hydroxyproline, since it is assumed that this amino acid occurs almost exclusively in the scleroproteins of the connective tissue (24). Collagen content of nine hepatomas, W256 carcinoma, R-2788 lymphosarcoma, and two fibrosarcomas in rats and two hepatomas in mice was measured by Gullino *et al.* (24-26). All hepatomas contained more collagen than liver did (Table 2). Unlike regenerating liver, collagen content per unit tissue weight remained constant during growth in eight of nine rat hepatomas. In Hepatoma 5123, during growth, collagen content per unit tissue weight decrease was similar to that for regenerating liver. The results shown in Table 2 do not agree with those of Grabowska (27) who found a rapid decrease in collagen content of Guerin rat carcinoma and sarcoma as the tumor grew to 1.5 g. However, the collagen content per unit weight remained constant between 2 and 30 g tumor weight,

Table 2 *Collagen content of hepatocarcinomas and liver^a*

Strain/sex	Tissue denomination	Wt (g)	Hydroxyproline (μ g/mg N)	Collagen (mg/100 mg protein)
Hepatocarcinomas^b				
Rat lines				
Sprague-Dawley (M)	Novikoff	4.1 ± 14.6 ^c	10.9 ± 0.8	1.30
Fisher-344 (F)	LC 18	2.9 ± 6.2	10.9 ± 0.9	1.30
A × C (F)	3683	3.3 ± 9.0	17.4 ± 0.6	2.06
A × C (F)	3924A	2.2 ± 7.4	30.5 ± 1.8	3.69
Buffalo (M)	T3-2	4.2 ± 11.1	32.4 ± 1.2	3.82
OM (M)	HC	4.0 ± 7.8	42.6 ± 2.0	5.04
OM (F)	LC	3.9 ± 6.6	20.9 ± 1.3	2.46
OM (F)	3	3.5 ± 7.8	28.1 ± 2.0	4.34
Buffalo (M)	5123	3.4 ± 9.6	36.6 ± 2.0	4.34
Mouse lines				
C3H (M)	129 solid	0.75 ± 3.6	8.9 ± 0.5	1.06
C3H (M)	129 ascites	0.40 ± 1.4	19.7 ± 1.3	2.30
C3H (F)	134 solid	0.60 ± 2.1	18.2 ± 1.1	2.16
C3H (F)	134 ascites	0.60 ± 1.5	17.3 ± 0.8	2.05
Normal liver				
Rat lines				
Sprague-Dawley (M)	Normal	190-210 ^d	8.6 ± 0.4	1.02
Fisher 344 (F)	Normal	130-145	9.0 ± 0.2	1.06
A × C (F)	Normal	180-200	8.5 ± 0.1	1.01
Buffalo (M)	Normal	190-210	5.9 ± 0.2	0.68
Sprague-Dawley (M)	14 days regenerating	190-210	5.3 ± 0.3	0.63
Buffalo (M)	14 days regenerating	190-210	3.4 ± 0.2	0.39

^a From Gullino *et al.* (24, 25); reproduced from paper of Gullino (16) by permission.

^b Additional information in the paper of Gullino and Grantham (26).

^c Mean ± SD.

^d Host weight.

similar to Gullino's findings. Gullino *et al.* (24) attributed the initial reduction in collagen content of tumors to the inability of Grabowska's technique to separate the small tumor from the collagen-rich s.c. tissue.

Gullino and Grantham (25) demonstrated that the tumor collagen is produced by the host and its synthesis is governed by the tumor cells. These authors also showed that collagen content per unit tumor weight remains constant in all transplant generations regardless of site or age of tumors. If tumor growth depends on collagen production to the extent that it depends on neovascularization, this characteristic of tumors may be exploited in arresting the tumor growth. However, this author is not aware of any such attempts.

Unlike collagen, no attempt has been made to quantify the elastic fiber content of tumors. These fibers account for ~2–5% of the dry weight of skin (28) and ~30–60% of the elastic arteries (*e.g.*, aorta) (15).

Due to internal structure of collagenous and elastic fibers, these fibers have water space within them which is probably accessible to small molecules and ions (*e.g.*, glucose, urea, sodium, and chlorine). The water contents of collagenous and elastic fibers are estimated to be ~0.6 ml/g collagen and ~0.56 ml/ml elastin, respectively (15, 29, 30). The implication of this space in interstitial transport is not understood.

C. Polysaccharides

Various *in vitro* and *in vivo* studies have shown that the stabilized polysaccharide network (hyaluronate and proteoglycans) enmeshed in the collagenous fibers offers considerable resistance to interstitial transport. While the insoluble fibrous proteins (collagen and elastin) impart structural integrity to a tissue, the polysaccharides are thought to govern the mass transfer characteristics of the tissue due to their high negative charge density and hydrophilic character. The mutual repulsion of negative charges on the chains leads to swelling in solution. In addition, these polysaccharides impart viscoelastic behavior to the interstitium. The viscosity of the polysaccharide solutions depends upon their molecular weight, pH, and binding between hyaluronate and proteoglycans and/or other tissue components. (For detailed review, see *e.g.*, Refs. 23, 31, and 32.)

Choi *et al.* (33) have reported hyaluronate content of 0.018% in a rat chondrosarcoma. Toole *et al.* (34) reported hyaluronate contents of 0.014 and 0.028% in a carcinoma and s.c. tissue of a nude mouse. In contrast, these authors found unusually high content of hyaluronate in V2 carcinoma in rabbits and attributed it to its metastatic properties. Fiszer-Szafarz and Gullino (35, 36) studied the relationship between HA² and hyaluronidase in the interstitial fluids of tumor and the s.c. tissue (TIF and SIF, respectively). They found HA concentration of SIF to be 53.5 µg/ml fluid in the scapular region, but only 37.2 µg/ml in the lumbar region. In W256 carcinoma HA concentration was 38 µg/ml when grown in the scapular region and 20 µg/ml when grown in the lumbar region. Further, the concentration of HA was ~25% lower in s.c. areas distant from the tumor. These investigators attributed the low HA concentration in tumors to the concomitant elevated TIF hyaluronidase activity, which was ~57% higher in TIF than in SIF. The increased hyaluronidase activity may make tumors a source of polysac-

charide fragments and may ultimately affect the immune response of the host (16). The data on HA bound to the collagen matrix in tumors are not available.

Unlike hyaluronate, the data on the proteoglycans (GAG) content of tumors are limited. Boas (37) and Pearce (38) found hexosamine content in mouse s.c. tissue to be ~0.18% and ~0.14%, respectively. Brada (39) measured it to be ~0.027% in both Ehrlich and Krebs tumors in mouse. In contrast, Sylven (40) found relatively high GAG content in various sarcomas (0.1–1.5%). Since in Sylven's investigations GAG content was measured in the fluid collected by blunt dissection and formation of small pouches in the tissues, the validity of results depends upon the extent of damage done to the tissue/cells. Due to the importance of these macromolecules in the solute and fluid transport in tumors, a definite need exists to measure their concentrations in various tumor types.

D. Composition of Tumor Interstitial Fluid

Methods. The results on interstitial fluid composition are controversial due to the methodological problems as well as the heterogeneity in the interstitium. Most commonly used methods include: direct sampling using needle (catheter) or micropipet; implanted wicks; and chronically implanted micropore chamber (or perforated capsule) technique. [All of these techniques were originally developed for interstitial pressure measurements (see Section IIIA).] The major problem with the direct sampling method is the cellular and vascular damage caused by puncturing the tissue; as a result the fluid collected may be a mixture of cellular and pericellular fluids. Although micropipets may reduce this damage, one is not sure whether the fluid withdrawn represents the free-fluid phase or the gel phase. Furthermore, the applied suction may increase net capillary filtration and lower interstitial fluid concentration. The major objection to the wick technique is that it may act as a colloid osmometer. Finally, the major objections to the chamber/capsule procedure are: (a) the chamber may influence the structure of the surrounding tissue; and (b) the chamber fluid may not represent interstitial fluid due to hindered transport across the micropore membrane or the surrounding connective tissue layer. Gullino *et al.* (41) presented the following data in support of the use of their chamber for TIF measurement: (a) the pore size (0.45 µm) is large compared to the molecules present in the TIF; (b) samples collected outside and inside a chamber immersed in plasma have identical compositions; (c) when two chambers are placed close to each other in a s.c. pouch or a tumor, proteins with enzymatic activity placed in one chamber can be found in the other chamber; and (d) histological examination shows neoplastic cells touching the chamber (16, 42, 43). There are three problems with this chamber which should be kept in mind: (a) implant of the chamber in the s.c. area leads to lactic acid production from glucose in ~1 week to ~1 month and formation of a fibrosarcoma in ~1 year; (b) it takes several days to fill up the chamber and hence it has a slow dynamic response; and (c) the ability of this chamber to measure the exchange of macromolecules has not been tested independently, especially in light of hindered diffusion offered by the connective tissue layer around the chamber.

Other procedures for sampling the interstitial fluid include: (a) sampling the lymph fluid [Note that the equality between interstitial free fluid and lymph is still unresolved (15).]; (b) determination of solute concentration in excised tissues; (c) intravital fluorescent microscopy; and (d) exchange kinetics of radiolabeled solutes. The advantages and disadvantages of each

² The abbreviations used are: HA, hyaluronic acid; GAG, glycosaminoglycans; MP, micropipet technique; TIF, SIF, NIF, tumor, s.c., and normal interstitial fluid, respectively; IFP, interstitial fluid pressure; TIFP, SIFP, pressure of TIF and SIF, respectively; BSA, bovine serum albumin; WIN, wick-in-needle technique; IF, interstitial fluid; 0, value in water.

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 Table 3 Composition of interstitial fluid of tumors^a compared with serum, lymph, and peritoneal fluid

	Units	Tumor interstitial fluid	Serum of blood		Peritoneal fluid		Lymph from thoracic duct	Normal s.c. interstitial fluid
			efferent from tumor	afferent to tumor	Normal	Ascites ^b		
Total proteins	g/100 ml	3.2 ± 0.1 ^c	5.2 ± 0.2	4.8 ± 0.1	3.8 ± 0.2	32 ± 0.1	2.5 ± 0.3	4.1 ± 0.2
Free amino-N	mg/100 ml	5.9 ± 0.4	5.1 ± 0.2	5.2 ± 0.2	6.3 ± 0.4	4.0 ± 0.2	5.5 ± 0.2	6.3 ± 0.2
Free glucose	mg/100 ml	Traces	123 ± 6	188 ± 8	108 ± 6	Traces	125 ± 5	105 ± 12
Lactic acid	mg/100 ml	161 ± 11	122 ± 4	90 ± 8	20 ± 3	158 ± 10	27 ± 4	28 ± 4
Total cholesterol	mg/100 ml	8 ± 2	64 ± 5	60 ± 4	28 ± 3	22 ± 4	150 ± 9	29 ± 4
Lipid phosphorus	mg/P 100 ml	1.1 ± 0.1	5.0 ± 0.3	5.0 ± 0.3	0.8 ± 0.1	1.0 ± 0.1	4.9 ± 0.4	2.0 ± 0.8
Density	g/ml	1.014		1.019		1.017		1.014

^a All data from Walker carcinoma 256 and Sprague-Dawley rats except those under "Ascites." Reprinted from paper of Gullino (42) with permission of S. Karger AG, Basel.

^b Data from Hepatoma 7974 in Japanese/N rats (44).

^c Mean ± SD.

of these techniques are discussed by Aukland and Nicolaysen (12).

Results. According to Starling's hypothesis, the driving force for transcapillary exchange of fluid is the difference between intra- and extravascular hydrostatic and osmotic pressures. Because of their low reflection coefficient and high permeability, most low molecular weight solutes do not contribute significantly to the osmotic pressure gradients, at least at steady state. Concentrations of various low molecular weight solutes (*e.g.*, ions, nutrients, waste products, and enzymes) have been measured by Gullino *et al.* (41, 42) in TIF, SIF, and plasma and are summarized in Table 3. TIF has high H⁺, CO₂, and lactic acid concentrations and low glucose and O₂ concentrations as compared to SIF. The differences in the concentrations of H⁺, CO₂, glucose, lactic acid, cholesterol, lipid phosphorus content, and free amino acids between plasma and TIF are significant, perhaps due to tumor metabolism (see also Ref. 45).

Based on the invasive characteristics and presence of necrotic areas in tumors, it is generally assumed that TIF has high levels of proteolytic and lysosomal enzyme activities. The experimental data on this subject are inconclusive. Sylven *et al.* (46–49), using the direct sampling method, have shown increased lysosomal and proteolytic activity in TIF, especially in necrotic areas. Gullino and Lanzerotti (50) have also reported increased activity of six lysosomal enzymes during mammary tumor regression (which involves digestion of dead cells similar to necrosis); however, they found the increase in activity in the cells and not in the pericellular fluid, either before or during regression. In a separate study, Fiszer-Szafarz and Gullino (36) did report increased activity of hyaluronidase in TIF.

The results on the protein concentration in TIF are as controversial as in the NIF. Based on high effective vascular permeability and effective interstitial diffusion coefficient in tumors (8) one would expect higher concentrations of plasma proteins in TIF than in NIF. In support of this hypothesis are the data of Sylven and Bois (47) who, using the direct sampling method, found TIF and SIF concentrations, respectively, ~67–97% and ~30–50% of the plasma concentration (TIF, ~3.9–5.7 g/100 ml; NIF, ~2.8 g/100 ml) (see also Ref. 51). On the other hand, using the chamber method Gullino *et al.* (41) found opposite results (Table 3). These results are surprising especially because extravascular deposition of fibrin is a prominent feature of neoplasia (52). Since a major fraction of the body's plasma proteins is found in the extravascular compartment and their concentrations in plasma and interstitial space govern transcapillary exchange, more work is needed on the composition of interstitial fluid for both normal and neoplastic tissues.

III. Interstitial Fluid Pressure and Flow in Tumors

The schematic shown in Fig. 1 depicts the current concept of fluid and solute movement in the interstitium of a normal

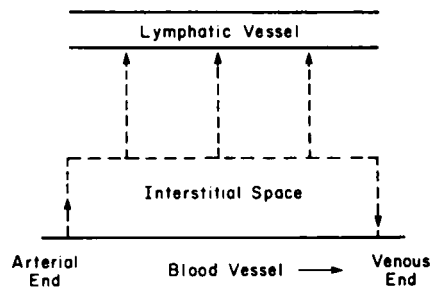


Fig. 1. Schematic of solute and fluid movement in the normal interstitial space. Solute refers to macromolecules and fluid refers to dilute solution of small hydrophilic molecules in water. Note that transcapillary filtration and reabsorption of fluid depend upon the hydraulic and osmotic pressure differences between intravascular and extravascular spaces, per Starling's hypothesis. The small amount of fluid which is not reabsorbed is collected by the lymphatic vessels. Movement of fluid through the interstitium and into the lymphatic vessels results from relatively small shifts in hydraulic and osmotic pressure gradients. Movement of macromolecules is due to diffusion as well as convection associated with fluid motion. Since anatomically well-defined, functioning lymphatic vessels may be absent in a neoplastic tissue, the excess fluid and macromolecules ooze out of the tumor and may be collected by the lymphatic vessels of the surrounding normal tissue.

tissue. According to the hypothesis of Starling (53), fluid movement across the vessel wall is governed by the transcapillary hydrostatic and osmotic pressure gradients. Most of the fluid filtered into the interstitial space is reabsorbed into the microvascular network by the Starling mechanism. The residual fluid is taken up by the lymphatic vessels. Since tumors may not have anatomically well-defined lymphatic vessels, this residual fluid may ooze out of the tumor periphery and may be reabsorbed by the lymphatics of the surrounding normal tissue thus aiding lymphatic dissemination of cancer cells. If the fluid reabsorption is not rapid enough and/or tumor cells continue to proliferate, the pressure in the interstitial space may increase. This elevated pressure may lead to vascular occlusion and ultimately necrosis and/or may facilitate intravasation and ultimately vascular dissemination of cancer cells. The objective of this section is to address these issues by examining the available data on the interstitial pressure and fluid flow in tumors. (The implications of fluid flow in the solute transport are discussed in Section IV.)

A. Interstitial Pressure in Tumors

Methods. Currently, there are three methods to measure local interstitial pressure, (a) needle, (b) WIN and (c) MP, and one method to measure average interstitial pressure, micropore chamber, also referred to as the perforated capsule method. Each of these methods has its advantages and limitations (see, *e.g.*, Refs. 54 and 55 for detailed comparison).

In the needle method, first used by Henderson in 1936, a

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Table 4 Interstitial fluid pressure of tumor and host normal tissues

Host	Tumor	Age/size	Host tissue	Pressure (mm Hg) mean ± SD (range)		Method and size of probe	Ref.
				Host	Tumor		
Rat	W256 carcinoma H5123 H7974 Novikoff hepatocarcinoma Fibrosarcoma 4956	5-10 g	s.c. tissue	(7-9)	(8-16)	CH ^a	41
	W256 carcinoma	3-5.1 g	s.c. tissue	(-0.1-+0.5)	(6-22)	CH	58
	W256 carcinoma	~1.3 g (5 days)	Testis	2.98 ± 0.2	21.2 ± 4.1	N (26-gauge)	59
	DMBA-induced mammary carcinoma	~0->5.5 g (6-8 wk)	Skin	-0.2 ± 1.2	(2.4 ± 2.4 ^b - 16.0 ± 4.8)	WIN (0.6 mm o.d.) MP (1-3 μm)	60
	DMBA-induced mammary carcinoma	3.4 ± 0.15 g (1.1-7.3 g)	Skin	Not measured	10.3 ± 1.3	WIN (0.6 mm o.d.)	61
	MCA-induced sarcoma	0.25-1.7 g (12-35 days)	Tail	4.7 ± 1.4	26.6 ± 11.5	WIN (0.6 mm o.d.)	62
	Hepatoma ascites AH 109A AH 272	(7-21 days)	s.c. tissue	-2.2	(~0-30)	N	63
Rabbit	Brown-Pearce carcinoma	(~12 days)	Testis	8.3 ± 1.3	19.3 ± 6.0	N (21-gauge)	57
	VX2 carcinoma	0.05-2 g (8-26 days)	s.c. tissue	~0	(~2-20) ^b	MP (1 μm)	Footnote 3

^a CH, micropore chamber; N, needle; o.d., outside diameter; DMBA, dimethylbenzanthracene; MCA, 3-methylcholanthrene.

^b Measured intratumor pressure gradients as a function of tumor size; see text for details.

needle filled with physiological saline coupled to a pressure-measuring device is inserted into the tissue, and pressure is increased until fluid flows into the tissue. The pressure at this point is considered to be equal to the interstitial fluid pressure. In the wick-in-needle technique, a wick made of polyester or other fibers is placed in the needle to provide a large surface area continuum with the interstitium and to reduce occlusion. Both needle and WIN methods can cause tissue distortion and trauma. Fluid injection in the needle technique may increase interstitial pressure. The fibrous wick may act as a colloid osmometer in the WIN technique in chronic measurements.

Micropipets, 1-3 μm in diameter, connected to a servo-null pressure-measuring system reduce the problems of needle and WIN techniques considerably. Most investigators have not been able to use this method for depths greater than 800 μm due to pipet breakage. We have been able to overcome this problem by choosing suitable glass capillaries to make pipets.³

The micropore chamber technique was introduced at about the same time independently by Guyton (56) and Gullino *et al.* (41) to sample the interstitial fluid of normal and tumor tissues, respectively. In this method, the chamber must be implanted in the tissue days in advance so that it is filled up with the interstitial fluid by the time of measurement. As a result, this method cannot be used for dynamic measurements. In addition, the connective tissue surrounding the chamber may act as a semipermeable membrane, excluding large molecules from the chamber fluid and consequently introducing osmotic effects. Gullino (43) has carefully examined several of these effects in his studies of tumor pathophysiology and found them to be negligible. (See Section IID.)

Results. Young *et al.* (57) were the first investigators to measure TIFP and found it to be higher than IFP in the normal host tissue. Since that time IFP has been measured in several

animal tumors using all four techniques (Table 4) and the results agree with Young's findings (58-63). The increased value of TIFP has been attributed to the absence of a well-defined lymphatic system in the tumor (58) and to increased permeability of tumor vessels (8).

Young *et al.* (57), Wiig *et al.* (60), Paskins-Hurlburt *et al.* (59), Hori *et al.* (63), and Misiewicz and Jain³ have examined the intratumor pressure as a function of tumor size. All these investigators found that as the tumor size increases, TIFP rises, presumably due to the proliferation of tumor cells in a confined area as well as high vascular permeability and possible absence of functioning lymphatic vessels in tumors. This increase in TIFP also correlates with reduction in tumor blood flow and development of necrosis in a growing tumor (9, 59, 60, 63).³ Wiig *et al.* (60) attributed the rise in TIFP to ischemic cell swelling as well.

Wiig *et al.* (60) and Misiewicz and Jain³ have also examined the intratumor pressure gradients. Using the WIN technique, Wiig *et al.* (60) found that in tumors <2.5 g, pressure in the outer one-third of the tumor is 6.0 ± 1.7 (S.D.) mm Hg and in the central one-third it is 11.4 ± 4.1 mm Hg, whereas in tumors >5.5 g, the values in these regions are 9.6 ± 3.5 and 16.0 ± 4.8 mm Hg, respectively. The highest TIFP measured was +23.3 mm Hg in the central region of a tumor >5.5 g. Using the MP technique, these authors found TIFP equal to 2.4 ± 2.4 mm Hg in the superficial layer (<800 μm) of small as well as large tumors. Our results using the MP technique for the entire tumor are in general agreement with those of Wiig *et al.* (60) (Fig. 2).

A limited number of investigators have attempted to modify TIFP with physical and chemical means. For example, Young *et al.* (57) reported that IFP in both normal and neoplastic tissues increases by injection of fluid (0.5 ml) into the tissue and by the application of digital compression. This fact should be kept in mind during the diagnosis and treatment of human

³ M. Misiewicz and R. K. Jain. Interstitial pressure gradients in VX2 carcinoma, manuscript in preparation.

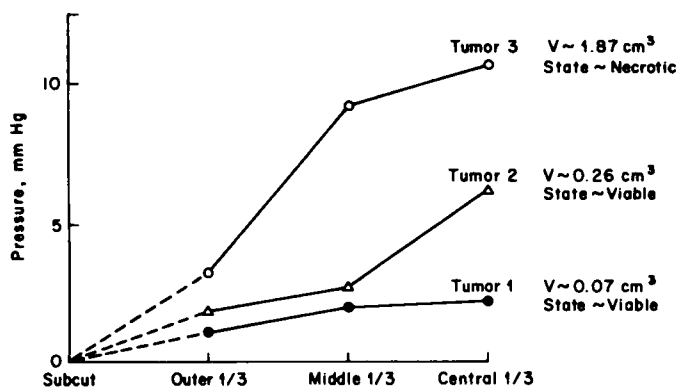


Fig. 2. Interstitial pressure gradients in VX2 carcinoma. Note the increase in pressure as the tumor grows. From Misiewicz and Jain.³

tumors, *e.g.*, palpation, especially if repeated or applied injudiciously; aspiration biopsy; and the local injection of anesthetics or other pharmacological agents. Similarly, Wiig and Gadeholt (62) were able to increase TIFP by increasing the venous pressure (which also resulted in skin and tumor blood flow reduction) and by plasma volume expansion with 5% BSA solution (which also resulted in skin and tumor blood flow increase). Finally, Tveit *et al.* (61) reported a decrease in the IFP of dimethylbenzanthracene-induced mammary carcinoma in rats from 10.3 ± 1.3 mm Hg to 7.0 ± 1.1 mm Hg during noradrenaline infusion. This agent, which is a potent vasoconstrictor, increased the systemic pressure by 30–40 mm Hg and decreased tumor blood flow. These studies do not support the hypothesis that reduction in tumor blood flow is a result of increased TIFP.

Whatever the key factor or factors for the increase in TIFP are, absence of functioning lymphatic vessels, increased permeability to macromolecules, tumor cell proliferation in a relatively rigid area, or ischemic cell swelling, the elevated TIFP has profound implications in tumor growth, detection, and treatment. For example, increased TIFP may facilitate the entrance of cancer cells into tumor blood vessels or into the surrounding normal tissue lymphatics, thereby aiding the metastatic process. Increased TIFP may reduce the Starling forces responsible for extravasation of fluid and various solutes, *e.g.*, cytotoxic agents, monoclonal antibodies, biological response modifiers, making it difficult to deliver these detection/treatment agents to large tumors. Increased TIFP may also hinder extravasation of leukocytes involved in immune response. All these effects are also influenced by the microvascular pressure in tumors which has been found to be low compared to that in the host tissue (64).

B. Interstitial Fluid Flow in Tumors

Methods. Increased pressure gradients in the tumor interstitium suggest the existence of significant convection in the tumor interstitium. To measure this fluid flow, Butler *et al.* (58) utilized two methods: (a) comparison of erythrocyte concentration (hematocrit) of tumor afferent and efferent blood in a tissue isolated preparation where tumor is connected to the host by a single artery and a single vein (43); and (b) continuous drainage of the interstitial fluid from micropore chambers embedded in normal and neoplastic tissues (43).

Note that both of these methods provide values of “net” interstitial flow (as measured by fluid loss), and not of the local convective velocity of the fluid. The latter has not been measured for normal or neoplastic tissues to date. (See Section IVA

for a novel method of measuring convective velocity of a solute.)

Results. Hemoconcentration measurements by Butler *et al.* (58) show that in four different mammary carcinomas, 2–5 g, the fluid loss, Q_{IF} , to the interstitial compartment is ~ 0.14 – 0.22 ml/h/g tissue (Table 5). This is approximately 5–10% of plasma flow rate through these tumors and significantly more than the lymph drainage in most normal tissues [0.0017 – 0.072 ml/h/g (12)]. The oozing out of this fluid from tumors may be responsible for the peritumor edema often seen in s.c. tumor implants and may play a role in the production of lymphatic metastasis. Although the direction and magnitude of these convective currents were not measured by these investigators, their results explain the observations of Reinhold (65) who found the spread of pyranine dye in tumor interstitium at rates faster than predictable by diffusion alone (66).

Although convective flow depends on the pressure gradients and hydraulic conductivity of the medium (see Equation A), these authors found a significant correlation between Q_{IF} and tumor blood flow rate per unit mass. Since tumor blood flow rate per unit mass decreases as a tumor grows (for review, see Ref. 9), Q_{IF} was found to be proportionately less for large tumors. It is worth noting here that these investigators found no difference in Q_{IF} in growing *versus* regressing hormone-dependent tumors (58).

Continuous drainage of interstitial fluid from micropore chambers showed that tumors oozed out ~ 4 – 5 times more fluid than the s.c. tissue (~ 3.5 – 4.75 ml/day *versus* ~ 0.9 ml/day). Furthermore, the fluid drained from tumors in ml/day remained fairly constant as the tumor grew from 2 g to 26.5 g in 5 days (58).

C. Theoretical Studies of Interstitial Pressure-Flow Relations

As discussed in Section II, the interstitial space is considered to have two compartments, the colloid-rich gel space containing the hydrophilic hyaluronate and proteoglycans at or near equilibrium with the colloid-poor, free-fluid space. In this model, the gel phase is considered to be immobilized, although the possibility of mobile hyaluronate cannot be excluded (35, 36, 67). In addition, whether these two compartments are arranged in series or in parallel is not known (15). Finally, although preferential fluid channels and rivulets have been reported in the interstitial space by Nakamura and Wayland (68) and Casley-Smith (69), to date no direct measurements of fluid velocity in the interstitium have been made. To this end, a limited number of investigators (*e.g.*, Refs. 70–72) have computed velocity and pressure profiles around single and multiple capillaries using Darcy’s law for flow through a porous medium:

$$u = -K \frac{\partial p}{\partial x} \quad (\text{A})$$

where u is the fluid velocity, p is the pressure, and K is the

Table 5 Interstitial fluid loss in mammary tumors^a

Tumor	MTW9 ^b	DMBA	NMU	W256
Wt (g) (mean \pm SE)	4.3 \pm 0.9	3.8 \pm 1.0	2.3 \pm 0.4	3.9 \pm 0.6
H ^c (mean \pm SE)	1.042 \pm 0.006	1.068 \pm 0.011	1.051 \pm 0.013	1.029 \pm 0.007
IF loss				
ml/hr/g ^c	0.22	0.19	0.14	0.18
% blood perfused	4.2	5.1	2.7	6.7
% plasma perfused	6.5	8.5	4.5	10.2

^a Data from Butler *et al.* (58).

^b MTW9, hormone-dependent tumor; DMBA, NMU, chemically induced tumors; W256, Walker 256 carcinoma; H, hematocrit of efferent blood/hematocrit of afferent blood ($P < 0.001$); IF, interstitial fluid.

^c Values of lymph flow for normal tissues range from 0.0017 ml/h/g (human skeletal muscle) to 0.072 ml/h/g (rabbit intestine). (From Table 4 of Ref. 12.)

hydraulic conductivity. (See Section IV for measurements and values of K .) These theoretical analyses show large pressure gradients near the capillary wall which die out at distances beyond a few capillary diameters (71). [Note that in addition to small scale pressure gradients around individual capillaries, large scale pressure gradients exist in tumors from its center to the periphery (Fig. 2). The relationship between these two pressure gradients has not been studied theoretically or experimentally.] These analyses also point out that the convective transport patterns in the interstitium are quite sensitive to intravascular pressures and intercapillary interactions (72). It must be mentioned here that these authors in their models assume fluid filtration and reabsorption to occur at the arterial and venous ends of capillaries, respectively. Zweifach and Lipowsky (73) on the other hand propose that filtration and absorption are also temporal (periodic) processes not just spatial ones. The basis of their conjecture is that under normal conditions the interstitial colloid osmotic pressure is ~ 8 – 10 mm Hg and the interstitial hydraulic pressure is ~ 0 mm Hg. Direct pressure measurements suggest that the pressure drop across a 600 – 1200 - μm path from precapillary to postcapillary is ~ 3 – 5 mm Hg. On the other hand, presumably due to arterial vasomotion, the pressure in capillaries fluctuates from as low as 10 – 15 mm Hg during near stasis to as high as 20 – 25 mm Hg above blood colloid osmotic pressure during maximal flow. Experimental measurement of fluid velocity in the interstitium is now needed to resolve the temporal and spatial contributions to the fluid flow.

IV. Transport Parameters Characterizing Interstitial Diffusion and Convection in Tumors

Transport of molecules in the interstitium is due to concentration gradients (diffusion) and the motion of interstitial fluid (convection). For one-dimensional transport, the diffusive flux, J_D , of a solute in a medium is given by Fick's law:

$$J_D = -D \frac{\partial C}{\partial x} \tag{B}$$

where D is the diffusion coefficient of the solute in the medium, and $\partial C/\partial x$ is the concentration gradient of solute (C is concentration and x is position or distance coordinate). Similarly, the convective flux, J_c , is given by:

$$J_c = CR_F u = -CR_F K \frac{\partial p}{\partial x} \tag{C}$$

where u is the convective flow velocity of the solvent resulting from pressure gradients in the medium (see Equation A), R_F is the retardation factor (solute convective velocity/solvent convective velocity), K is the tissue hydraulic conductivity for convective flow of solvent through the medium (k/η , where k is Darcy's constant, and η is solvent viscosity) and $\partial p/\partial x$ is the pressure gradient (p -hydrostatic pressure). The convective and diffusive transport may be in the same direction or in opposite directions depending upon the pressure and concentration gradients.

In what follows we will present published values of each of the transport coefficients, D , K , and R_F , for the normal and tumor interstitium. The pressure gradients and convective fluid flow in the tumor interstitium were discussed in Section III.

A. Interstitial Diffusion Coefficients

Methods. Various methods of measuring diffusion coefficients in a (liquid) medium are summarized by Cussler (74).

Most methods require measuring solute flux at a known concentration gradient or measuring relaxation of concentration gradients as a function of time in the medium and then fitting the steady or the unsteady state diffusion equation to the concentration data to extract the diffusion coefficient.

Due to the difficulties involved in measuring the concentration gradients in the interstitial space *in vivo*, most diffusion measurements to date have been carried out in tissue slices *in vitro* or in various gels/solutions as a model of the interstitium. Only recently have the developments in quantitative fluorescent microscopy allowed measurements of the effective interstitial diffusion coefficients *in vivo* (12, 22, 68, 75–81). There are three major problems with the intravital fluorescent microscopy methods: (a) these methods can be used only for thin tissues or for the superficial layer of thick tissues; (b) the effective diffusion coefficients include both diffusive and convective components; and (c) several biologically useful molecules, e.g., O_2 , CO_2 , are not fluorescent, and their molecular weight is less than that of currently available fluorescent tags. The first problem has been addressed by Goldstein *et al.* (82) who monitored transport in tumors using fiber optic microfluorometry; however, poor spatial resolution and fluorescence quenching do not permit one to extract diffusion coefficient from their approach. Regarding the second problem, we have recently proposed the use of fluorescent recovery after photobleaching to discriminate interstitial convection from diffusion *in vivo* (83). In this method, which is used routinely by cell biologists, a well-defined concentration gradient of a fluorescent tracer is artificially imposed in the extravascular region of a tissue by photobleaching with a laser beam. The relaxation of the concentration profile is monitored and analyzed to yield the diffusion coefficient and the convective velocity (83, 84).

When the diffusing species (e.g., O_2 , CO_2 , antibodies) binds to or reacts with any component of the tissue, the estimation of its diffusion coefficient becomes even more complex. If reaction and binding are not properly accounted for, the values of diffusion coefficients may be incorrect. One solution to this problem is to study transport of a nonreacting species which is similar in size, structure, charge, and configuration to the species in question.

Results. Effect of Tissue Water Content. Vaupel (51) has recently compiled the tissue diffusion coefficients of various small molecular weight species: O_2 , CO_2 , N_2 , H_2 , glucose, and inulin (Table 6; Refs. 66 and 85–91). All D values presented were corrected to 37°C (310°K) by the following correlation based on the Stokes-Einstein equation (74):

$$D_{310} = D_T \cdot \frac{\eta_T}{\eta_{310}} \cdot \frac{310}{T} \tag{D}$$

Table 6 Diffusion coefficient of glucose and inulin (cm^2/s)

Tissue	Glucose	Inulin	Ref. ^a
Connective tissue membrane	2×10^{-6}		85
Human aorta-intima media	1.7×10^{-6} 1.3×10^{-6}		86
Human articular cartilage	2.2×10^{-6}	2.5×10^{-7}	87, 88
Saline	9×10^{-6}	3×10^{-6}	89
Brain	6.7×10^{-7}		90
Plasma	8.75×10^{-6}		
Any tissue ^b	3.6×10^{-6}	2.8×10^{-7}	66
DS-carcinosarcoma			91
Tissue slices	2.6×10^{-6}		
Ascites cells	4.3×10^{-6}		

^a Courtesy of P. Vaupel.

^b Estimated using a correlation based on published data.

where T is the absolute temperature ($^{\circ}\text{K} = 273 + ^{\circ}\text{C}$) during measurement and η is the viscosity of water. The subscripts of D and η refer to the temperature in $^{\circ}\text{K}$. Vaupel found that the diffusion coefficients of small solutes (O_2 , CO_2 , N_2 , and H_2) decreased exponentially with the percentage of water content, z , of the tissue:

$$D = a \exp(-bz) \quad (\text{E})$$

These results are not surprising since the polysaccharide network composed of hyaluronate and proteoglycans dispersed in the interstitial collagen and elastic fibers offers little resistance to these gaseous molecules. For larger molecules, however, hydrodynamic and steric interactions with the solute may be as important as or more important than the water content (see below).

Molecular Weight Dependence. Diffusion coefficients of macromolecules, primarily dextrans, in water and in normal tissues have been measured by several investigators and can be described by the power law expression (92)

$$D = a(M_r)^{-b} \quad (\text{F})$$

The coefficients a and b for water and various tissues are summarized in Table 7. Note that the value of the exponent, b , is ~ 0.5 for water and ranges from ~ 0.75 to ~ 3 for various tissues, suggesting that the dependence of the diffusion coefficients on molecular weight in tissues deviates from that for free diffusion in water. These results are consistent with the hypothesis that hydrodynamic and steric interactions among the solute, solvent, and the interstitial matrix determine the transport properties of a solute in tissues, and not just the water content.

Despite rapid progress in this field, there is a paucity of interstitial diffusion data in tumors. Nugent and Jain (22) and Gerlowski and Jain (80) obtained the effective interstitial diffusion coefficients of various dextrans and albumin in VX2 carcinoma *in vivo* (Fig. 3). Note that the macromolecular transport in this tumor is hindered to a lesser extent than in nontumorous (mature granulation) tissue. Whether this significant difference is solely due to the physicochemical characteristics of the interstitial matrix of these tissues or due to increased interstitial convection needs to be answered (see below). Whatever the cause of this difference is, it favors the use of macromolecules in cancer detection and treatment (8, 93). Similar studies in various animal and human tumors are needed to exploit monoclonal antibodies and drug-macromolecule conjugates optimally in the management of neoplastic diseases.

Dependence on Configuration, Charge, and Binding. Since dextrans are linear molecules and albumin is a globular molecule, it is more reasonable to compare their transport properties on the basis of their molecular size than their weight. Usually the Stokes-Einstein radius, a_E , of a molecule is chosen as a measure of its size (92):

$$a_E = k/6\pi\eta D_0 \quad (\text{G})$$

where k is the Boltzmann constant, and D_0 is the free diffusion coefficient of the molecules in water at absolute temperature T and viscosity η .

Shown in Fig. 4 are ratios of effective diffusion coefficient to free diffusion coefficient (D/D_0) for sodium fluorescein, BSA, and dextrans *versus* their Stokes-Einstein radii (94). Note that in both normal and neoplastic tissues BSA diffusion is significantly lower than that of a dextran of the equivalent Stokes-Einstein radius. This effect has been observed in several normal tissues; however, no effects of this nature have been reported for diffusion in tumors by other investigators. For example, Fox and Wayland (77) found diffusive transport of BSA in the rat mesentery to be hindered more than dextrans of the same molecular size.

The deviation of diffusion coefficients from a strict molecular size basis could be explained in terms of configuration, charge, and binding of the test molecule. The dextrans used in our study are linear polymers with a slight degree of branching (5%), and the albumin molecule is loosely coiled in an ellipsoidal shape in aqueous solution (92, 95). Various *in vitro* and *in vivo* studies have shown greater transport rates of linear molecules than that of globular molecules of equivalent Stokes-Einstein radius (96–98).

Electrostatic repulsion of negatively charged albumin by negative charges of the interstitial matrix would lead to a smaller effective volume for diffusion. Works by various investigators on capillary permeability to charged proteins support this hypothesis as reviewed previously (99, 100). The reduction in D has been related to the fixed charge density of matrix by Maroudas (87). Similarly, the effects of electrochemical potential on interstitial transport in connective tissues have been reviewed by Grodzinsky (101).

Finally, binding of BSA to the tissue components could further reduce the diffusivity of albumin. However, Rutili (102) found no measurable binding between dextrans and proteins, *in vivo*. The precise role of configuration, charge, and binding in macromolecular diffusion still needs to be determined for normal or neoplastic tissue.

Pore and Fiber-Matrix Models. The differences between normal and neoplastic tissue diffusion coefficients were related to the size of the solute molecules and to the physicochemical properties of the interstitial matrix of these tissues using a pore model and a fiber-matrix model (94, 103). It must be pointed out here that there is little evidence based on electron micrographs that well-defined pores or fiber-matrix structures exist in the interstitial space. However, these two models provide an empirical framework within which to examine the transport relationships of different solutes and to obtain some important and useful correlations.

Table 7 Molecular weight dependence of diffusion coefficient in water and tissues

Medium	Solute (M_r)	$D = a(M_r)^{-b} \text{ cm}^2/\text{s}$		Ref.
		a	b	
Water	Dextran (10,000–147,000)	1.26×10^{-4}	0.478	92
Human articular cartilage (<i>in vitro</i>)	Dextran (5,000–40,000)	6.17×10^{-2}	1.34	88
Various normal tissues (<i>in vitro</i>)	Various solutes (32–69,000)	1.778×10^{-4}	0.75 (0.65–0.81)	66
Mesentery (<i>in vivo</i>)				
Cat	Dextran (3,400–393,000)	2.75×10^{-3}	0.758 ^a	68
Rat	Dextran (3,450–41,200)	5.5×10^{-3}	1.09	77
Rabbit ear (<i>in vivo</i>)				
Mature granulation tissue	Dextran (19,400–150,000)	10^a	2.96	22, 80
VX2 carcinoma	Dextran (19,400–150,000)	2.51×10^{-2}	1.14	

^a Diffusion coefficients are higher in the cat mesentery than in the rat mesentery due to possible diffusion in the superperfusate in the former preparation.

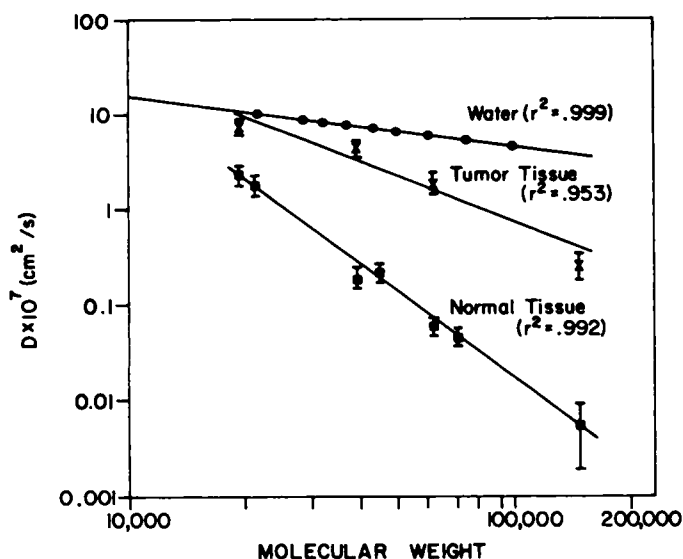


Fig. 3. Dependence of dextran diffusion coefficients on molecular weight. ●, data of Granath and Kvist (92) for aqueous diffusion corrected to 37°C. ×, ■, data for VX2 carcinoma and mature granulation tissue, respectively; —, best fits to the expression $a(M_r)^b$ ($r^2 = 0.999, 0.953, 0.992$ for water, tumor, and granulation tissue, respectively); bars, SD. Reproduced from the paper of Gerlowski and Jain (80), with permission.

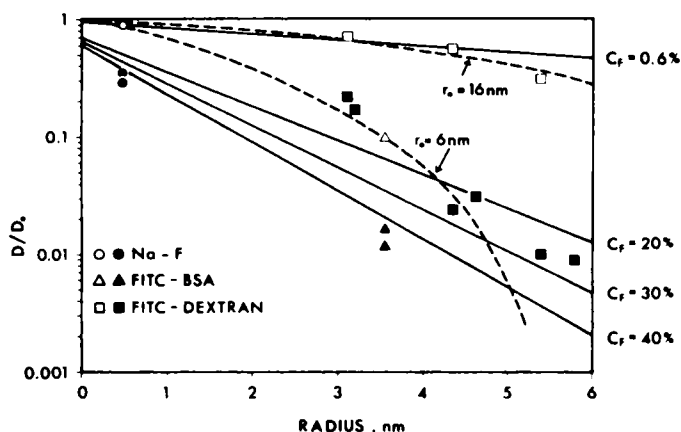


Fig. 4. Ratios of effective diffusion coefficient to free diffusion coefficient for sodium fluorescein (Na-F), fluorescein isothiocyanate (FITC)-BSA, and fluorescein isothiocyanate-dextran versus Stokes-Einstein radius of the molecules; ■, ▲, ●, tumor; □, △, ○, normal tissue; —, fiber-matrix model; —, pore model; r_0 , pore radius; C_F , fiber concentration. Reproduced from the work of Nugent and Jain (94) with permission.

When the interstitial matrix of a tissue is modeled as a circular cylindrical pore of radius r_0 , pore radii of 6 and 16 nm, respectively, describe the dextran data in normal and neoplastic tissues adequately. Considerably smaller pore radii (4 nm for normal and 6 nm for neoplastic) are required to account for the restriction of BSA (Fig. 4).

When the tissue interstitial space is modeled as a random matrix of straight fibers of radius r_F , fiber concentrations C_F of 20 and 0.6%, respectively, account for restriction of dextran by normal and neoplastic tissues. Considerably higher values of C_F (~40% for normal and ~20% for neoplastic) are required to explain the BSA data (Fig. 4). It is of interest to note here that Fox and Wayland (77) calculated the values of C_F to be 6 to 28% to explain their diffusion data for dextran and albumin in the mesentery. Our results show that the granulation tissue of the rabbit ear offers a greater restriction to molecular transport than the mesentery. Although the fiber matrix model is in qualitative agreement with the data, values of C_F are consider-

ably higher compared to measured values of HA. It is possible that collagen fibers also offer resistance to the solute transport (104). Further work is now needed to improve these models to predict diffusion coefficient in tissues based on physicochemical characteristics of solute-tissue system.

B. Hydraulic Conductivity and Retardation Factor

Methods. Hydraulic conductivity of tissues is normally obtained by applying Darcy's law to *in vitro* filtration data. In these experiments, flow rate, Q , of fluid is measured across a tissue slice of thickness Δx and cross-sectional area A for known applied pressure difference, Δp , across the tissue. For steady flow the hydraulic conductivity, K , is given by

$$K = \frac{(Q/A)}{(\Delta p/\Delta x)} \quad (H)$$

Extreme care must be exercised during excising, slicing, and holding (clamping) to avoid tissue compression and damage.

Measurement of interstitial K *in vivo* is extremely difficult. Swabb *et al.* (66) have measured K of s.c. tissue and Hepatoma 5123 in the rat. In these experiments, pressure is suddenly decreased in a micropore chamber placed in the interstitial space, and the resulting flow of interstitial fluid into the chamber is measured as a function of time. The unsteady state analysis of these data provides the pressure diffusivity, E , which is equal to $K/\alpha\theta$. (Here K is the hydraulic conductivity, α is the interstitial space compressibility, and θ is the interstitial space volume fraction.) For details, see the paper of Swabb *et al.* (66).

The retardation factor, R_F , is normally determined from ultracentrifuge experiments by measuring the ratio of the sedimentation coefficient of solute in the desired medium to that in physiological saline solution. The interstitial fluid is usually simulated by hyaluronic acid or proteoglycan solutions (105).

Results. Hydraulic Conductivity. Hydraulic conductivity, K , of a tissue, similar to that of a porous bed, should depend on tissue interstitial space volume fraction, cell diameter, and architecture of the interstitial matrix. In the absence of data on these parameters, Swabb *et al.* (66) proposed the following correlation to describe the *in vitro* values of K for various tissues in terms of their glycosaminoglycan concentration, C_{GAG} (g/100 g tissue):

$$K = 4.6 \times 10^{-13}(C_{GAG})^{-1.202} \quad (I)$$

These authors estimated tissue GAG content as twice published values of hexosamine content for normal tissues, and 0.01–0.05 g/100 g for Hepatoma 5123 based on the liver content of 0.088 g/100 g.

Support for this correlation comes from the qualitative studies of Day (106, 107) and Hedbys (108) who showed an increase in flow across mouse fascia and cornea, respectively, due to the application of hyaluronidase. Similarly, Maroudas (109) found a decrease in K with increasing fixed charge density of articular cartilage. Note that the fixed charge density is related to GAGs. [For more details on the role of polysaccharides on fluid flow, see the reviews by Fatt (110) and Granger (111).]

Contrary to the above hypothesis, Jackson and James (112) found that hyaluronate accounts for only part of the flow resistance. Levick (104) proposes that collagen fibrils can contribute significantly to the interstitial resistance due to their volume occupancy and net surface area. The water content, z , of tissue has been also related to its hydraulic conductivity (109–111). Perhaps one of the most comprehensive power law

correlations between K and z was developed by Bert and Fatt (113), which fits K data over 9 orders of magnitude.

$$K = az^b \quad (J)$$

This dependence of K on water content means that during *in vitro* or *in vivo* experiments water content of a tissue may change due to applied pressure and may lead to erroneous results. In this context it is worth noting that K for dog s.c. tissue was found to be about 2 orders of magnitude higher by Guyton *et al.* (114) than that for rat s.c. tissue found by Swabb *et al.* (66). (1.8×10^{-9} versus 6.4×10^{-12} cm⁴/dyn.s). Similarly, Swabb *et al.* (66) found K for Hepatoma 5123 about 1 order of magnitude higher *in vitro* than *in vivo* (31×10^{-12} versus $2.9 - 8.4 \times 10^{-12}$). More *in vitro* and *in vivo* studies are needed to resolve the effect of tissue composition on K .

Retardation Factor. The relative velocity of a solute with respect to the solvent velocity, R_F , has been studied extensively in model and biological membranes, both experimentally and theoretically (see, *e.g.*, Ref. 115). There is a paucity of such studies for tissues. The data of Laurent and Pietruszkiewicz (105) on R_F in HA solution were described by Swabb *et al.* (66) by the equation

$$R_F = 1.5 \exp[-8.64 \times 10^{-3}(M_r)^{0.366}(C_{HA})^{0.5}] \quad (K)$$

for $6.9 \times 10^4 < M_r < 2.8 \times 10^9$ and $0 < C_{HA} < 0.35$ g/100 g solution.

By assuming that tissue has the same retardation properties as the HA solutions, Swabb *et al.* (66) proposed the following empirical correlation after correcting for the interstitial space fraction (0.43):

$$R_F = 1.5 \exp[-1.318 \times 10^{-2}(M_r)^{0.366}(C_{GAG})^{0.5}] \quad (L)$$

where C_{GAG} ranges from 0 to 0.813 g GAG/100 g tissue.

More rigorous experimental and theoretical studies along these lines are needed to improve our understanding of convective transport of solute in the interstitium.

C. Ratio of Convection versus Diffusion in the Interstitium

Despite overwhelming evidence for significant interstitial convection in normal and neoplastic tissues, there is no direct measurement of the magnitude and direction of convective velocity of a solute or solvent in the interstitium (see Section III). In the absence of such hard data one can only hypothesize about the relative contribution of convection to the interstitial transport. For one dimensional transport, using Equations B and C, one can obtain

$$\lambda = \frac{\text{Diffusive flux}}{\text{Convective flux}} = \frac{D}{R_F K} \cdot \frac{\Delta C}{C} \cdot \frac{1}{\Delta p} \quad (M)$$

in which λ is J_D/J_C (flux ratio). To obtain an order of magnitude of λ , Swabb *et al.* (66) assumed $\Delta C/C \sim 1$ and $\Delta p \sim 30$ mm Hg and simplified Equation M to

$$\lambda \approx 2.5 \times 10^{-5} D/R_F K \quad (N)$$

Using the empirical correlations for D (Table 7), K (Equation I), and R_F (Equation L), these authors estimated diffusion to convection ratios for solutes of given molecular weights moving through tissues with known GAG content (Fig. 5). Note that the transport of low molecular weight substances is diffusion dominated while convection becomes important at higher molecular weights.

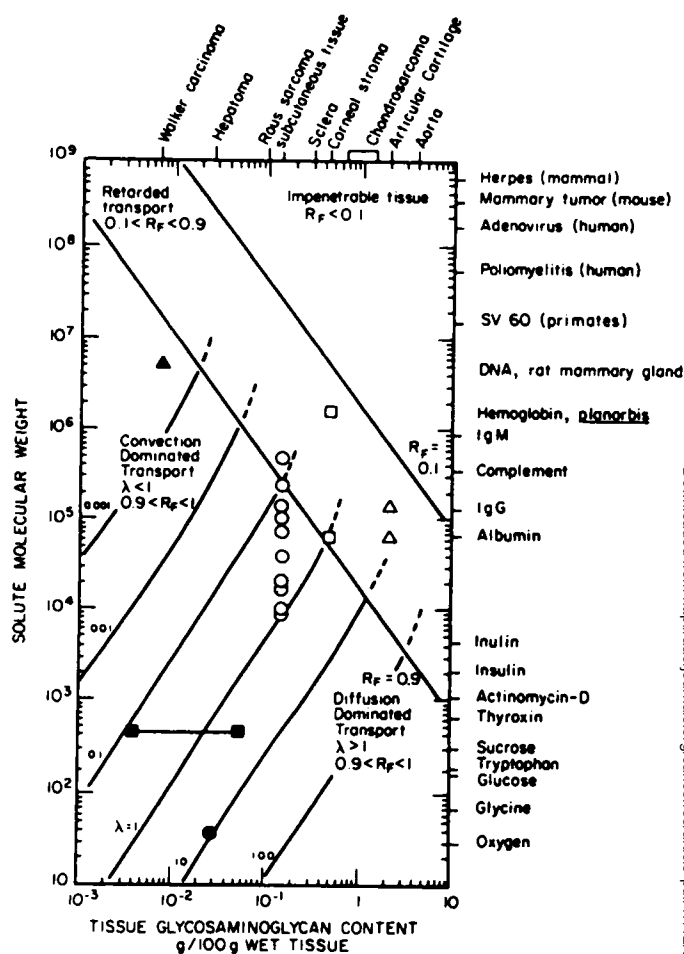


Fig. 5. Importance of diffusive and convective mass transport in the extravascular space of normal and neoplastic tissue at 37–38°C. The parameter λ is the ratio of solute diffusive flux to convective flux. The position of the lines of constant λ is determined by choosing $\Delta c/(c\Delta p) = 1/(30 \text{ mm Hg})$, where c is extravascular solute concentration and p is interstitial fluid hydrostatic pressure. The retardation factor R_F is the solute convective flow velocity/solvent convective flow velocity for flow through a polysaccharide network. Published data validating the analysis are: ●, mouse mammary adenocarcinoma BICR/SA1 and human bronchial carcinoma (116, 117); ■, mouse mammary tumors C3HBA (65, 118); ▲, Walker carcinoma 256 (119); ○, dog paw (120); □, ox corneal stroma (121); △, human articular cartilage (122). Indicated tumor glycosaminoglycan contents are approximate. Reproduced from Swabb *et al.* (66) with permission.

The analysis of Swabb *et al.* (66) assumes that diffusion and convection occur in the same direction making $\lambda > 0$. If these processes occur in the opposite direction λ would be negative. In addition, due to spatial heterogeneity in the interstitial pressure, solute concentration, interstitial volume fraction, polysaccharide concentration, charge distribution, binding, fluid viscosity, and tissue hydration, λ may differ from one location to another in the tissue. The need for experimental and theoretical investigations to address these issues is urgent.

V. Conclusions and Future Perspective

The objective of this review article was to summarize our current understanding of transport of fluid and solute molecules in the tumor interstitium. To this end, we have discussed various experimental and theoretical approaches to quantify interstitial transport in tissues. The data available in the literature suggest that the tumor interstitium is significantly different in structure and function from the interstitium of most normal tissues. In general, the tumor interstitial compartment is characterized by a large interstitial space, high collagen content, low proteogly-

can and hyaluronate concentrations, and absence of anatomically well-defined functioning lymphatic network compared to most normal tissues. These structural differences are presumably responsible for high interstitial fluid pressure and bulk fluid flow and high effective interstitial diffusion coefficient of macromolecules as well as large hydraulic conductivity in tumors.

Despite rapid progress in this area in recent years, there is a paucity of quantitative data on interstitial transport parameters in tissues and several questions remain unanswered. Throughout the text, these unresolved problems were pointed out in hopes of stimulating multidisciplinary research in this area. In what follows, some of these problems are summarized.

Despite the overwhelming evidence and importance of increased interstitial convection in tumors, there are no direct measurements of magnitude and/or direction of convective velocity in normal and tumor tissues. Recent development in fluorescent microscopy should permit measurements of convective *versus* diffusive transport in the interstitium. Availability of such information would help in determining optimal size of macromolecules for tumor detection and treatment.

Although macroscopic interstitial pressure gradients have been measured from the center of a tumor to its periphery, there are no measurements to date of microscopic pressure gradients around individual vessels. This information is needed to relate convective velocity of fluid around vessels with the bulk flow of fluid in the tumor interstitial compartment.

While high interstitial diffusion coefficients of macromolecules favor movement of large molecules in the tumor interstitium, high interstitial pressure and low microvascular pressure may retard extravasation of cells and molecules, especially in large tumors. Methods must be developed, therefore, to modulate these pressures to increase extravasation without significantly reducing interstitial transport in large tumors.

Most data on the composition of and transport in the tumor interstitium are available for animal tumors. Recent developments in various noninvasive techniques, *e.g.*, nuclear magnetic resonance, positron emission tomography, should permit collection of tissue uptake data in patients. Availability of such information should help in determining the transport parameters for human tissues and ultimately in predicting uptake and distribution of pharmacological agents in patients using various mathematical models. The need for research in this area is urgent.

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