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Bulk Transfer of Fluid in the Interstitial Compartment of Mammary Tumors

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SUMMARY

Venous blood leaving a solid tumor showed higher erythrocyte concentration than did aortic blood. Net fluid loss of efferent blood as calculated from hematocrit differences was 2.7 to 6.7% of flow volume, 4.5 to 10.2% of perfusing plasma volume, or 0.14 to 0.22 ml fluid per hr per g in 2 to 5 g transplanted MTW9 and Walker 256 mammary carcinomas, and primary *N*-nitrosomethylurea- and 7,12-dimethylbenz(α)anthracene-induced mammary carcinomas of rats. Net fluid loss was directly related to blood flow but inversely related to tumor size. Increased hydrostatic pressure in tumor interstitial space was a consistent finding. Micropore chambers embedded in transplanted tumors drained 4 to 5 times more interstitial fluid than did identical chambers in the s.c. tissue. It is concluded that: (*a*) convective currents are present within the interstitial spaces of tumors; (*b*) the magnitude of fluid transfer can be measured by the difference in hemoconcentration between afferent and efferent tumor blood; and (*c*) the volume of this fluid transfer is not altered by hormone-induced tumor regression. The increased hydrostatic pressure of tumor interstitial spaces is interpreted as being due to absence of an anatomically well-developed lymphatic network. The bulk transfer of fluid within interstitial spaces is comparable to lymphatic drainage and should be considered in assessing drug concentration and distribution in solid tumors.

INTRODUCTION

Fluid balance among vascular, interstitial, and cell compartments is maintained by hydrostatic and colloido-osmotic pressures. When the venous capillary bed differs in size from the arterial bed as, for instance, in muscle (11), an asymmetry of the exchange area may occur and a lymphatic drainage is required to maintain fluid balance. Thus, a lymphatic network is demonstrable in most normal tissues. In growing neoplastic tissues, the vascular network undergoes extensive modifications in vessel number, length, and surface area (21, 22) with consequent asymmetry of the exchange area. One would, therefore, expect that maintenance of fluid balance within the tumor would require an active lymphatic system. Nonetheless, anatomic evidence of a lymphatic network in solid tumors is lacking. The general conditions under which transport of molecules of different size may occur by diffusion or convection have been described and the likelihood of convective currents in tumor interstitial spaces has been postulated (19, 20). In this paper, evidence is presented to show that: (*a*) such convective currents indeed exist; (*b*) an estimate of their magnitude can be obtained experimentally; and (*c*) this estimate is not substantially changed during hormone-dependent tumor regression.

MATERIALS AND METHODS

Experimental Approach. Convective transfer of fluid within the tumor was evaluated by comparing erythrocyte concentration in the afferent and efferent tumor blood using a preparation in which the whole blood perfusing the tumor was collected by a single vein. From the hematocrit difference and from the value of blood flow determined for each tumor, calculations of the amount of fluid oozing out through the tissue were made.

The presence of a high hydraulic pressure and/or an elevated convective transport within the neoplastic tissue was demonstrated by measuring directly the hydrostatic pressure of the tumor interstitial fluid and the difference between the amount of interstitial fluid drained from the tumor and from the normal s.c. tissue where the transplant was done. Drainage of the interstitial fluid was obtained from micropore chambers embedded within the tissues.

Animals and Tumors. Female rats, 161 to 305 g, were fed Wayne Lab Blox (Allied Mills, Inc., Chicago, Ill.) with water *ad libitum* and kept under identical housing conditions. Transplantable tumors studied were: hormone-dependent MTW9 mammary carcinomas in Wistar-Furth rats (13) and hormone-independent W256[2] in random-bred Sprague-Dawley rats (18). Primary, carcinogen-induced tumors studied were hormone-dependent 7,12-dimethylbenz(α)anthracene mammary carcinomas in Sprague-Dawley rats (12) and hormone-responsive *N*-nitrosomethylurea mammary carcinomas in Buffalo/N inbred line (10). Regression was readily induced in MTW9 tumors by surgical removal of the mammotropin source, a

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[2] The abbreviation used is: W256, Walker 256 mammary carcinoma.

tumor from the hypophysis transplanted into the same host bearing MTW9. Physiopathological characteristics of MTW9 carcinomas during growth and regression have been described elsewhere (8). Castration resulted in regression of most 7,12-dimethylbenz(α)anthracene tumors and growth retardation of most *N*-nitrosomethylurea tumors.

Surgical Procedures. All surgery was performed under urethan anesthesia (1 mg/g i.p.). Tissue-isolated tumors, connected to the host by a single artery and vein were used following a procedure previously described (4, 5). Three PE 50 polyethylene catheters (Clay-Adams, Parsippany, N. J.) were placed into each tumor-bearing rat; 1 was inserted into the right external jugular vein, 1 into the aorta via the left common carotid artery, and 1 into the tumor vein. The latter catheter drained the blood flowing out of the whole tumor. The aortic catheter was used to measure pressure and sample arterial blood. Through the jugular catheter, whole blood was infused into the host to balance the blood removed from the tumor vein and to maintain constant arterial pressure during sampling. This experimental procedure, which permits tumor flow measurement while blood samples are collected, has been reported in detail elsewhere (5).

Sampling. Efferent tumor blood was continuously collected (1) for 1 hr in 6 successive samples of 10 min each. At midpoint of each 10-min venous sample, 0.5 to 0.7 ml of blood was removed from the aorta. Samples were weighed and hematocrits were immediately determined with a Drummond microhematocrit apparatus (Drummond Scientific Co., Broomall, Pa.). Thus, a comparison between aortic and efferent tumor blood was made at each sampling.

^{51}Cr Labeling and Sampling. Five ml arterial blood, anticoagulated with acid-citrate-dextrose solution, were centrifuged, the buffy coat was removed, and erythrocytes were washed twice in 0.15 M NaCl and then diluted to 5 ml with phosphate-buffered saline (0.15 M NaCl + 0.01 M phosphate buffer, pH 7.4) containing 50 μCi of ^{51}Cr (New England Nuclear, Boston, Mass.). The mixture was incubated at 20° for 1 hr with occasional gentle swirling. Unbound ^{51}Cr was removed by 2 washings in phosphate-buffered saline, and then the erythrocytes were diluted to 5 ml in 0.15 M NaCl. Four ml of the mixture were added to the infusion reservoir (40 ml of whole blood) and equilibrated within the perfusion system. The remaining 1 ml of tagged erythrocytes was injected into the tumor-bearing animal through the jugular catheter 15 min before sampling started. ^{51}Cr γ emissions from 200-μl aliquots of afferent and efferent blood were counted. Unbound ^{51}Cr was determined in additional 200-μl aliquots of plasma from each sampling. On the average, only 1.3% of the total blood counts were found in plasma.

Interstitital Fluid Drainage. Tumor interstitial fluid was collected by a micropore diffusion chamber incorporated within the tumor tissue as described (6). The chamber's components were 2 Millipore filters (Millipore Corp., Bedford, Mass.) of 0.45 μm average pore diameter, sealed to a polystyrene ring 2 mm thick, 19 mm outside diameter, and 5 mm inside diameter. Interstitital fluid was collected to the exclusion of cells (6) and drained through a PE 90 polyethylene tubing, 40 cm long (Clay-Adams). The micropore chambers were placed in the s.c. tissue of the interscapular region of Sprague-Dawley rats. Six control animals received a single chamber with no further treatment; their chambers drained s.c. interstitial fluid. Six experimental rats received 1 s.c. chamber, as the controls, as well as 200 mg of W256 carcinoma fragments that were placed around the chamber. For each chamber the draining catheter, heat sealed at the distal end, was pulled through a tunnel of the s.c. tissue to the sacral region where it was coiled and retained in a s.c. pouch. Four or 5 days later, when the tumor had grown to encompass the chamber, the catheter was brought out of the sacral pouch, sutured to the skin, and opened at the distal end. The rat was placed in a restraining cage, and fluid draining from the s.c.- or tumor-surrounded chamber was collected in weighed tubes containing 200 μl of mineral oil and a few crystals of thymol, to prevent evaporation and infection, respectively. Five samples, each consisting of a 24-hr collection of fluid, were taken from both control and tumor-bearing animals. Tumor sizes were measured daily and their weights computed by the formula length x width x height x (π/6). The 24-hr fluid samples were weighed and the average flow rates of s.c. and tumor interstitial fluids were computed.

Since the hydrostatic pressure of the s.c. fluid is very low (Table 5) drainage sometimes does not occur unless the catheter of the chamber is full of fluid and the collecting vial is about 15 cm lower than the chamber, *i.e.*, a siphon action is probably necessary to drain s.c. interstitial fluid.

Interstitial Fluid Pressure. Each of 4 animals received 2 micropore chambers, 1 in the left and 1 in the right lumbar region. Fragments of W256 carcinomas were placed around the left chamber and fluid draining from both chambers was collected for 3 days. Then, both catheters from the chambers of each animal were connected to a water manometer and the pressure needed to stop fluid drainage was measured simultaneously for 3 days. Thus, in the same animal, the hydrostatic pressure of the tumor interstitial fluid was compared with that of the s.c. tissue.

RESULTS

Hemoconcentration of Blood Flowing through Tumors. Table 1 reports the values obtained in a representative experiment where hemoconcentration was measured in the same sample of afferent and efferent tumor blood by 2 methods, hematocrit and ^{51}Cr counts. There was no difference between the data obtained with both methods.

Blood leaving the 4 mammary carcinomas studied consistently showed an increase in hemoconcentration compared with the aortic sample (Table 2). Two of the tumors were transplanted and 2 were primary, carcinogen induced; thus, the findings pertain to solid neoplastic tissue, regardless of its origin.

Hematocrits and blood flow were measured for each tumor and net fluid loss was calculated (Table 3). The range of average values was 0.14 to 0.22 ml/hr/g, which represents a relatively small variation for tumors of different

Table 1

Hemoconcentration in afferent and efferent blood of a growing MTW9 tumor (4.2 g)

Each sampling consisted of efferent blood collection from the tumor vein for 10 min, and of arterial blood collection from the aorta at mid-interval of the venous sampling.

	Concentration in Sample						
	1	2	3	4	5	6	Av.
^{51}Cr counts							
Vein	8,653	11,485	12,487	11,473	10,884	9,621	
Artery	7,990	11,033	11,758	11,348	10,830	9,288	
Vein/artery	1.083	1.041	1.062	1.011	1.005	1.088	1.038
Hematocrits							
Vein	36.8	36.8	37.1	38.0	39.5	37.9	
Artery	36.0	35.0	35.8	36.5	37.2	36.3	
Vein/artery	1.023	1.050	1.037	1.041	1.063	1.043	1.043

Table 2

Hemoconcentration in transplanted and primary mammary carcinomas

Tumor			
Type	Wt (g)	E/A[a]	p
MTW9	4.3 ± 0.9[b]	1.042 ± 0.006	<0.01
W256	3.8 ± 1.0	1.068 ± 0.011	<0.01
7,12-Dimethylbenz(α)anthracene	2.3 ± 0.4	1.051 ± 0.013	<0.01
N-Nitrosomethylurea	3.9 ± 0.6	1.029 ± 0.007	<0.01

[a] E/A, hematocrit of tumor efferent blood/hematocrit of tumor afferent blood.
[b] Mean ± S.E. (5 to 10 tumors in each group).

Table 3

Extravascular fluid loss and tumor perfusion rate

Tumor	FL[a]	Fluid loss (% blood perfused)[b]	Fluid loss (% plasma perfused)
MTW9	0.217	4.2	6.5
7,12-Dimethylbenz(α)anthracene	0.192	5.1	8.5
N-Nitrosomethylurea	0.137	2.7	4.5
W256	0.183	6.7	10.2

[a] FL, [ExH] − [E] where FL = fluid loss (ml/hr/g); E, flow rate of efferent blood (ml/hr/g); H, hemoconcentration factor, ratio of efferent/afferent values obtained from ^{51}Cr counts and hematocrit.
[b] Percentage of blood and plasma perfused were calculated from blood flow and hematocrit values.

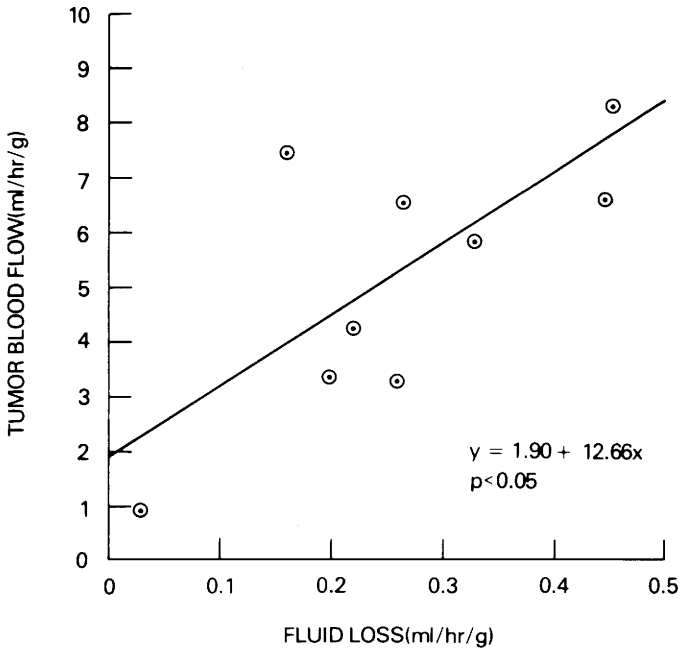

Chart 1. Comparison between fluid loss and blood flow. The amount of fluid loss by tumors was proportionate to blood flow in a weight range of 1 to 8 g (MTW9 carcinomas).

weight, growth rate, and histology. Attention is called to the fact that fluid loss through the interstitial compartment is a considerable amount, 5 to 10% of the input (Table 3). The fraction of fluid supply that did not return into the venous circulation and oozed out of the tumor through the interstitial spaces increased proportionately to the supply of blood (Chart 1). Since blood supply decreases as the tumor mass increases (7), large tumors lost proportionately less fluid per unit mass.

Hemoconcentration was compared in growing and regressing MTW9 carcinomas in an attempt to determine whether differences could be observed during rapid cell loss produced by hormonal deprivation of the host. In 4 out of 6 experiments, regressing tumors had a slightly higher hemoconcentration than did growing tumors; however, the Student t test failed to reveal a significant difference at the 5% level between growing and regressing tumors (Table 4).

Table 4

MTW9 carcinomas: comparison of hemoconcentrations in growing and regressing tumors

	E/A[a]
^{51}Cr counts	
Growing	1.027 ± 0.013[b]
Regressing	1.042 ± 0.031
Hematocrits	
Growing	1.038 ± 0.007
Regressing	1.048 ± 0.011

[a] E/A, hematocrit of tumor efferent blood/hematocrit of tumor afferent blood.

[b] Mean ± S.E.

Hydrostatic Pressure and Drainage of Tumor Interstitial Fluid. Elimination of about 0.2 ml of fluid per hr, which failed to return to the general circulation from each g of tumor (Table 3), should occur through the extracellular spaces. Because anatomical evidence of a lymphatic network is lacking in tumors, drainage should occur through convective currents within the interstitial compartment. The direction of these currents will depend on tissue hydraulic conductivity which in turn depends on glucosaminoglycan content (20). Changes of direction in these currents are probably also produced by a random increase in resistance to flow due to asymmetry of growth in different areas of the tumor. This suggests that hydrostatic pressure should change within the tumor interstitial spaces and probably would be increased as compared to tissues with proper lymphatic drainage.

This hypothesis was tested by connecting the catheter draining the micropore chamber to a manometer and measuring the level of the water column that blocked interstitial fluid drainage. The pressure required to stop drainage of the tumor fluid was consistently larger than the pressure needed to arrest drainage of s.c. fluid (Table 5); both measurements were made in the same rat. When the fluid drained was measured, an amount 4 to 5 times larger was collected from the neoplastic than from the s.c. tissue (Chart 2). Moreover, during 5 days of collection, the flow rate of interstitial fluid changed relatively little for both the tumor and the s.c. tissue. This was consistent with the data on the hydrostatic pressure and suggested that the hydrostatic pressure of tumor interstitial fluid was stabilized at a level several-fold higher than in the s.c. area receiving the transplant (Table 5).

Table 5

Hydrostatic pressure of interstitial fluid

Determinations in W256 carcinoma and s.c. tissue of same animal. Tumor weight, 3.0 to 5.1 g. The catheter of each micropore chamber was connected to the manometer for 3 days and the values reported were determined during 3rd day when equilibrium of pressure was reached.

	Pressure (cm H_2O)			
	TIF[a]		SIF	
Rat	Minimum	Maximum	Minimum	Maximum
1	+26.0	+30.0	−0.2	+0.2
2	+8.0	+8.6	−0.1	+0.2
3	+18.0	+19.0	−0.2	+0.6
4	+14.0	+18.0	−0.1	+0.1

[a] TIF, tumor interstitial fluid; SIF, s.c. interstitial fluid.

DISCUSSION

The fluid movement within tumor interstitial spaces has been evaluated as 0.15 to 0.20 ml/hr/g or 5 to 10% of plasma passing through the vascular network of 4 mammary carcinomas, 2 transplanted and 2 primary. These values were obtained by measuring the change in erythrocyte concentration between afferent and efferent tumor blood. No blood from surrounding tissues could mix with that draining from the tumor since the tumor grew encased in a paraffin bag (5). This certainty is indispensable to the credibility of the results. Venous blood was sampled for 10 min and the arterial sample was taken at midvenous sampling times. Moreover, at least 6 samples were taken from the same tumor over a period of 1 hr; thus, hematocrit changes due to intermittency of circulation in different areas of the tumor were probably compensated for. Hemolysis does occur in tumors (14), but it is a relatively long-term event which does not interfere in a 1-hr-experiment as shown by ^{51}Cr counts in plasma. An increase in erythrocyte volume could also give hematocrit values equal to that measured; thus, loss of fluid might not be the cause of hemoconcentration. We are unaware of any observation showing volume increase in erythrocytes passing through a tumor. However, this theoretical possibility is excluded in our experiments by the data on ^{51}Cr counting since radioactivity is independent of any change in erythrocyte volume. An increase in pressure of tumor interstitial fluid was

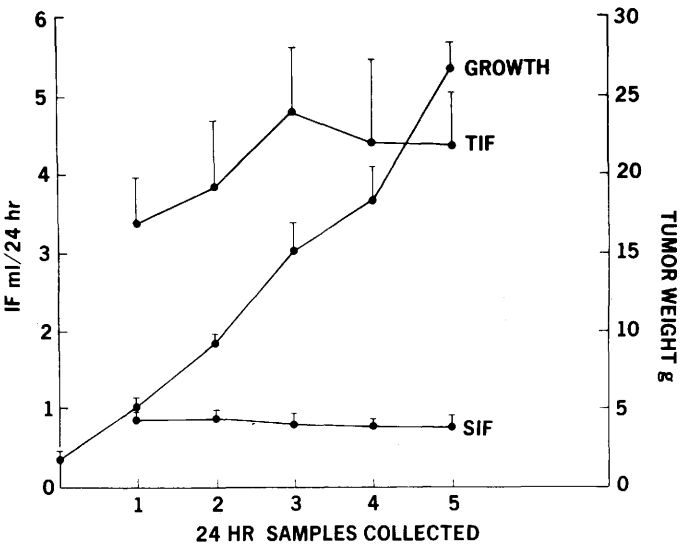

Chart 2. Outflow of interstitial fluid (*IF*) from micropore chambers embedded in W256 tumors (*TIF*) or in the s.c. tissue (*SIF*). Each *point* represents the quantity collected over 24 hr from a group of 5 animals. The collection period lasted 5 days while tumors grew from 2 to 26.5 g (*growth*). *Vertical bar*, S.E.

postulated when drainage from a micropore chamber could be stopped only by pressures of 8 to 30 cm H_2O, much larger than that needed for the s.c. fluid. The data reported here of a 3- to 4-fold increase in the amount of fluid drained from a tumor, as compared with the s.c. tissue when identical micropore chambers were used, further supports the conclusion that a high hydrostatic pressure exists in solid tumors. This condition is probably related to an asymmetry in the diffusion-absorption processes of the tumor vascular network. Since the neoplastic tissue lacks a lymphatic network, only convective transfer of fluid within interstitial spaces can substitute for it. Previous work (9) emphasized the finding that interstitial compartment sizes were much larger in tumors than in normal tissues. This condition may compensate for the lack of an efficient lymphatic drainage.

To our knowledge, the values reported here represent the 1st direct measurement of extravascular fluid transfer in a solid tumor. Indirect estimates have been obtained: values from 0.08 to 0.31 ml/hr/g were calculated for W256 (16, 17) and 0.06 to 0.31 ml/hr/g for Novikoff and hepatocarcinoma 5123 (19). From vascular-extravascular exchange of ^{131}I-plasma proteins, values of fluid loss from the vascular spaces of 2.7% of perfusing plasma have been calculated (2).

The actual amount of fluid oozing out of the tumor depends not only on the hydrostatic pressure of the interstitial fluid but also on the hydraulic conductivity of the tissue. This, in turn, correlates with the amount of glucosaminoglycans present in the interstitial compartment. The conditions influencing diffusion and convection in neoplastic tissues have been discussed in a previous work (20).

Net fluid loss correlated best with the rate of tumor perfusion. A loss of 5 to 10% of supply represents a relatively large amount of fluid to be drained. Often tumors have an edematous halo, especially when growing in the loose s.c. tissue. Usually, this edema is interpreted as being secondary to the mechanical disturbance of the tumor mass. However, equally sized spheres of inert material fail to produce the kind of edema observed, for instance, around 4- to 10-g W256 carcinomas. Accumulation of fluid oozing out of the solid mass is the most likely cause of peritumoral edema.

The presence of convective currents plays a role in the production of lymphatic metastasis and must be considered when measurements on drug transport within tumors are made. However, we are unaware of the directions of these currents and therefore are unable to make a prediction about their role in bulk transport. The importance of these currents is further underlined by observations such as the spread of pyronine dye in tumor intracellular spaces at rates greater than predictable by diffusion alone (15) or presence of dyes in necrotic areas where vascular supply is lacking and transfer by diffusion should occur at a much lower speed (3).

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