Regional Measurements of Blood Flow in Experimental RG-2 Rat Gliomas

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ABSTRACT

Regional measurements of blood flow (F) were performed in transplanted intracerebral RG-2 rat gliomas using [14C]iodoantipyrine, Kety-Schmidt blood flow equations, and quantitative autoradiography. Twenty-nine intracranial tumors in ten rats were analyzed by location; 18 intraparenchymal, seven meningeal, two third-ventricular, and two fourth-ventricular tumors were studied. For all tumors, averaged mean F was 91 ± 33 (S.D.) ml/hg/min. In all but one tumor, mean F was intermediate between normal cortex and corpus callosum values. There was moderate regional variation: averaged mean F was lower in tumor center (78 ± 47 ml/hg/min) than in tumor periphery (93 ± 30 ml/hg/min). Within individual tumors, F showed moderate variation which correlated to some extent with histological features; a regional F of <10 ml/hg/min was observed in only one tumor within an area of necrosis. F in regions of brain immediately surrounding the tumor was higher than in tumor periphery. Blood flow to RG-2 tumors seems unlikely to limit drug delivery any more than to normal brain, and the consistent levels from tumor to tumor and within individual tumors make the RG-2 model an excellent one with which to study drug delivery in experimental brain tumors.

INTRODUCTION

The intracerebral location of malignant gliomas has impeded acquisition of quantitative information about their biological behavior. Nonetheless, the available information suggests that they are remarkably heterogeneous. Their variable histology is well known (23), as is their variable pattern of contrast enhancement on computerized tomography scans (19, 25). Bigner et al. (2) have argued that heterogeneity at the cellular level may be an important factor in their well-known resistance to treatment. In experimental gliomas, variability has also been the common rule. Those induced by ASV5 (7) and ENU (16) exhibit marked variability in latency to time of tumor induction, cellular morphology, and location. Striking variability in the physiological parameters of blood flow and permeability has also been demonstrated in both of these tumor models (5, 6, 10, 11, 20).

The extent to which an animal model can be used to study the factors that regulate drug delivery to human brain tumors is especially problematic considering the variability of both tumor systems and the paucity of quantitative information about blood flow and capillary permeability in human brain tumors. New methods for measuring regional blood flow (1, 18) and vascular permeability (28) in human patients are being developed. At the same time, it may be possible to minimize the problems of experimental tumor variability and heterogeneity by careful selection of models. We selected the RG-2 rat glioma model for these studies because its known attributes suggested that it may be a particularly useful model with which to explore the problem of drug delivery in brain tumors. In this series of studies, we used quantitative autoradiographic methods (4) to measure blood flow and blood-to-tissue transport (12) in RG-2 rat gliomas produced by intracerebral injection of tumor cells. These parameters, which to a large extent determine drug delivery, will be discussed as they relate to the usefulness of RG-2 transplanted gliomas for brain tumor research.

MATERIALS AND METHODS

Tumor Induction and Animal Preparation. The RG-2 tumor cell line was originally derived from an ENU-induced glioma (26). Cells from the same passage level, kept frozen until used for the experiments, were thawed and grown for 48 hr in Eagle’s minimal essential medium with 10% fetal calf serum and gentamicin. They were injected at a concentration of 10^6 cells in 2 μl into the right hemisphere of ten 250-g male Fischer 344 rats (11).

Animals were anesthetized with halothane:nitrous oxide:oxygen [1:5:7.5:30 (v/v)]. Bilateral femoral artery and vein catheters (PE-50 polyethylene tubing) were inserted, and a single extracorporeal A-V shunt was formed with 2 cm of silicon rubber tubing (inside diameter, 1.3 mm; outside diameter, 3 mm; LKB Instruments, Inc., Rockville, Md.). The animals recovered from anesthesia, and body temperature was maintained at 35–37° using heat lamps. Arterial blood pressure, pO2, pCO2, and pH were monitored during the recovery period and immediately prior to the experiment. Blood pressure was monitored continuously during the experimental period. All values were in normal physiological ranges when the experiments were performed.

Experimental Procedures. Regional blood flow was measured using [14C]iodoantipyrine (4-[N-methyl-14C], 40 to 60 mCi/mmol; New England Nuclear, Boston, Mass.) as described previously (24). Isotopic purity was determined with 2 solvent systems (4). Greater than 97% of the radioactivity was localized to the appropriate chromatographic region.

Regional blood flow (F) was determined from experiments in which 40 μCi of [14C]iodoantipyrine in 1 ml 0.9% NaCl solution were administered into the femoral vein contralateral to the A-V shunt for over 35 sec with a variable speed infusion pump, according to an infusion schedule that resulted in continuously increasing blood levels. Serial blood samples, obtained by puncture of the extracorporeal A-V loop with a 23-gauge needle attached to a 1-ml open-barrel syringe, were drawn over each 5-sec period and centrifuged, and plasma radioactivity was measured by β-liquid scintillation counting in a Packard 300C liquid scintillation spectrometer (Packard Instruments, Downers Grove, III.) using external standard quench corre-
tions. Animals were decapitated 30 sec after start of the infusion; the brains were removed within approximately 1 min and frozen in liquid Freon which was cooled to −40°C. The frozen brains were dipped in embedding matrix, tightly wrapped in a plastic freezing bag, and stored at −80°C prior to sectioning.

Quantitative Autoradiography and Histology. Tissue sections were prepared for histology and quantitative autoradiography as described previously (4). Brains were mounted on planchets, and 8 serial sections were cut at 20-μm thickness at −20°C in a cryostat at intervals of 600 μm through the entire brain. Sections 3 to 6 were placed on glass coverslips, rapidly dried on a slide warmer at 85°C, and glued to pressed cardboard for autoradiography. Sections 1, 2, 7, and 8 were placed on microscopic glass slides, fixed in a formalin:ammonium bromide solution (2 g NH4Br:100 ml 10% formaldehyde solution), and stained with hematoxylin and eosin.

The dried tissue sections were placed in an X-ray cassette with [14C]methylmethacrylate standards precalibrated to reference 20-μm-thick brain sections of known radioactivity. The sections and standards were overlaid with single-coated X-ray film (MR-1; Eastman Kodak Co., Rochester, N.Y.) for a 6-week period of exposure. To convert the X-ray film images to tissue radioactivity (nCi/g), the absorbances of the images produced by the [14C] standards were measured, and a standard curve was generated for each film. Sequential measurements of absorbance within 50-μm sections of the tissue autoradiographic image were made with a scanning microdensitometer. The absorbance data were stored by a computer, converted to tissue radioactivity by means of the standard curve, and displayed on a video monitor as described previously (4, 9).

Calculations and Measurements. The calculation of blood flow in these studies depends on a working equation developed by Kety (14, 15)

\[ C_i(T) = X/c \int C_d(t)e^{-kr}dt \]

(A)

where \( C_i(T) \) is the tissue concentration of radioactive IAP as determined by quantitative autoradiography at time, \( T \), after introducing the tracer into the circulation, \( \lambda \) is the tissue:blood partition coefficient, \( C_d \) is the concentration of the tracer in arterial blood, and \( t \) is the variable time. Equation A was solved for \( k \) using the experimentally determined data for the other variables; \( k \) equals a constant that incorporates the rate of blood flow in the tissue as follows

\[ k = mF/\lambda \]

(B)

In Equation B, \( F \) is the rate of blood flow per unit mass of tissue, and \( m \) is a constant between 0 and 1 that represents the extent to which diffusional equilibrium between blood and tissue is achieved by the marker material during its passage from the arterial to the venous end of the capillary (24).

Measurements of \( \lambda \) were made in 5 tumor-bearing rats by the method of Sakurada et al. (24). Anesthetized, tracheotomized rats were given injections of an i.v. bolus of 20 μCi [14C]IAP after both renal arteries and veins, hepatic artery, superior mesenteric artery, and hepatic vein had been ligated. Arterial plasma samples were collected throughout the experiment. The brain was processed as described above, and values of \( \lambda \) were calculated from final arterial plasma [14C]IAP concentrations and brain [14C] concentration as determined by quantitative autoradiography.

Regional measurements of \( F \) were obtained using a computer-controlled cursor-outlining routine on a video monitor that could demarcate selected tissue areas for measurement; the results were expressed as the mean and S.D. of all values for the 50-μm sections within the outlined area. The selected tissue areas were defined on the basis of the histological and quantitative autoradiography images (4). Whole-tumor measure-

ments represent the average of all measurements within the histologically defined tumor cross-sectional area. High and low values represent the range of variation within the tumor, which is also indicated by the S.D. of the whole tumor measurements. Central tumor values represent 20 to 40% of the measurements of the cross-sectional area of the tumor taken from the geographic center of the tumor. Peripheral tumor values represent 20 to 40% of the measurements of the cross-sectional area of the tumor at the tumor edge extending around the circumference of the tumor. The BAT was an arbitrary narrow rim of brain tissue, 200- to 300-μm wide, immediately surrounding the tumor. BST represented a zone, 200- to 300-μm wide, immediately outside BAT. Measurements of \( F \) were also obtained in GLC and GLCC that did not contain tumor.

RESULTS

Patterns of Tumor Growth. Tumors that arose from intracerebral injection of RG-2 tumor cells resulted in large confluent masses, and almost all were intraparenchymal (Table 1). Meningeal subarachnoid tumors, when they did occur, were small (Table 2). Some RG-2 cells apparently spread through cerebrospinal fluid pathways, resulting in tumors that grew independently near the lateral ventricles (Table 1; Fig. 1), third ventricle, or fourth ventricle (Table 3; Figs. 1 and 2). Tumors in the fourth ventricle grew as independent masses that developed intimate contact with but did not invade the choroid plexus (Fig. 2). Near the lateral ventricles, tumors masses invaded surrounding brain and did not grow intraventricularly (Fig. 1). In the third ventricle, the tumors invaded the ependymal lining but were largely intraventricular (Fig. 1).

Microscopic Behavior. Regardless of location, the microscopic appearances of the tumors were similar. Tumor cells were spherical without prominent processes and had moderate cytoplasm and large nuclei with moderate pleomorphism. Areas of necrosis were unusual and, when present, were small; pseudopalisading was uncommon. The edge of the tumors was characterized by an abrupt transition from tumor to brain (Figs. 1 and 2), although occasional, minor perivascular infiltration occurred. BAT in RG-2 tumors represented a 200- to 300-μm zone of brain immediately outside the tumor edge, usually tumor free, and has been discussed previously (11). BST was a 200- to 300-μm zone immediately outside BAT and was always tumor free.

Blood Flow. Blood flow was measured in tumors and brain as outlined above. In fourth-ventricular tumors, the flow in adjacent cerebellum and brainstem were measured. Tables 1 to 3 present data for individual tumors in intraparenchymal (Table 1), meningeal (Table 2), and intraventricular locations (Table 3). In each table, tumors are ordered on the basis of increasing size as determined by the maximum cross-sectional area of the tumor in serial sections. Blood flow in the section with maximum cross-sectional area of a given tumor was found to present a representative pattern of \( F \) for the entire tumor. This was validated by determining \( F \) in a sequential analysis of the histological and autoradiographic sections cut along the rostrocaudal axis of the tumor in several experiments. The lower limit of \( F \) that could reliably be measured in these experiments, represented by X-ray film background, was 1 to 2 ml/kg/min.

Tissue:Blood Partition Coefficient. In 5 intracerebral tumors, \( \lambda \) varied from 0.95 to 1.34 (1.15 ± 0.15). The corresponding value of \( \lambda \) for frontal cortex in the same 5 experiment animals was 0.99 ± 0.13. For the purposes of the calculations reported in this paper, \( \lambda \) was taken to be 0.8, as reported by Sakurada et al. (24), for normal rat cortex. These small variations in \( \lambda \) result
Table 1
Blood flow values in intraparenchymal RG-2 tumors

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Location</th>
<th>Size (sq mm)</th>
<th>Blood flow (ml/hg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole tumor</td>
<td>Low-high</td>
<td>Center</td>
</tr>
<tr>
<td>1</td>
<td>Periventricular</td>
<td>2.27</td>
<td>45-162</td>
</tr>
<tr>
<td>2</td>
<td>Thalamus</td>
<td>2.38</td>
<td>24-103</td>
</tr>
<tr>
<td>3</td>
<td>Cortex</td>
<td>3.17</td>
<td>91-380</td>
</tr>
<tr>
<td>4</td>
<td>Cortex</td>
<td>3.33</td>
<td>28-108</td>
</tr>
<tr>
<td>5</td>
<td>Cortex</td>
<td>3.45</td>
<td>28-207</td>
</tr>
<tr>
<td>6</td>
<td>Thalamus</td>
<td>3.76</td>
<td>40-157</td>
</tr>
<tr>
<td>7</td>
<td>Subcortical white</td>
<td>4.36</td>
<td>32-94</td>
</tr>
<tr>
<td>8</td>
<td>Periventricular</td>
<td>5.48</td>
<td>34-200</td>
</tr>
<tr>
<td>9</td>
<td>Hippocampus</td>
<td>5.54</td>
<td>62-235</td>
</tr>
<tr>
<td>10</td>
<td>Cortex</td>
<td>7.23</td>
<td>57-296</td>
</tr>
<tr>
<td>11</td>
<td>Thalamus</td>
<td>10.81</td>
<td>16-158</td>
</tr>
<tr>
<td>12</td>
<td>Periventricular</td>
<td>11.95</td>
<td>40-216</td>
</tr>
<tr>
<td>13</td>
<td>Thalamus</td>
<td>19.93</td>
<td>16-150</td>
</tr>
<tr>
<td>14</td>
<td>Hippocampus</td>
<td>21.26</td>
<td>14-176</td>
</tr>
<tr>
<td>15</td>
<td>Thalamus</td>
<td>27.8±</td>
<td>22-169</td>
</tr>
<tr>
<td>16</td>
<td>Cortex</td>
<td>34.34</td>
<td>5-125</td>
</tr>
<tr>
<td>17</td>
<td>Thalamus</td>
<td>38.11</td>
<td>37-293</td>
</tr>
<tr>
<td>18</td>
<td>Thalamus</td>
<td>44.12</td>
<td>34-320</td>
</tr>
</tbody>
</table>

a Maximum cross-sectional area.

b Mean ± S.D.

Table 2
Blood flow values in meningeal RG-2 tumors

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Location</th>
<th>Size (sq mm)</th>
<th>Blood flow (ml/hg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole tumor</td>
<td>Low-high</td>
<td>Center</td>
</tr>
<tr>
<td>1</td>
<td>Piriform cortex</td>
<td>0.15</td>
<td>97 ± 9</td>
</tr>
<tr>
<td>2</td>
<td>Temporal cortex</td>
<td>0.33</td>
<td>110 ± 12</td>
</tr>
<tr>
<td>3</td>
<td>Superior colliculus</td>
<td>0.50</td>
<td>125 ± 22</td>
</tr>
<tr>
<td>4</td>
<td>Piriform cortex</td>
<td>0.59</td>
<td>112 ± 16</td>
</tr>
<tr>
<td>5</td>
<td>Superior colliculus</td>
<td>0.99</td>
<td>79 ± 24</td>
</tr>
<tr>
<td>6</td>
<td>Occipital cortex</td>
<td>1.23</td>
<td>91 ± 14</td>
</tr>
<tr>
<td>7</td>
<td>Superior colliculus</td>
<td>1.46</td>
<td>143 ± 35</td>
</tr>
</tbody>
</table>

a Maximum cross-sectional area.

b Mean ± S.D.

Fig. 1. a, histological section of brain with 2 separate tumor foci, one (Table 1, Tumor 5) in cortex and one (Table 1, Tumor 9) in hippocampal fornix; H & E. b, computer-reconstructed image of blood flow. Blood flow values (ml/hg/min), gray scale on right. Note the relative homogeneity of blood flow values throughout these medium-sized tumors.

in negligible (=5%) differences in the values of F calculated by Equations A and B for a 30-sec experiment when the true blood flow is <100 ml/hg/min, and λ varies between 0.8 and 1.2 (8, 22).

Intraparenchymal Tumors. All intraparenchymal tumors had a maximum cross-sectional area >2 sq mm (Table 1). Mean tumor flow ranged from 43 (Table 1, Tumor 16) to 182 ml/hg/min (Table 1, Tumor 3). Mean tumor F was intermediate between...
Blood Flow in RG-2 Gliomas

Fig. 2. a, histological section of brainstem containing 2 distinct RG-2 tumor foci. Bilobed midline tumor corresponds to Tumor 3 in Table 3. Lateral tumor corresponds to Tumor 4 in Table 3; H & E. b, blood flow values for computer-reconstructed image (ml/hg/min), gray scale on right. Although the lateral tumor has a high focal increase in F (207 ml/hg/min), the bulk of both tumors have blood flow values similar to that of surrounding brain.

Table 3
Blood flow values in third and fourth ventricular RC-2 tumors

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Location</th>
<th>Size* sq mm</th>
<th>Whole tumor</th>
<th>Low-high</th>
<th>Center</th>
<th>Periphery</th>
<th>BAT</th>
<th>BST</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Third ventricle</td>
<td>1.58</td>
<td>50 ± 20</td>
<td>22-100</td>
<td>37 ± 8</td>
<td>50 ± 22</td>
<td>78 ± 4</td>
<td>75 ± 4</td>
</tr>
<tr>
<td>2</td>
<td>Third ventricle</td>
<td>4.07</td>
<td>68 ± 24</td>
<td>26-150</td>
<td>69 ± 13</td>
<td>74 ± 25</td>
<td>80 ± 6</td>
<td>81 ± 11</td>
</tr>
<tr>
<td>3</td>
<td>Fourth ventricle</td>
<td>2.71</td>
<td>113 ± 15</td>
<td>71-153</td>
<td>91 ± 12</td>
<td>120 ± 15</td>
<td>97 ± 13</td>
<td>123 ± 10</td>
</tr>
<tr>
<td>4</td>
<td>Fourth ventricle</td>
<td>2.95</td>
<td>130 ± 47</td>
<td>63-207</td>
<td>95 ± 15</td>
<td>135 ± 38</td>
<td>97 ± 13</td>
<td>123 ± 10</td>
</tr>
</tbody>
</table>

* Mean ± S.D.

F in CLC and CLCC in all but one instance (Table 1, Tumor 2), in which F was lower in tumor than in CLCC. Averaged mean whole tumor F (84 ± 34 ml/hg/min) was significantly less than F in CLC (184 ± 80 ml/hg/min) and greater than F in CLCC (41 ± 11 ml/hg/min); p < 0.01, paired t tests.

Tumor location did not correlate with F; however, the number in each location was small. The only tumor located completely in white matter (Table 1, Tumor 7) had a mean F of 51 ml/hg/min. The 3 periventricular tumors (Table 1) had an averaged mean F of 87 ml/hg/min. The 2 hippocampal tumors (Table 1) had an averaged mean F of 98 ml/hg/min. The 5 cortical tumors (Table 1) had an averaged mean F of 99 ml/hg/min, and the 7 thalamic tumors (Table 1) had an averaged mean F of 72 ml/hg/min. None of these groups differed significantly from another.

Moderate regional variation in F was present, as indicated by the range of values observed in individual tumors and the size of the S.D. of whole tumor F. F was higher to tumor center than to tumor periphery in 4 tumors and lower in the center in the remainder (Table 1). Averaged mean F in tumor center was significantly lower than in tumor periphery (p < 0.05, paired t test). F in BAT was higher than in tumor periphery in 9 tumors but, in the other 9, the converse relationship was observed (Table 1); the difference between the 2 was not significantly different (p > 0.05, paired t test). F in BST was higher than F in tumor periphery in 12 tumors (Table 1). Averaged mean F in BST was significantly higher than F in either tumor periphery or BAT (p < 0.05, paired t test). In 11 tumors (Table 1, Tumors 2 to 8, 10, 11, 13, and 17), the highest values of F recorded in each tumor were in tumor adjacent to gray matter structures and, in 3 tumors (Table 1, Tumors 3, 6, and 9), the lowest values were in cortical tumors where they extended to the surface of the brain. In 9 tumors (Table 1, Tumors 2, 8, 11 to 13, and 15 to 18), the lowest values were in the center (Fig. 1). In none were the highest or lowest values of F found adjacent to choroid plexus, even though many of these tumors abutted upon choroid plexus. When tumor abutted on the ventricular surface, values of F were usually intermediate.

The only histological feature that correlated with local variation in F within a tumor was necrosis. All tumors with necrotic foci were over 10 sq mm in maximum cross-sectional area. In 4 tumors, (Table 1, Tumors 11, 13, 14, and 18), the foci of necrosis were <50 μm in diameter. In tumors (Table 1, Tumors 15 to 17), the areas of necrosis were larger (50 to 200 μm in diameter), but in none were large confluent areas of necrosis present. Local values of F in areas of necrosis were below 20 ml/hg/min in 4 of these tumors (Table 1, Tumors 11, 13, 14, and 16), but in only one (Table 1, Tumor 16) was a value of F <10 ml/hg/min measured.

Meningeal Tumors. These tumors generally grew flattened along the brain surface. All meningeal tumors were small; aver-
age size in terms of maximum cross-sectional area was 0.8 ± 0.5 sq mm (Table 2). Mean tumor F was high (Table 2); averaged mean tumor F was 108 ± 22 ml/hg/min. This was accompanied by moderate regional variation in F as indicated by the range of F and S.D. of the mean tumor value (Table 2). None of the tumors had areas of necrosis, and none of the tumors had local values of F <40 ml/hg/min. F in tumor center could be compared to F in tumor periphery in only 4 tumors; in 2 tumors (Table 2, Tumors 5 and 6), F was higher in periphery and, in 2 tumors (Table 2, Tumors 4 and 7), F was higher in tumor center.

F in BAT was higher than mean tumor F in 5 of the tumors and, in 2, it was lower. Averaged mean tumor F was significantly lower than F in contralateral gray matter (p < 0.05, paired t test) and higher than F in contralateral white matter (p < 0.01, paired t test).

Third-Ventricular Tumors. Two tumors were located in the third ventricle (Table 3; Fig. 1). In both, mean tumor F was lower than CLC and higher than CLCC. F in BAT and BST were both higher than mean tumor F or F in tumor periphery. In both tumors, the lowest values were in central regions of the tumor, and the highest values were at the edge where tumor invaded hypothalamus.

Fourth-Ventricular Tumors. Two tumors were located in the fourth ventricle (Table 3; Fig. 2). In both, mean tumor F was lower than CLC and higher than CLCC. F in BAT was similar to mean tumor F in each tumor. In each tumor, the highest values were recorded next to the choroid plexus of the fourth ventricle (Fig. 2).

Tumor Comparisons. The averaged mean values of F for each area of tumor and brain examined in each of the 4 tumor groups, and for all tumors combined, are shown in Table 4. Since these tumors are all RG-2, the principle variable should be location. However, the mean size of tumors in each location is different, so comparisons of F between groups may not be valid. However, when tumor size was compared to whole tumor F, only a weak negative relationship was observed (linear regression correlation coefficient, r = -0.27) which was not significant (p < 0.05; t test).

For the averaged values of all tumors, F increased progressively from tumor center to tumor periphery to BAT, with an abrupt increase at the tumor edge (Table 4). However, the averaged mean differences in F between tumor, BAT, and BST were not significant (p > 0.05; Student's t test). Averaged mean tumor F for all tumors was intermediate between contralateral gray and white matter and significantly different from both (p < 0.001; Student's t test). The values of F in cortex and corpus callosum in tumor-free brain are similar to those reported for normal rats (24). Although there are large and obvious differences between the averaged mean tumor F of the intraparenchymal tumors and those of each of the other groups, the small size of the other groups precludes significant comparisons.

DISCUSSION

Four major observations can be drawn from this study. (a) Mean F to individual RG-2 brain tumors was intermediate between that measured in contralateral gray and white matter in all but one instance. (b) Mean tumor F was remarkably independent of tumor size or location. (c) Moderate regional variation in F was observed, with lowest values in tumor center. Nonetheless, averaged mean F to tumor center in the 8 largest intraparenchymal tumors was still higher than contralateral tumor-free white matter. (d) Regional variation in F could to some extent be correlated with histological features. Where necrosis occurred, F was locally reduced, although a value of F <10 ml/hg/min was measured only once. Where tumor abutted upon gray matter, local F was often the highest and, conversely, the lowest values were often recorded where the tumor was adjacent to white matter. F in tumor adjacent to choroid plexus was not markedly increased; RG-2 tumors did not invade choroid plexus but seemed to push it aside.

These determinations of blood flow in RG-2 tumors can be compared to similar measurements in other brain tumor models. Two autochthonous models have been studied: ENU- and ASV-induced gliomas. ENU-induced gliomas were studied by Blasberg et al. (5, 6), using methods identical to those in the present study, and by Yamada et al. (27), who used [14C]antipyrine as the blood flow marker. In the 33 ENU-induced gliomas reported by Blasberg et al. (5), averaged mean tumor F was 45 ± 20 ml/hg/min. A relationship between tumor size and F was not observed, nor was there an apparent relationship between F and tumor location or histological classification. In general, blood flow values were closer to white matter than were cortical F values. Yamada et al. (27) measured F in 57 ENU-induced tumors. However, their data are not expressed in terms of whole tumor blood flow but, rather, in terms of "viable part, peripheral edge, and necrotic center." They reported a direct relationship between tumor size and F, although this is difficult to discern from their data.

In ASV-induced brain tumors, a completely different pattern was observed (10). Regional measurements of F were made in 46 individual tumors. Mean tumor F varied markedly, with a
range of 9 to 175 ml/hg/min in individual tumors, and an averaged mean F of 60 ± 34 ml/hg/min for all tumors. Variation of F within an individual tumor could also be extreme; a range of 142 ml/hg/min was encountered in one tumor. Blood flow did not correlate with tumor size, location, or histological classification, but it did correlate with histological evidence of necrosis and tumor invasion into choroid plexus. There was also a global reduction in F in animals with hydrocephalus or with large tumor burdens.

Determination of blood flow, similar to those reported here, have been made in transplanted gliomas with the RT-9 cell line (21) and in a "nitrosourea-induced malignant glioma" (13). This latter study, by Hossman et al. (13), used [14C]antipyrine and reported few details; averaged mean F in solid parts of the tumor was 57.8 ± 2 ml/hg/min, with gradual reduction toward zero in the center, where large confluent necrosis was present. In the RT-9 tumors, a weak inverse relationship between tumor size and F was observed; mean F was 67 ± 9 ml/hg/min for 7 tumors with a maximum cross-sectional area <1.5 sq mm and 52 ± 19 ml/hg/min for tumors between 22 and 21 sq mm (21). A consistent reduction in F was present in the center of larger tumors or when necrosis was present. An additional study of F in Walker 256 metastatic tumors has reported findings similar to those in the RT-9 tumors; an inverse relationship between tumor size and F was present, and F values over 40 ml/hg/min were seldom seen in Walker 256 tumors over 2 mm in diameter (6).

In conclusion, some general observations about blood flow to brain tumors in experimental models can be made with reference to the RG-2 tumor results presented here and the results from other tumor models discussed briefly above. In general, mean tumor blood flow values have ranged from 30 to 90 ml/hg/min. Occasional exceptions above and below this range occurred, particularly in ASV-induced tumors but, in general, mean tumor blood flow values were in the range of normal white matter or slightly above. This implies that, for an initial approach, blood flow is not likely to limit drug delivery to experimental brain tumors any more than to white matter of normal brain. Within individual tumors, low blood flow values are particularly likely to be encountered in tumor center or in necrotic areas, but not at the tumor edge, which has been considered to be critical in terms of drug delivery (17). Although BAT has not been specifically discussed with reference to the other tumor models above, in all instances, F to BAT was similar to or higher than that in tumor periphery, as it was in many RG-2 gliomas, suggesting that blood flow to BAT is not likely to impede drug delivery to this region any more than to tumor proper.

In this paper, we presented the results of regional blood flow determinations in RG-2 experimental gliomas and have shown that blood flow in RG-2 gliomas is intermediate between that of normal gray and white matter, and that moderate regional variation F is consistent with the other brain tumor models studied to date. However, the delivery of chemotherapy drugs to tumor is a function of both blood flow and capillary permeability, among other variables (3). In the following paper (12), we will present the results of regional measurements of blood-to-tissue transfer of a model compound in RG-2 gliomas.

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REFERENCES

Regional Measurements of Blood Flow in Experimental RG-2 Rat Gliomas


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