Accessibility of Circulating Immunoglobulin G to the Extravascular Compartment of Solid Rat Tumors

Stephen W. O'Connor and William F. Bale

ABSTRACT

We have measured the rate of influx ($k_m$) of normal rat immunoglobulin G (IgG) from the blood into the fluid surrounding the cells of three syngeneic rat fibrosarcomas as well as rat skin, muscle, lung, and kidney. Also measured was the rate of efflux ($k_{ou}$) of IgG from the tumor and tissue back into the circulation. The value of $k_m$ ranged from 0.11 to 0.90% of the blood value (activity/ml blood) transferred/hr/g for the nonmalignant tissues and from 6.2 to 7.9% of the blood value transferred/hr/g for the three tumors. Dividing $k_m$ by the plasma volume of the tissue gave a measure of the permeability of the vascular bed of that tissue. This ratio was rather constant for the normal tissues studied; however, it was at least an order of magnitude larger in all three tumors, indicating that the vasculature of the tumors was very permeable to IgG.

The interstitial fluid volume (IF) of the tumor and tissue was calculated. The IF of the three tumors contained approximately 0.5 ml of fluid per g, while the IF of normal tissues had values that ranged from 0.14 to 0.34 ml of fluid per g. Knowledge of the IF, $k_m$, and $k_{ou}$ allowed a calculation of the concentration of IgG in the fluid surrounding the cells of tumors and tissues. The concentration of IgG in the IF of the tumors was found to be 50% of the plasma concentration; this was larger than the concentration of IgG in the IF of normal tissues, where the values ranged from 9 to 28% of the plasma concentration.

A model for Ab localization onto solid tumors was developed. The model was used to discuss the mechanism of localization as well as the physiological limits of drug- or isotope-coupled Ab localization.

INTRODUCTION

Autochthonous and transplanted tumors are vascularized by a nonmalignant endothelium derived from the host. This vascular bed separates the fluid bathing the tumor parenchyma from the plasma and thus isolates the majority of malignant cells from the blood supply of the host. In order for antibodies circulating in the plasma to combine with the majority of tumor cells, they must first pass from the plasma across the vascular endothelium into the fluid bathing the tumor cells. Since the vasculature of normal tissues prevents the free movement of proteins from the plasma into the IF, it is conceivable that the vasculature of solid tumors prevents potentially cytotoxic circulating antibodies from reaching target antigens.

The difficulty in obtaining antibody localization onto solid tumors can be caused by many factors. If antibody localization is low, it could be a result of a poor antibody preparation, the binding of injected antibody by circulating antigen, the inaccessibility of injected antibody to solid tumor cells, or other factors. This study will concentrate on only one of these factors, the accessibility of circulating antibody to solid rat tumors. Although the values reported here will represent the accessibility of non-immune IgG to the extravascular compartment of solid tumors, these values should be the same for antitumor antibody as well.

Peterson et al. (9, 10), studying 2 syngeneic rat sarcomas, determined the rate of influx of immunoglobulin into the extravascular fluid of these tumors. Dewey (3), studying primarily normal tissues, also measured the accessibility of γ-globulin to the extravascular fluid of the Walker and Murphy carcinomas, while Song and Levitt (12), studying the Walker carcinoma, measured the permeability of the vasculature of the tumor to rat albumin. This paper extends these studies by measuring the accessibility of circulating IgG to the extravascular fluid of 3 syngeneic rat fibrosarcomas. It will also measure the concentration of IgG in the fluid bathing tumor and tissues by the use of the exchange constants $k_m$ and $k_{ou}$.

The advent of monoclonal antibody has increased the possibility of using radiolabeled or drug-coupled antibody for tumor detection and therapy (13). It is hoped that the values reported here will be of use to those researchers.

MATERIALS AND METHODS

Animals and Tumors. Female Fischer-344 (F-344) rats were supplied by Microbiological Associates. All tumors studied were induced in our laboratory in female F-344 rats with the use of chemical carcinogens. The A-MC and C-MC tumors were induced with methylcholanthrene, while the BP-II tumor was induced with benzpyrene. Histological examination revealed that all 3 tumors were anaplastic fibrosarcomas. After induction, the tumors were maintained by serial, s.c. trocar transplants into recipient F-344 rats. All tumors had been carried for at least 1 year after their induction before they were used in this study.

Iodination and Purification of Normal F-344 IgG. The γ-globulin fraction of F-344 sera was obtained by sodium sulfate precipitation (15). Four mg of γ-globulin were labeled with approximately 1 mCi of $^{125}$I by the ICI method (2). The procedure was performed in such a way as to give 1.5 to 3 iodines/IgG molecule. Forty $\mu$Ci of the $^{125}$I-γ-globulin were mixed with 0.5 ml of serum and further purified by gel filtration on a Sephadex G-200 column (14). The labeled protein separated into a small, high-molecular-weight peak near the void volume, and a second large peak at $M_\text{r}$ 150,000 which was pooled and used in all subsequent experiments. Reduction and sodium dodecyl sulfate-polyacrylamide electrophoresis by Laemmli's method (8) confirmed that the second peak was a pure preparation of $^{125}$I-IgG.

$^{51}$Cr Labeling of Erythrocytes. F-344 whole blood was added to an equal volume of Alsever's solution. The erythrocytes were pelleted and the buffy coat removed. The erythrocytes were washed 2 additional times. One-tenth ml of Na$^{51}$CrO$_4$ was added to a buffer (pH 7.4) consisting of 2.1 ml of Ringer's solution, 0.6 ml of 3% sodium tricitrate,
and 0.3 ml of 5% glucose. Two ml of packed RBC were added dropwise to this solution. After 1-hr incubation at room temperature with occasional stirring, the cells were centrifuged and washed twice.

**Exchange Rates of IgG.** The accessibility of circulating IgG to the extravascular fluid of solid tumors and tissues was measured by injecting $^{125}$l-IgG into the femoral vein of tumor-bearing rats and measuring the radioactivity in the tissues as a function of time after injection. The injected $^{125}$l-IgG rapidly mixes in the blood of the rat. This rapid dilution is followed by a slower accumulation of the $^{125}$l-IgG into the extravascular fluid of the tumor and tissues of the rat. The experiment was performed in the following way. A group of F-344 rats was given a small s.c. implant of tumor 8 to 12 days before injection. The day before injection, the animals were given 625 μM KI in their drinking water to facilitate the excretion of inorganic iodine and prevent the uptake of iodine-125 by the thyroid. One-half ml of $^{125}$l-IgG or 0.5 ml of $^{125}$l-IgG and $^{51}$Cr-labeled RBC diluted in 0.9% NaCl solution (saline) was injected into the femoral vein of tumor-bearing rats. At times, from 5 min to 4 days after injection, a treated animal was anesthetized, weighed, and blooded by cardiac puncture, and sacrificed. The tumor and organs were removed, and together with the blood, they were weighed and counted in a dual-channel, well-type scintillation counter. The tumors were on the order of 1 g in weight. If the tumors were much larger than 1 g, only nonnecrotic areas of the tumor were weighed and counted. Normal tissue study was taken from either normal or tumor-bearing animals and gave similar results in either case. Curves for normal and tumor tissue were generated using approximately 30 animals. Both ether asphyxiation and decapitation of anesthetized animals were used to sacrifice animals. Both methods gave similar results for lung, skin, muscle, and tumor. The kidney had a lower blood volume in decapitated animals but the same value for $k_i$ and $k_o$.

**Extracellular Fluid Volume: Inulin.** Following a procedure similar to that of Boyle ef al. (1), tumor-bearing rats were sacrificed, and about 1 g of the tumor was removed, weighed, and minced in a small cup. Two ml of minimal Eagle’s medium containing $[^3H]$inulin were added to the cup, and at times, from 0 to 60 min, after the addition, 10-μl aliquots of media were removed for liquid scintillation counting in a Nuclear Chicago scintillation counter. The $[^3H]$inulin diffuses into the tumors ECF and at equilibrium the ECF of the tumor can be calculated as follows:

$$\frac{ECF}{g} = \frac{(\text{Volume of media})}{(\text{Wt of tumor})} \times \frac{(\text{initial cpm} - \text{final cpm})}{(\text{final cpm})} \quad (A)$$

**Extracellular Fluid Volume: Sodium.** A tumor-bearing rat was anesthetized and bled by cardiac puncture. The rat was then sacrificed, and the tumor and tissues were removed, weighed, and then dried. The dried tissues were dissolved in concentrated HNO$_3$ and their sodium content determined in a flame photometer. Assuming the concentration of sodium is much higher outside the cell than inside, and that the concentration of sodium in the IF is the same as the concentration of sodium in the serum, the ECF of the tissue can be estimated by the following formula:

$$\frac{\text{Volume of ECF (ml)}}{\text{Wt of tissue (g)}} = \frac{\text{sodium/g tissue}}{\text{sodium/ml plasma}} \quad (B)$$

If the ECF is very small, then the contribution of intracellular sodium may be sizable, yielding an incorrect value for the ECF.

**Plasma Volume of Tissue.** Just after i.v. injection of $^{125}$l-IgG, all the radioactivity in the tissue is intravascular. The plasma volume can be calculated from graphs which will be discussed later as

$$\text{ml of plasma/g of tissue} = Y \text{-intercept} \times \left(\frac{100\text{-hematocrit}}{100}\right) \quad (C)$$

**Calculations.** The change in radioactivity in the extravascular compartment of the tissue with time is caused by 2 factors: (a) the influx of radioactive material from the blood across the capillaries into the extravascular compartment of the tissue; and (b) the efflux of radioactive material out of the tissue:

$$\Delta \text{cpm/\text{g}} = (\text{influx of } ^{125}\text{l-IgG from the blood}) - (\text{efflux of } ^{125}\text{l-IgG out of the tissue})$$

Letting $T(t)$ equal extravascular cpm/g of tissue at time $t$, $B(t)$ equals cpm/g of blood at time $t$, $k_i$ equals influx rate constant, and $k_e$ equals efflux rate constant:

$$\frac{dT(t)}{dt} = k_iB(t) - k_eT(t) \quad (D)$$

(a) at early time points, $k_eT(t) = 0$, and Equation D integrates to:

$$k_i = \int_0^t B(t)dt \quad (E)$$

(b) at later time points, $B(t)$ can be approximated by $A_0e^{-kt}$, and Equation D integrates to:

$$T(t) = \frac{A_0k_1}{k_2 - \alpha} (e^{-\alpha t} - e^{-kt}) \quad k_2 > \alpha \quad (F)$$

(c) letting $t \to \infty$ and dividing by $B(t)$ and its equivalent $A_0e^{-kt}$ yields:

$$T(t) = \frac{k_1}{k_2 - \alpha} \quad (G)$$

**RESULTS**

$^{125}$l-IgG injected i.v. rapidly mixes in the blood of the rat, and initially all the radioactivity in the tissue is a result of $^{125}$l-IgG in the plasma volume of the tissue. At later times, the total radioactivity in the tissue is the sum of both the $^{125}$l-IgG in the plasma of the tissue and the $^{125}$l-IgG which has passed across the vascular endothelium into the IF. The difference between the total radioactivity and the radioactivity in the plasma volume of
the tissue is the radioactivity which is present in the IF. After i.v. injection of $^{125}$I-lgG into tumor-bearing rats, there is a time-dependent accumulation of radioactivity into the IF of the A-MC tumor (Chart 1). The initial time points of this figure can be used in Equation E to calculate the influx rate constant $k_w$. By taking the percentage of injected dose in the IF of 1 g of tumor at an early time point $t_0$ (Chart 1) and dividing this by the area under the curve of the percentage of injected dose in 1 g of blood plotted from zero to $t_0$ (Chart 1), $k_w$ was calculated as the percentage of the blood value (activity/g blood) transferred to the IF of 1 g tumor/hr (Table 1). Similar experiments were also performed on the C-MC and BP-II tumors as well as skin, muscle, lung, and kidney; their influx rate constants are also reported (Table 1).

These values, however, are not an accurate measure of the permeability of the capillary bed of the tissue, since some tissues are much more highly vascularized than others. Using the plasma volume of a tissue as a measure of the extent of its vascularization, $k_w$ was divided by the plasma volume of the tissue to give a ratio reflecting the relative permeability of the capillaries of that tissue (Table 1). Correcting for the plasma volumes of the tissues gave similar ratios for the 4 normal tissues studied. The tumor ratios, however, were an order of magnitude larger than those of normal tissues (Table 1), reflecting the higher permeability of the capillaries of the tumors.

Chart 2 reflects the time-dependent increase in the ratio of total $^{125}$I-lgG per g of tumor to $^{125}$I-lgG per g of blood. The $Y$-intercept of this graph reflects the radioactivity in 1 g of tumor at zero time, before any $^{125}$I-lgG has been able to pass into the IF, and can be used to calculate the plasma volume of the tumor by Equation C. The value of the intercept is small and illustrates the fact that A-MC tumor is poorly vascularized. The plasma volumes of the 3 tumors and 4 normal tissues are given in Table 2 and are in good agreement with the RBC volumes obtained using $^{51}$Cr-labeled erythrocytes. The difference between the $Y$-intercept and the ratio at later times equals the ratio of extravascular cpm/g tumor to cpm/g blood. The figure demonstrates an initial influx of radioactivity into the IF of the tumor. As the $^{125}$I-lgG accumulates in the IF, the return of $^{125}$I-lgG to the blood becomes increasingly important, until a steady-state ratio is reached. This plateau ratio minus the $Y$-intercept of Chart 2 along with the value of $k_2$, and $\alpha$ (the half-life of $^{125}$I-lgG; value, 0.0064) were used to calculate the efflux rate constant, $k_{ex}$, using a rearrangement of Equation F. Table 1 reports the efflux rate constants as the percentage of the tissue value (activity/g tissue) lost per hr.

Table 1

<table>
<thead>
<tr>
<th>Tumor</th>
<th>$k_w$</th>
<th>$k_w$/plasma (ml)</th>
<th>$k_{ex}$</th>
<th>$k_{ex}$/plasma (ml)</th>
<th>$\frac{T[125I-lgG]/[plasmal]}{[lgG]}$</th>
<th>$\frac{125I-lgG}{lgG}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-MC tumor</td>
<td>6.2</td>
<td>± 0.5</td>
<td>0.49</td>
<td>± 0.02</td>
<td>13.44 ± 3.0</td>
<td>9 ± 6</td>
</tr>
<tr>
<td>C-MC tumor</td>
<td>7.9</td>
<td>± 0.6</td>
<td>0.48</td>
<td>± 0.03</td>
<td>16.68 ± 6.0</td>
<td>16 ± 8</td>
</tr>
<tr>
<td>BP-II tumor</td>
<td>6.7</td>
<td>± 0.6</td>
<td>0.38</td>
<td>± 0.05</td>
<td>18.43 ± 8.0</td>
<td>9 ± 5</td>
</tr>
<tr>
<td>Lung</td>
<td>0.43</td>
<td>± 0.05</td>
<td>0.225 ± 0.007</td>
<td>2.50 ± 1.00</td>
<td>0.76 ± 0.10</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.108</td>
<td>± 0.03</td>
<td>0.055 ± 0.003</td>
<td>3.19 ± 0.50</td>
<td>0.304 ± 0.07</td>
<td>0.044 ± 0.005</td>
</tr>
<tr>
<td>Skin</td>
<td>7.6</td>
<td>± 0.8</td>
<td>0.22</td>
<td>± 0.01</td>
<td>27.00 ± 0.50</td>
<td>0.46 ± 0.10</td>
</tr>
<tr>
<td>Kidney</td>
<td>9.0</td>
<td>± 1.0</td>
<td>0.078 ± 0.008</td>
<td>96.28 ± 2.00</td>
<td>0.36 ± 0.13</td>
<td>0.0775 ± 0.01</td>
</tr>
</tbody>
</table>

$^a$ Percentage of the blood value (activity/ml) transferred/hr/g tissue.

$^b$ Ratio of extravascular cpm/g tissue to cpm/g blood.

$^c$ Percentage of tissue value (activity/g) returned/hr.

$^d$ Ratio of the concentration of IgG in extravascular fluid to the concentration of IgG in the plasma.

$^e$ Mean ± S.E.

Note: The mean ± S.E. of the blood plasma volume and hematocrit, and this may have contributed to low values of the skin and kidney. Analysis of the fluid compartments of the tumors shows them to be poorly vascularized tissues with large IFs.

ECF content of the 3 tumors was measured by 2 methods. Both $^3$Hinulin and the total sodium content of the tissue gave similar tumor ECF volumes (Table 2). Only the total sodium content of the tissue was used to estimate the ECF of normal tissues (Table 2). Because the muscle ECF was small, an overestimate of its value might have been made. The 3 tumors studied gave surprisingly large values for their ECFs, but these values are in good agreement with those reported by Gullino (5, 6). The IF of the tissue is the total extracellular fluid minus the plasma volume and is also given in Table 2. The RBC volume of the tissues was determined with $^{51}$Cr-labeled RBC (Table 2) and, like the plasma volume determinations, gave very small values for the 3 tumors. Knowing the plasma volume and RBC volume allowed a calculation of average hematocrit of the tissue (Table 2). Except for the kidney, ether asphyxiation and decapitation yielded the same RBC volume, plasma volume, and hematocrit. It is known that the hematocrit of blood within tissue is smaller than that of a circulating blood sample, and decapitation will cause the hematocrit within the tissue to be even lower (4). In addition, an overestimate of the plasma volume will lower the calculated hematocrit, and this may have contributed to low values of the skin and kidney. Analysis of the fluid compartments of the tumors shows them to be poorly vascularized tissues with large IFs.
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Table 2
Fluid compartments of tumors and tissues

<table>
<thead>
<tr>
<th></th>
<th>ml RBC/g</th>
<th>ml plasma/g</th>
<th>$[^3]H][inulin$</th>
<th>Sodium$</th>
<th>Total H2O/g</th>
<th>IF (ECF-plasma)</th>
<th>Hema- tcrit</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-MC tumor</td>
<td>0.0028 ± 0.0001$^a$</td>
<td>0.0070 ± 0.0033</td>
<td>0.61 ± 0.044</td>
<td>0.63 ± 0.02</td>
<td>0.88 ± 0.005</td>
<td>0.60 ± 0.05</td>
<td>28.6</td>
</tr>
<tr>
<td>C-MC tumor</td>
<td>0.0026 ± 0.0004</td>
<td>0.0051 ± 0.0011</td>
<td>0.56 ± 0.06</td>
<td>0.58 ± 0.06</td>
<td>0.89 ± 0.002</td>
<td>0.55 ± 0.006</td>
<td>33.8</td>
</tr>
<tr>
<td>BP-11 tumor</td>
<td>0.0041</td>
<td>0.0076 ± 0.0070</td>
<td>0.34</td>
<td>0.41</td>
<td>0.65 ± 0.02</td>
<td>0.33</td>
<td>35.0</td>
</tr>
<tr>
<td>Skin</td>
<td>0.0013 ± 0.0001</td>
<td>0.0054 ± 0.0005</td>
<td>0.35 ± 0.03</td>
<td>0.55 ± 0.02</td>
<td>0.34 ± 0.03</td>
<td>19.4</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>0.0019 ± 0.0001</td>
<td>0.0046 ± 0.0004</td>
<td>0.15 ± 0.02</td>
<td>0.76 ± 0.02</td>
<td>0.14 ± 0.02</td>
<td>29.2</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>0.0915 ± 0.005</td>
<td>0.1269 ± 0.025</td>
<td>0.42 ± 0.06</td>
<td>0.82 ± 0.02</td>
<td>0.293 ± 0.08</td>
<td>41.9</td>
<td></td>
</tr>
<tr>
<td>Kidney (ether)</td>
<td>0.0655 ± 0.002</td>
<td>0.1173 ± 0.005</td>
<td>0.48 ± 0.05</td>
<td>0.77 ± 0.01</td>
<td>0.34 ± 0.06</td>
<td>23.2</td>
<td></td>
</tr>
<tr>
<td>Kidney (decapitation)</td>
<td>0.0258 ± 0.002</td>
<td>0.054 ± 0.004</td>
<td></td>
<td></td>
<td></td>
<td>20.5</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Extracellular fluid (ml/g tissue).
$^b$ Mean ± S.E.

The values of $k_{in}$, $k_{out}$, and the IF of the tissue can be used to calculate the concentration of IgG in the fluid bathing the cells of the tumors and tissues. At equilibrium the ratio of $k_\text{in}$ to $k_\text{out}$ is equal to the ratio of extravascular IgG/g tissue to IgG/g blood. This calculation assumes no local production of IgG and that the concentration of IgG is held constant in the blood. It gives a value slightly lower than the plateau ratio minus the Y-intercept of Chart 2 where the $[^{125}]$lgG in the blood is constantly dropping. Using the IF volume of the tissue, the ratio of the concentration of IgG in the fluid bathing the tumor cells to the concentration in the plasma is determined and given in the last column of Table 1.

DISCUSSION

Previously reported values for the influx rate constant of tumors have ranged from 1.3 to 2.7% of the plasma activity transferred/hr/g tissue for the Murphy and Walker tumors, respectively (3), and from 1.2 to 1.9% for the 2 syngeneic tumors studied by Peterson et al. (10). By multiplying the influx rate constants of Table 1 (which are based on activity per ml of blood) by 1-hematocrit, this study determined influx rate constants that range from 3.2 to 4.1% of the plasma activity transferred/hr/g tumor. These values are slightly larger than those reported previously and may simply reflect differences in the extent of vascularization of the tumors.

The efflux rate constant $k_{out}$ represents the loss nonimmune IgG from the IF of the tissue. The efflux of IgG takes place both across the vascular endothelium and through the lymphatic system. Although there is no proof of lymphatic drainage in these tumors, the fact that the concentration of IgG is lower in the IF than in the plasma might indicate its presence. If antitissue antibody is used instead of nonimmune IgG, the value of $k_{out}$ will be smaller because of its binding to tumor.

Only short- and long-term data have been used to estimate $k_{in}$ and $k_{out}$. However, when these values of $k_{in}$ and $k_{out}$ along with $B(t)$ are used in Equation D, the curve generated by a numerical solution of the resulting differential equation fits all the time points. The rate constants for the A-MC tumor produced a theoretical curve which fit the experimental data with a correlation coefficient of 0.98, thus supporting the simple kinetics assumed here.

Although the rate constants reported here represent the influx of normal IgG, the values should hold for immune IgG as well and should reflect the accessibility of circulating antibody to solid tumor cells. The localization of antibody onto solid tumor cells is a complex problem. In order for circulating antitumor antibody to reach the extravascular fluid of the tumor, it must first pass into the vasculature of the tumor and then across the vascular endothelium into the fluid bathing the tumor cells. Antibody entering the bloodstream from lymph nodes, spleen, or by injection will enter the IF of normal tissue as well as that of the solid tumor. While this process may be important in killing metastatic tumor cells, until this extravascular antibody in the IF of normal tissues reenters the bloodstream, it will be unable to bind cells of the implanted tumor. Extrapolating the linear portion of the blood decay curve in Chart 1 back to the ordinate demonstrates that at equilibrium 50% of the IgG is extravascular, predominantly in the IF of normal tissue. Thus, the kinetics of the accumulation of antibody onto a solid tumor must at least include its distribution into normal tissues, its metabolic decay, and its accessibility to solid tumor cells.

The accumulation of immune IgG onto the A-MC tumor was determined using a simple model for the distribution and metabolism of normal IgG, and the value of $k_{out}$ for the A-MC tumor. As illustrated in Chart 3, the rat can be considered as consisting of 3 compartments: (a) the tissues into which $\gamma$-globulin exchanges slowly, such as skin and muscle; (b) those in which $\gamma$-globulin exchanges quickly, such as lung and kidney; and (c) the blood compartment. The rate constants of this model were determined by a variety of techniques. Whole-body counting (data not shown) was used to determine the metabolism of $^{125}$I-IgG. Since 50% of the IgG is extravascular, this decay constant was doubled to give $k_w$, while $k_1$, $k_2$, and $k_4$ were determined by adjusting their values to fit the blood decay curve of a normal rat. The value of the influx rate constant for a 1-g A-MC tumor was used to calculate $k_w$. Since antitumor antibody will bind tumor cells, $k_{out}$ cannot be used to determine $k_7$. Instead, a 1-day half-life of antibody bound to tumor cells, as determined by Izzo and Bale (7), was used to calculate $k_7$. If we consider a 1-g tumor in a 100-g rat and assume that all of the bound $\gamma$-globulin remains at the site of the tumor ($k_7 = 0$), then the model predicts that 15% of the injected $^{125}$I-IgG reaches the tumor in 24 hr, and approximately 45% of the injected $^{125}$I-IgG eventually finds its way to the tumor before being metabolized. On the other hand, if it is assumed that $^{125}$I-IgG has a 1-day half-life at the site of the tumor and that once localized it will be unable to relocalize, bound radioactivity reaches a peak in 1.5 days and has a peak value of 12% of the injected dose. In either case, the ratio of tumor size to animal size will of course alter the extent of localization.

The predicted amount of localization can be compared with that actually obtained using labeled antitissue antibodies. The extent of localization predicted by the model will be too high if antibody binding circulating antigen inhibits localization. The interesting fact is that instead of being too high, the actual
localization that Izzo and Bale (7) obtained using immune alloantibody in suppressed animals with a growing tumor was in fact higher than that allowed by the model. That is to say that more antibody localized than ever reached the extravascular space of the tumor. Three possible explanations for this discrepancy are: (a) Fischer tumors growing in Buffalo rats have vascular trees that are of a poor architecture, and more 125I-IgG antibody is able to enter the IF than is true of this syngeneic system; (b) an inflammatory or immune response on the part of the host might allow greater influx of 125I-IgG; and (c) antibody may in fact not have to leave the blood or the vascular compartment to localize; it may be binding to tumor cells lining the blood vessels.

In conclusion, while the vascular tree of the 3 tumors studied here inhibits the accessibility of IgG to tumor cells, poor localization is as much a result of the low vascularization of the tumor as it is a result of the low permeability of their capillary beds, since normalizing $k_5$ for the blood volume of the tissue showed the tumors to be very permeable to IgG. This result is consistent with the idea that tumors secrete a vascular permeability factor (11). Lack of accessibility of antibody to the tumor is probably not the only explanation for poor in situ deposition of rat antibody, and factors such as circulating antigen must be included for a full explanation.

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