SUMMARY

$^{133}$Xe dissolved in 0.9% NaCl solution was injected directly into KHT sarcomas in C3H mice in order to study tumor blood flow. The rate of blood flow in ml/100 g/min was calculated from the clearance half-time and the partition coefficient. The mean rate of blood flow in the tumors of unanesthetized control mice was 21.2 ± 0.8 ml/100 g/min, which was significantly faster than the blood flow in the tumors of pentobarbital-anesthetized control mice. $^{133}$Xe was cleared exponentially in virtually all tumors, and rate of clearance decreased with increasing tumor volume in tumors larger than approximately 500 to 600 cu mm; in smaller tumors there was no conclusive evidence of dependence of clearance rate on size. Clearance rate was unaffected by multiple daily injections of $^{133}$Xe. At 3 hr after localized tumor irradiation (250 kV; half-value layer, 5.9 mm Al) with doses greater than 1,000 rads, blood flow tended to decrease with increasing radiation dose. At 3 to 4 days postirradiation, rate of blood flow was significantly increased in tumors receiving 1,000 rads; blood flow was also increased in tumors exposed to 2,000 and 4,000 rads, but not until approximately 7 days. Rate of blood flow decreased at 3 hr and 3 to 4 days in tumors irradiated with 8,000 to 16,000 rads. The postirradiation increases in blood flow were not correlated simply with gross tumor shrinkage, although tumor growth was slowed proportionally to radiation dose. Thus, irradiation brought about changes in rate of blood flow in the KHT sarcoma, and these changes were dependent on both radiation dose and postirradiation time. The observed time course of radiation-induced blood flow changes may partially account for the tumor reoxygenation shown in our previous studies and those of others.

INTRODUCTION

In a prior series of radiobiological experiments, we have studied functional cellular changes that occur in tumors after irradiation (9-11, 24). As most tumors are heterogeneously supplied with oxygen, irradiation preferentially kills those cells that contain oxygen, while anoxic or severely hypoxic tumor cells are spared. The events that supervene during the 1st 1 to 2 days after moderate radiation doses have been termed "reoxygenation," and this is characterized by a return of the tumor to the net oxygenation status approximating that which it exhibited prior to irradiation. Although "reoxygenation" is a designation which cannot rigorously be supported by direct measurements, it is difficult to conceive of any other phenomenon that would account for the observed data. Angiographic studies (18) have established that irradiation is followed by alterations in the intravascular volume of certain experimental tumors, and it has been inferred that such alterations "may be translated into a relative increase of tumor tissue oxygenation" (19).

The use of radioactive inert gases $^{133}$Xe and $^{85}$Kr for studying circulatory physiology is now well established (4, 12-14). Tumor blood flow has been studied by the rate of clearance of $^{85}$Kr from tumors in rabbits; here, the isotope had entered the tumor from the lungs via the arterial circulation. With the introduction of the direct injection technique (13), the placement of dissolved $^{133}$Xe directly into tumors has been used to measure blood flow in both human (7) and transplanted (15-17) tumors. We used this technique to determine whether the reoxygenation which occurs in irradiated tumors might be related to the effects of irradiation on tumor blood flow.

MATERIALS AND METHODS

Tumors and Irradiation. The KHT sarcoma in syngeneic C3H mice was used for these experiments. Tumors were transplanted from single cell suspensions prepared as described previously (11): $10^6$ viable tumor cells were inoculated intradermally in a volume of 0.05 ml. These inocula reached sizes appropriate for clearance studies (about 100 to 200 cu mm) after approximately 8 to 20 days. For irradiation, the mice were anesthetized with i.p. sodium pentobarbital in a dosage of 0.06 mg/g body weight. The tumors, together with overlying skin, were retracted gently and sandwiched between the halves of special Plexiglas localizers (8) from which air was excluded by the use of a tissue-equivalent bolus made of rice paste. The localizers were fitted over a brass tube that is part of a 6-place cone; this permits the simultaneous irradiation of 6 tumors in localizers. Thus assembled, the tumors were fixed at a position 35 cm from the target of a 250-kV constant...
potential X-ray machine, operated at 15 ma, without added filtration and with a half-value layer of 5.9 mm Al. The radiation dose rate was 301 rads/min. Each irradiated tumor received a single dose ranging from 1,000 to 16,000 rads.

Radioisotope Techniques. $^{133}$Xe was dissolved in 0.9% NaCl solution with a minimum concentration of 2 mCi/ml. For injection into tumors, a 250-μl syringe was filled with the isotope solution from a rubber-stoppered injection vial; precautions were taken to keep the solution free of air in both the vial and the syringe. The procedure for tumor injection was usually as follows: the point of the needle (30-gauge; outside diameter, 0.3 mm) was made to enter through the skin and into the tumor near its base; the needle was advanced halfway across the diameter of the tumor, and 10 μl of radioactive solution were deposited. The needle was then withdrawn along the same track, but not removed from the tumor, and then angled about 45° to the right or left; the needle was advanced about halfway to the opposite margin of the tumor and another 10 μl were deposited; this procedure was repeated after the needle had been angled approximately 45° to the opposite direction. The total volume of 0.9% NaCl solution injected was 30 μl, which contained 60 to 150 μCi and resulted in maximum counting rates of 100,000 to 300,000/min. Immediately after injection of the radioisotope, the mouse was placed in a plastic box of inside dimensions 3 x 3 x 7.4 cm; this was fitted with a thin Mylar lid that was perforated for ventilation, as were the 2 end panels of the box. The box was sufficiently large so that a mouse could assume a normal rest position and could move a few mm in any direction. The longest dimension of the box was approximately the same as the diameter of the collimator of the scintillation detector. The box was placed on a table top with its lid about 2 cm from the end of the collimator.

For experiments with anesthetized mice, the animals were placed on a 0.25-inch-thick lead diaphragm with a central 1-cm diameter hole through which the isotope-injected tumor was allowed to hang pendants over the scintillation detector. This lead diaphragm prevented counting of tissues other than the tumor and overlying skin. Only a limited number of mice was anesthetized for radioisotope clearance trials. All clearances performed in irradiated tumors were done without subjecting the mice to subsequent anesthesia after they had recovered from their irradiation anesthetic. As the anesthetic was effective for 0.5 to 1 hr and complete recovery required up to 1.5 hr, the shortest postirradiation time used was 3 hr.

Radioactivity was detected by a 1.5-inch NaI crystal with a cylindrical collimator. The output of the detector was processed with a rate meter, with a time constant of 3 sec, and traced continuously on a linear recorder (Chart 1). The radioactivity from each animal was recorded for at least 8 min, which represented at least 1, but usually several, half-times. These data were replotted on semilogarithmic graph paper and fitted with straight lines for facilitation of computation of the half-times of radioisotope clearance. With very few exceptions, clearance was a single exponential function for as long as 30 min after injection. The rare instances in which more complex functions occurred could be traced to faulty injection technique; these data were not used in any analyses. The half-times could be converted to rates of blood flow by the relationship: blood flow (in ml/100 g/min) = 100 λ (ln 2/τ₁/₂).

The partition coefficient, λ, was determined for samples of the tumor under investigation with a slight modification of the
method described by Veall and Mallet (25). Homogenates of KHT tumor were prepared by grinding weighed fresh tissue in a glass homogenizer with a close-fitting Teflon pestle. A measured amount of distilled water was added during homogenization so that the final mixture would have the consistency of heavy cream. Fresh mouse blood was drawn from the infraorbital sinus and was hemolyzed by rapid freezing and thawing. Samples of tumor homogenate, hemolyzed blood, and 0.9% NaCl solution were introduced into sample tubes, 7 cm long, which were made from soft glass tubing with an outside diameter of 7 mm and an inside diameter of 5 mm. The tubes were half-filled with sample, and to this was added approximately 0.1 ml of air containing 30 to 50 µCi of $^{133}$Xe; immediately afterwards the tubes were sealed with an oxygen torch. These tubes were then fixed in the horizontal position to a shaker that was immersed in a water bath at 37°. They were shaken continuously for 2, 4, and 24 hr before being removed for radioactivity measurements. Immediately before measurement, each tube was vigorously shaken by hand and then briefly centrifuged in 50-ml centrifuge buckets filled with water at 37°. This procedure effectively separated the gaseous and liquid phases in the sample tubes. For radioactivity measurements, sample tubes were clamped to a lead diaphragm that was attached to the end of the collimator of a waterproofed scintillation detector; the scintillation detector was immersed in a 37° water bath. Measurements in the gaseous and liquid phases of the end of the collimator of a scintillation detector was immersing in a 37° water bath. Measurements in the gaseous phase in blood sample tubes.

RESULTS

Partition Coefficient. Incubation of the sample tubes for 2 hr was sufficient to achieve equilibration of the $^{133}$Xe in all of the samples tested.

The partition coefficient, $\lambda_T$, was obtained from the solubility of $^{133}$Xe in tumor, $S_T$, relative to the solubility in mouse hemolyzed blood, $S_B$:

$$\lambda_T = \frac{S_T}{S_B}$$

As $S_T$ was determined to be 0.1288 and $S_B$ was 0.1446, the partition coefficient was 0.891. This value was used to convert all clearance half-times to rates of tumor blood flow in ml/100 g/min.

$S_T$ was calculated from the relationship:

$$S_T = \frac{[C_T(W_T + V_{sal}) - V_{sal}(S_{sal})]}{[W_T]}$$

where $C_T$ is taken from the count rate of the tumor homogenate and expressed as a fraction of the count rate of its gaseous phase, $W_T$ is the volume of tumor tissue in its homogenate (weight of tumor/tumor-specific gravity), $V_{sal}$ is the volume of 0.9% NaCl solution added to the fresh tumor in preparing the homogenate, and $S_{sal}$ is the solubility of the isotope in 0.9% NaCl solution, i.e., counts in NaCl solution relative to counts in the gaseous phase. $S_B$ was obtained from counts made from hemolyzed blood relative to counts in the gaseous phase in blood sample tubes.

Blood Flow in Tumors of Anesthetized Mice. At the outset of this study, it was intended that measurements of radioisotope clearance from tumors be made by use of anesthetized animals as described above. It became obvious that the state of anesthesia itself significantly perturbed the rate of tumor blood flow; there were no differences in the shape of the $^{133}$Xe disappearance curves—only the slope was affected. Because of these findings and the difficulties in achieving a reproducible level of anesthesia in these animals, all subsequent measurements were made on unanesthetized mice. The frequency distributions of blood flows in the tumors of anesthetized and unanesthetized mice are shown in Chart 2. The 36 determinations made with anesthetized mice showed a mean blood flow of 13.8 ± 1.2 ml/100 g/min, and the 133 tumors of unanesthetized mice showed a significantly ($p < 0.001$) greater mean blood flow, 21.2 ± 0.8 ml/100 g/min.

Single versus Multiple Injections. As the principal goal of this study was to measure changes in blood flow as a function of time after tumor irradiation, it was necessary to determine whether the ability of a tumor to clear a given injectate was influenced or in any way compromised by the same tumor having been injected previously. The data listed in Table 1 provide no evidence of change in blood flow with multiple injections of $^{133}$Xe. These 1 to 4 injections were administered at daily intervals. For this analysis it was necessary to use blood flow data obtained with unirradiated tumors because irradiation per se might be expected to change the blood flow (see below); but it was possible separately to examine selected data from irradiated tumors, groups of which had been injected with $^{133}$Xe for a 1st or 2nd injection at the same time after a given dose of radiation. These data also gave no
evidence for changes in blood flow as a result of prior injection.

Radioisotope Clearance in Tumors of Different Sizes and with Different Injection Volumes. For tumors covering a wide range of volumes and receiving multiple-locus injections, there was no statistically significant difference in rate of blood flow until the volume of approximately 560 cu mm was exceeded (Table 2). Mean blood flow was slower in tumors greater than this size. The variability in blood flow rates calculated from $^{133}$Xe clearances and the tendency for flow rate to be slower in larger tumors are illustrated by the data in Table 3. In this experiment, the injected material was deposited in a single locus, i.e., at only 1 point rather than the usual 3 loci as described above. Although for the 3 different injection volumes the mean blood flows of the smaller tumors were always greater than the larger tumors, none of these differences was statistically significant, owing presumably to the small sample size and the relatively great variability of these data. Pooling these 3 sets of data for the 3 injection volumes (Table 3, Line 4) gave a significantly ($p < 0.05$) greater mean blood flow in the small tumors than in the large ones. Within the relatively narrow range of injection volumes tested, therefore, this experiment provided no clear-cut evidence of dependence of radioisotope clearance rate on the amount of 0.9% NaCl solution injected.

For the collected data of Table 2, the best-fitting polynomial curve was of the form $Y = 0.525 + 0.869 X - 0.234 X^2$, where $X$ is log tumor volume and $Y$ is log blood flow. This curve (Chart 3) shows that, within the range of volumes studied and on the basis of clearances following the standard multiple-locus radioisotope injection technique, blood flow did not change appreciably up to volumes of about 500 cu mm and then became increasingly slower as tumor size increased further. Because of this finding, no irradiation experiments were performed with tumors larger than 500 cu mm at the time of irradiation; also, the data obtained from tumors that reached these higher volumes during the course of postirradiation growth were not used in the calculations presented below.

Effect of Radiation. The blood flows listed in Table 4 may be examined against a mean blood flow of $21.2 \pm 0.8$ ml/100 g/min in all 113 control mice, with the use of unirradiated tumors in unanesthetized mice. As it was necessary to anesthetize the mice for localized irradiation and no clearance studies were to be done in animals under anesthesia, it was impossible to determine blood flows promptly after irradiation, e.g., within 1 to 2 hr. While animals were usually

### Table 1

<table>
<thead>
<tr>
<th>Day of injection</th>
<th>BF $\pm \sigma_{BF}$ (ml/100 g/min)</th>
<th>$\overline{Vol} b \pm \sigma_{Vol}$ (cu mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$17.7 \pm 1.2$</td>
<td>$127 \pm 17.6$</td>
</tr>
<tr>
<td>2</td>
<td>$20.8 \pm 2.0$</td>
<td>$163 \pm 21.5$</td>
</tr>
<tr>
<td>3</td>
<td>$17.7 \pm 1.9$</td>
<td>$182 \pm 17.7$</td>
</tr>
<tr>
<td>4</td>
<td>$18.9 \pm 1.9$</td>
<td>$234 \pm 22.8$</td>
</tr>
</tbody>
</table>

$^a$ Mean rate of tumor blood flow (BF) for 14 tumors.

$^b$ Mean tumor volume for the same 14 tumors.

### Table 2

<table>
<thead>
<tr>
<th>Tumor volume (cu mm)</th>
<th>$n^a$</th>
<th>BF $\pm \sigma_{BF}$ (ml/100 g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35-70</td>
<td>11</td>
<td>$22.1 \pm 1.8$</td>
</tr>
<tr>
<td>71-140</td>
<td>46</td>
<td>$22.8 \pm 1.0$</td>
</tr>
<tr>
<td>141-280</td>
<td>31</td>
<td>$19.0 \pm 1.3$</td>
</tr>
<tr>
<td>281-560</td>
<td>30</td>
<td>$19.9 \pm 2.0$</td>
</tr>
<tr>
<td>561-1120</td>
<td>19</td>
<td>$13.7 \pm 1.5$</td>
</tr>
<tr>
<td>1121-2240</td>
<td>3</td>
<td>$6.7 \pm 0.9$</td>
</tr>
</tbody>
</table>

$^a$ The number of tumors tested.

$^b$ Mean rate of tumor blood flow (BF).

### Table 3

<table>
<thead>
<tr>
<th>Injection volume (cu mm)</th>
<th>Small tumors$^b$</th>
<th>Large tumors$^c$</th>
<th>All tumors (small + large)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>$26.0 \pm 3.1$</td>
<td>$20.6 \pm 1.1$</td>
<td>$23.3 \pm 1.8$</td>
</tr>
<tr>
<td>40</td>
<td>$23.6 \pm 4.0$</td>
<td>$16.5 \pm 5.6$</td>
<td>$20.1 \pm 3.5$</td>
</tr>
<tr>
<td>60</td>
<td>$33.5 \pm 0.9$</td>
<td>$23.6 \pm 8.3$</td>
<td>$29.2 \pm 3.9$</td>
</tr>
</tbody>
</table>

$^a$ Shown as mean blood flow, BF, in ml/100 g/min ± $\sigma_{BF}$.

$^b$ Tumors with mean volume of 97 cu mm. Range is 59 to 126 cu mm; $\sigma_{Vol}$ is 7 cu mm.

$^c$ Tumors with mean volume of 310 cu mm. Range is 207 to 498 cu mm; $\sigma_{Vol}$ is 26 cu mm.

$^d$ $n = 3$ tumors. All other groups contained 4 tumors. The pooled data in Column 4 represent 8, 8 and 7 tumors; the pooled data in Line 4 represent 12, 11, and 23 tumors.

![Chart 3](chart3.png)

Chart 3. Rate of tumor blood flow (log scale) as a function of tumor volume (log scale) for 140 determinations.

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Blood Flow in Irradiated Mouse Tumors

Table 4

<table>
<thead>
<tr>
<th>Dose (rads)</th>
<th>3 hr BF ± sBF</th>
<th>Volb ± sVol</th>
<th>3 to 4 days BF ± sBF</th>
<th>Volb ± sVol</th>
<th>7 days BF ± sBF</th>
<th>Volb ± sVol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(ml/100 g/min)</td>
<td>(cu mm)</td>
<td>(ml/100 g/min/mm)</td>
<td>(cu mm)</td>
<td>(ml/100 g/min)</td>
<td>(cu mm)</td>
</tr>
<tr>
<td>1,000</td>
<td>22</td>
<td>20.3 ± 1.8</td>
<td>165 ± 16</td>
<td>11</td>
<td>30.2 ± 3.2</td>
<td>138 ± 11</td>
</tr>
<tr>
<td>2,000</td>
<td>12</td>
<td>19.0 ± 0.7</td>
<td>197 ± 31</td>
<td>5</td>
<td>23.6 ± 1.5</td>
<td>291 ± 48</td>
</tr>
<tr>
<td>4,000</td>
<td>14</td>
<td>15.7 ± 1.8</td>
<td>179 ± 21</td>
<td>52</td>
<td>19.0 ± 1.1</td>
<td>294 ± 11</td>
</tr>
<tr>
<td>8,000</td>
<td>13</td>
<td>17.4 ± 1.3</td>
<td>207 ± 33</td>
<td>10</td>
<td>13.8 ± 2.5</td>
<td>278 ± 21</td>
</tr>
<tr>
<td>12,000</td>
<td>12</td>
<td>15.4 ± 2.2</td>
<td>195 ± 20</td>
<td>9</td>
<td>11.4 ± 2.1</td>
<td>343 ± 47</td>
</tr>
<tr>
<td>16,000</td>
<td>9</td>
<td>10.9 ± 1.1</td>
<td>291 ± 31</td>
<td>7</td>
<td>12.2 ± 1.7</td>
<td>310 ± 34</td>
</tr>
</tbody>
</table>

a Mean rate of tumor blood flow (BF).
b Mean tumor volume.

awake within 1 hr after irradiation, the anesthetic effect was not completely absent for approximately another hr; therefore, 3 hr was chosen as the earliest time at which the tumors could be studied without complications arising from anesthesia. At this time there was no clinical evidence of anesthetic effect: respiration, heart rate, and behavior were entirely normal. Table 4 shows that, whereas there was no 3 hr effect after 1,000 rads, blood flows at this early time tended to be significantly and increasingly lower with higher radiation doses.

The gross shrinkage that occurs in this tumor, especially at 4 days and later and after doses greater than 4,000 rads, precluded the investigation of blood flow changes at longer postirradiation intervals for the 3 higher doses. As summarized in Table 4, the irradiated tumors behaved in either of 2 ways at the 3- to 4-day interval as contrasted with their acute response at 3 hr: with 1,000 to 4,000 rads, the blood flow was greater at the longer time, but only 1,000 rads increased the blood flow significantly above that of unirradiated controls at the 3- to 4-day interval only that we observed were dependent upon radiation dose and postirradiation time. Within the first few hours after irradiation, high doses appear to cause a slowing of blood flow, which is more pronounced as the dose is increased. The effects of single doses of 8,000 to 16,000 rads, however, the data for the 2 postirradiation times are from the same experiments; n is lower at the 3- to 4-day interval only because of deletion of individual blood flow data on the basis of tumor volumes exceeding 500 cu mm or for purely technical reasons. The data in Table 4 illustrate that, even at these dose levels that would be expected ultimately to bring about virtually 100% regression, the average volume at 3 to 4 days was greater than at the time of irradiation.

DISCUSSION

To the extent to which the clearance of interstitially deposited 133Xe is a valid measure of the state of the circulation, irradiation induces changes in the rate of blood flow in the tumors studied. It must be recognized, however, that data provided by radioisotope clearance methods constitute only indirect indicators of blood flow. The changes that we observed were dependent upon radiation dose and postirradiation time. Within the first few hours after irradiation, high doses appear to cause a slowing of blood flow, which is more pronounced as the dose is increased. The effects of single doses of 8,000 to 16,000 rads must be regarded as of only theoretical interest, rather than of practical importance, as this kind of treatment regimen is not used in modern clinical radiotherapy. Also, it is unlikely that fractionated regimens reaching total doses of 6,000 rads, for example, would elicit the same vascular changes as single doses of this magnitude. The early decreased blood flow could result from the release of vasoconstrictor substances resulting from local tissue radiation injury. Particularly in the case of low to moderate radiation doses, such effects would be expected to be short-lived and of relatively little consequence in curative cancer radiotherapy. The longer term reductions in blood flow brought on by the very high doses may indeed be provoked by...
the same mechanism. An alternative explanation for the observed reductions in blood flow is the fragmentation in capillary architecture suggested by Rubin and Casarett (19). However, the absence of relevant, specific evidence to document these explanations identifies a need for further investigation.

The improved blood flow found to occur up to 7 days after the lower doses is of greater practical importance. Increased blood flow tended generally to occur at later postirradiation times with higher doses. In view of the variability reflected by the standard errors of the mean blood flows, it is difficult to detect unequivocal evidence of dose dependence. The maximum mean blood flow was 34.6 ± 3.5 ml/100 g/min; this was observed at 7 days after 4,000 rads. The next-highest rate was 30.2 ± 3.2 ml/100 g/min; this occurred at 3 to 4 days after 1,000 rads. Contrasted with the mean control rate of blood flow of 21.2 ± 0.8 ml/100 g/min, the blood flow rate of irradiated tumors appears to have been capable of being increased by approximately 50%. Additional experiments in the future are needed to establish, for several X-ray doses, the exact time when blood flow is maximal and the height which is attained at that time.

Provisionally, it is proposed that a mechanism that may underlie the later increases in blood flow involves an altered balance between tumor cell parenchyma and vascular stroma. Thus, with increasing radiation dose, cell death and breakdown may occur over a longer period, and this could account for finding maximal flow rates at a slightly earlier time in the case of the lowest dose tested.

Increased blood flow also may result from the relief of a type of circulatory obstruction which is typical of rapidly growing tumors (21, 22). Owing to its well-documented effect of reducing parenchymal growth rate, irradiation should allow the circulation to utilize such preexisting and relatively radioresistant vascular channels more efficiently. Without additional information, such as that afforded by careful histological analyses, it is impossible to invoke any mechanism with confidence.

Enhancement of tumor blood flow rates by irradiation would be expected to improve tumor oxygenation by making oxygen available to sites otherwise deprived of it, presumably because of the growth momentum of the tumor itself. Whether improved oxygenation is in fact achieved in these irradiated tumors and whether this accounts for the kind of tumor reoxygenation determined radiobiologically can be established only by other experimental approaches (9, 11, 24). Although presently available methods are not capable of providing answers to these all-important questions, the likelihood that increased blood flow plays an important causative part in improving oxygenation of tumors is strengthened by polarographic measurements of tumor oxygenation (2, 3); namely, tumor pO2 tended to be higher after radiotherapy than before.

It seems reasonable that changes in blood flow as reported herein could account for postirradiation reoxygenation. The diffusion radius around blocked or collapsed capillaries might be imagined as substantially less than 100 μm, the critical maximum diffusion radius suggested originally by the data and extrapolations of Thomlinson and Gray (23). Opening all capillary channels would at least ensure that all pericapillary tumor cells would be subject to the same amount of oxygen to a distance of approximately 100 μm. Although this reasoning is attractive, the time scale of the blood flow changes makes these findings inconsistent with separate observations on the reoxygenation of this same tumor. The present experiments give no evidence that significant improvements in blood flow occur before approximately 3 days after irradiation, whereas the radiobiological experiments suggest the reoxygenation process (see “Introduction”) to be more than half-completed by 3 hr and fully completed in this same tumor by approximately 12 to 24 hr (9–11, 24). Although the present report does not include observations between 3 hr and 3 days, our incomplete data at intermediate times such as 1 day do not indicate significant increases in blood flow before approximately 3 days.

The present findings are at variance with those of Song and Levitt (20), who worked with Walker Carcinoma 256 in rats, but the different results might be entirely attributable to differences in tumor, species, and experimental method (131Cr-labeled red blood cells). It is more difficult to reconcile our findings with those of Robert et al. (16), who used 133Xe in a manner similar to ours and reported marked reductions in blood flow rate (débit vasculaire spécifique) between 1 and 9 days after irradiation with 2,000 R in a rhabdomyosarcoma transplanted and studied syngeneically in C3H mice. They emphasized that this effect was obtained only in well-vascularized tumors, i.e., with blood flows of at least 5 ml/100 g/min; however, their more completely documented publication (17) shows that they never found blood flows of greater than double this rate. Considering that our mean flow rate was 21.2 ml/100 g/min (see Charts 2 and 3 for distributions of flow rates in our KHT sarcoma), it seems likely that the discrepancy is related to those differences in blood flow that are uniquely a function of differences in histological structure of their rhabdomyosarcoma and our undifferentiated anaplastic sarcoma. Should this be the case, one can readily appreciate the need for caution in extrapolating either set of findings to other tumors, either animal or human.

The xenon clearance method used in these experiments constitutes a useful and satisfactory means of studying the circulation of experimental tumors, provided that the rate of clearance of this radioisotope is sufficiently independent of tumor size so that it can be used over a range of volumes which are palpable, measurable, and of practical importance. As there was little or no systematic change in clearance rate over a wide and usable range of tumor sizes, application of this method to radiobiological questions is amply justified. Especially at the upper portion of our size range, there was an inverse relationship between blood flow and tumor size; this is similar to the findings of Gullino and Grantham (5). Their study, however, was confined to larger tumors, usually greater than 2 g, wet weight. The disagreement between the present results and those of Cataland et al. (1) and Robert et al. (17) is probably more illusory than real, as most of the tumors that they used were larger than the ones that we used; furthermore, their tumors were characterized by considerable necrosis and were of different histological types.
Cullino and Grantham (5), 0.14 to 0.17 ml/hr/mg of nitrogen, average flow rates in rat and mouse tumors reported by blood flow of the KHT mouse sarcoma is similar to blood flows found in other tumors. For example, in rabbit tumors diffuse through tissue spaces to be picked up in vascular channels; then these dissolved molecules are carried into venous drainage and then to the lungs, where they are expired and not recirculated. This assumption is made all the more reasonable when the method is applied in tissues in which the components (tissue cells, interstitial fluid, and blood) partition approximately equally. That approximately equal partitioning is achieved in the present experiments is strengthened by finding the partition coefficient to be 0.891, a value slightly higher than that associated with the liver, in which circulation is routinely studied by this method.

A lower range of tumor volume acceptable for this kind of experiment is established by a size which is conveniently and reliably measurable (by calipers) in 3 dimensions. In addition, this minimum tumor volume should be at least the 2nd or 3rd such measurement on a given tumor, in order that the tumor might be measured at least twice prior to treatment, thereby ensuring the availability of a useful estimate of its pretreatment growth rate. These requirements set the minimum usable size of a tumor at a mean diameter of approximately 5 mm (mean volume, 65 cu mm). The KHT tumor grows nearly exponentially, but with continuously decreasing slope from the lower limit just mentioned to a volume of approximately 1000 cu mm (Chart 4). At least until it reaches volumes approaching those close to the end of the period of near-exponential growth, this KHT tumor does not exhibit a simple gross distribution of necrosis that is anything like the usual "orange" analogy, i.e., a central necrotic core surrounded by a relatively thin, firm, and viable "rind." Rather, necrotic foci which are frequently grossly apparent are randomly distributed throughout the tumor mass. It is difficult to identify readily the appreciable volumes of necrosis in tumors of approximately 9 mm mean diameter (volume, 382 cu mm).

Despite great differences in species, size of different animals, tissue studied, and experimental techniques, the blood flow of the KHT mouse sarcoma is similar to blood flows found in other tumors. For example, in rabbit tumors the blood flow rate was reported as 29 ml/100 g/min (6). The average flow rates in rat and mouse tumors reported by Gullino and Grantham (5), 0.14 to 0.17 ml/hr/mg of nitrogen, are qualitatively similar to the average value established for the

KHT mouse sarcoma (0.0126 ml/hr/mg of tumor), after allowance is made for the expected nitrogen content of wet tumor tissue (approximately 10 to 20%). The report of slightly lower flow rates for 3 other mouse tumors (15–17) may easily be reconciled with the present findings: (a) a lower partition coefficient was assumed by these other groups, namely, 0.72, the value found for dog liver; (b) radioisotope clearance measurements in 1 study (15) were made with anesthetized animals; and (c) larger tumors were used, as noted above. Although the direct introduction of a quantity of 0.9% NaCl solution into these tumors must certainly cause some disruption in tumor architecture, this disruption must be minimal and easily reversible, as there were no obvious changes in tumor growth or biological behavior. This is consistent with the close agreement in flow rates between 1st and subsequent injections of the radioisotope (Table 1; Ref. 17). As detailed by earlier workers who have developed methods of radioactively labeled inert gas clearance, the validity of this approach rests on the assumption that dissolved gas molecules diffuse through tissue spaces to be picked up in vascular channels; then these dissolved molecules are carried into venous drainage and then to the lungs, where they are expired and not recirculated. This assumption is made all the more reasonable when the method is applied in tissues in which the components (tissue cells, interstitial fluid, and blood) partition approximately equally. That approximately equal partitioning is achieved in the present experiments is strengthened by finding the partition coefficient to be 0.891, a value slightly higher than that associated with the liver, in which circulation is routinely studied by this method.

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Blood Flow in Irradiated Mouse Sarcoma as Determined by the Clearance of Xenon-133

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