Mechanisms Underlying Reduced Growth Rate in C3HBA Mammary Adenocarcinomas Recurring after Single Doses of X-rays or Fast Neutrons

Janet S. R. Nelson, Rita E. Carpenter, and Dianna Durboraw

Division of Radiation Oncology, University of Washington School of Medicine, Seattle, Washington 98195

SUMMARY

C3HBA mammary tumors were irradiated with 3000 rads of 250-kVp X-rays or 1000 rads of 8-MeV neutrons, doses of radiation matched for producing equal growth delay. At 14 days postirradiation, tumors were regrowing at a reduced rate relative to controls. Cell kinetic parameters were examined using percentage of labeled mitoses techniques, and blood vessel spacing and tumor architecture were examined histologically to determine whether the mechanisms underlying growth rate changes were the same after neutron as after photon irradiation.

The tumor volume-doubling time at 14 days posttreatment is similar in both irradiated groups (\(T_D = 117\) hr for neutron-irradiated tumors, 132 hr for X-irradiated tumors) and is approximately twice as long as the doubling time of 61.4 hr in control tumors in the same size range. Control and X-irradiated tumors have median cell cycle durations of 19.3 and 18.5 hr, respectively; the more slowly growing X-irradiated tumors have a reduced growth fraction and increased cell loss factor. Regrowing neutron-irradiated tumors have a median cell cycle of 27.2 hr, with calculated growth fraction and cell loss factor values intermediate between those for control and X-irradiated tumors. Scatter in the percentage of labeled mitoses data makes it difficult to determine whether the cell cycle durations are significantly different.

The average distance from tumor parenchymal interphase cells to the nearest recognizable blood vessel is nearly identical in the two irradiated groups and for both groups is significantly greater than interphase to vessel distance in controls. The average distance in irradiated tumors approaches the maximal distance for \(O_2\) diffusion in mouse adenocarcinomas of a corded structure surrounding a central blood vessel. Both neutron- and X-irradiated tumors contain more necrosis and fewer viable-appearing parenchymal cells than do control tumors of the same size. The similar growth rate and growth delay in this tumor after 3000 rads of X-rays or 1000 rads of neutrons occur in the face of possibly different cell cycle durations and seem related to similar circulatory system inadequacies which limit growth and are expressed as greater average cell-to-blood-vessel distance and increased cell loss leading to necrosis, indicating oxygen or nutrient deprivation.

INTRODUCTION

Growth delay in experimental animal tumors has been a widely used end point of radiation effect, particularly in RBE\(^+\) studies comparing high linear energy transfer radiations such as fast neutrons with photons (11, 12). In many cases, growth delay is assumed to be principally due to cell killing because, after a period of no growth or decrease in volume, tumors regrow at a rate very similar to that in controls (11). In some rodent solid tumors, radiation-induced growth delay has another important component, a dose-dependent reduction in growth rate of recurring tumors (29, 30).

The growth rate reduction has been described as in vitro small colony formation (2, 22, 30). Small colony formation in vitro appears to be the same after high or low linear energy-transfer radiation; doses matched for equal cell killing give the same size distribution of colonies produced by the surviving cells (18, 31). These observations have been applied back to the in vivo situation, and, along with observations on the rate of healing of skin damage and tumor growth rate after high linear energy-transfer radiation (9, 11), indicate that repopulation rates do not differ as a function of linear energy transfer. The RBE for growth rate effects should be the same as the RBE for clonogenic cell killing in the same tumor system (31).

There is some evidence to the contrary. Berry (2, 3) examined the RBE for cell killing and for growth rate reduction in the P388 mouse ascitic leukemia. He found that, for 14-MeV neutrons and cyclotron neutrons (mean energy, 6 MeV), the cell-killing RBE was less than the RBE for reduction in growth rate. Hendry (13) reported a slower repopulation rate after 14-MeV neutrons than after X-rays, for mouse bone marrow and spleen CFU's regrowing in the irradiated host. He implicated a host-specific rather than a CFU-specific (i.e., cell cycle change) factor.

Growth delay in C3HBA mammary tumors has been used as an end point for determining the RBE for cyclotron neutrons (17). Since growth delay in this tumor is due in part to a reduced postirradiation growth rate, it is an appro-
appropriate system for examining the mechanisms underlying
growth rate changes following X-rays and neutrons.

MATERIALS AND METHODS

Tumor Transplants and Growth Determinations. The C3HBA mammary adenocarcinoma of the C3H/HeJ mouse
has been carried as a transplantable tumor by The Jackson
Memorial Laboratory, Bar Harbor, Maine, since 1949. For
these experiments, tumors were transplanted by placing 2-
to 3-mm (average diameter) pieces of nonnecrotic tumor
s.c. on the upper back of 6- to 12-week-old male C3H/HeJ
mice (The Jackson Memorial Lab). For growth rate determina-
tions, tumors were measured in 3 dimensions through
the skin with calipers 3 times weekly, from the time they
became palpable until they reached a volume in excess of
1000 cu mm or until 35 days after receiving X-rays or neu-
trons. Volumes were calculated using the formula (length \times
width \times depth)/2. Measurements were corrected for skin
thickness. Growth delay was used as an end point of radia-
tion effect and was determined as (number of days required
for an irradiated tumor to reach 5\times starting volume) –
(number of days required for control tumor to grow to 5\times
starting volume). The volume of each irradiated tumor was
normalized to 1.0 on the day it was irradiated, and volumes
on subsequent days were related to this starting volume.

Irradiation Sources and Methods. At 13 days postrans-
plant, those tumors with an average diameter of 3.7 to 5.7
mm (9 to 90 cu mm) were irradiated with X-rays or cyclotron
neutrons. This size range of tumors, while large, has been
shown to have very similar radiation response, as deter-
mined by using growth delay as an end point and normaliz-
ing the beginning tumor volume to 1.0 (17). X-irradiations
were performed with a GE Maxitron 250; the settings were
250 kVp, 30 ma, 1 mm Al + 0.5 mm Cu added filtration, half-
value layer = 1.6 mm Cu, target-to-skin distance = 33 cm,
dose rate approximately 200 R/min. A horizontal X-ray beam
was defined by an 8 x 8-cm field cut in a vertical lead shield,
6.4 mm thick. Dosimetry was performed with a Victoreen
condenser R meter, and a rad-entgen conversion factor
of 0.95 was used.

Tumor-bearing mice were anesthetized by i.p. injection
of sodium pentobarbital (60 \mu g/g body weight) in 0.9% NaCl
solution and irradiated while fastened to a vertical Lucite
animal holder. The tumors on the animals' backs were
oriented across the 4 corners of an 8 x 8-cm square marked
on the animal holder, which was aligned directly with the
square cut in the lead shield. The lead protected the rest
of the animals' bodies from irradiation; dose to the whole body
was well below 1% of the tumor dose, as determined with
LiF dosimeters.

Neutron irradiations were performed at the University
of Washington cyclotron. The neutron beam production and
dosimetry have been described previously (32). Briefly, a Be
target 1.5 mm thick was bombarded with 21-MeV deuterons,
producing a fixed horizontal neutron beam with an energy
spread of 0 to 25 MeV and a mean energy of 8 MeV. Dosim-
etry was performed with Shonka type A150 plastic tissue-
equivalent ionization chambers filled with tissue-equivalent
gas. Doses measured were rads of neutrons plus gammas. Tumor-bearing mice were irradiated at a target-to-skin dis-
tance of 108 cm and a dose rate of 40 to 50 rads/min. The anesthetized animals were fastened to the same holder
used in the X-irradiations, with their tumors oriented across
the corners of a 16 x 16-cm square. The holder was placed
3 cm from the exit of a borated, water-extended plastic
collimator which defined a 16 x 16-cm field. The dose to the
whole body approximated 5 to 7% of the tumor dose, as
determined from measurements made with the tissue-equiva-
lent ionization chamber in a mouse phantom.

Cell Kinetic Studies and Histology. Tumors from control
animals were taken for percentage of labeled mitoses experi-
ments at 14 days postransplant, while X- or neutron-irradi-
ated, tumor-bearing mice were taken at 14 days after receiv-
ing 3000 rads X-rays or 1000 rads neutrons. Tumor-bearing
mice received i.p. injections at 9:00 a.m. of 1 \mu Ci of
\[ ^3H \] thymidine per g body weight (Schwartz/Mann, Orange-
burg, N. Y., specific activity, 2.0 Ci/mmmole). At regular inter-
vals thereafter, 3 mice/sample time were sacrificed and the
tumors were excised, weighed, and fixed 24 to 48 hr in 3
parts ethanol/1 part glacial acetic acid. Prior to embedding
in Paraplast, all tumors larger than approximately 25 mg
were cut in half, so that sections could be made from the cut
surface and therefore would be taken from the center of the
tumor. For autoradiography, 4-\mu m sections were dipped in
Kodak NCT-2 emulsion (Eastman Kodak, Rochester, N. Y.)
diluted 50/50 with distilled water at 40°, dried, and stored in
black boxes with silica gel for 4 weeks at 4°. The autoradi-
ographs were developed in Kodak D-19 and stained with
hematoxylin and eosin. For each tumor, 200 metaphase and
anaphase cells were scored as labeled or unlabelled. All
sections were scored by repeated regular passages over the
full length of the section to avoid selecting cells from the
periphery or center of the tumor. Background averaged less
than 1 grain/nucleus; mitoses were considered labeled if
there were 3 or more grains over the nucleus. The average
number of silver grains per metaphase or anaphase from
tumors sacrificed late in these experiments was 8.2 \pm 0.5;
underexposure therefore was not considered a problem.

For determining mitotic index and labeling index, 1000 or
more parenchymal cells were counted. At least 1 complete
passage across an equatorial tumor section was made in
doing these counts; thus in some cases well in excess of
1000 cells were counted.

For general histological study, for determining distances
between cells and circulatory system elements, and for
mapping tumors for the relative proportion of parenchymal
cells, stroma, necrosis, blood vessels and blood-filled
space, 8-\mu m sections were cut from tumor centers and
stained with the Luxol fast blue-PAS-hematoxylin method
of Tannock and Steel (28). Luxol fast blue stains emythmocytes,
facilitating the identification of PAS-positive blood vessels
among hematoxylin-stained parenchymal and stromal cells.
Cell-to-blood-vessel distances were determined by locating
at random 20 interphase and 20 mitotic parenchymal cell
nuclei per tumor and measuring the distance from each cell
to the nearest identifiable blood vessel with an eyepiece
reticle fitted with a micrometer scale. Blood vessels were
identified as PAS-positive elements containing Luxol fast
blue-stained erythrocytes and/or lined with endothelial cells with typical elongate nuclei (24). Tumor sections also were mapped by the method of Chalkey (5) to determine the proportion of different cell types and acellular necrosis. An eyepiece reticle marked into a grid was used, and 4 points near the 4 corners, where grid lines crossed at right angles, were used as the observation points. As the section was moved on the microscope stage, "hits" scored by these 4 points were classified as to cell type, and each category was expressed as percentage of total hits. Each tumor was mapped until a minimum of 500 "hits" in parenchymal cells had been made.

RESULTS

The growth of control tumors and regrowth of tumors following single doses of X-rays or neutrons are shown in Chart 1. These growth curves show clearly that growth delay in reaching, for example, 5x starting volume has a component of reduced growth rate as well as a period of no growth or decrease in volume immediately after irradiation, due presumably to cell death. For single fractions of both X-rays and neutrons, there was no local tumor control at any of the doses indicated in Chart 1 (17).

To study this reduced postirradiation growth rate, tumors were irradiated with 1000 rads of 8 MeV (mean energy) neutrons or 3000 rads of 250-kVp X-rays which, as shown by the data given in Chart 2, are doses that produce equal growth delay. Chart 3 shows the volume response of tumors to 1000 rads of neutrons and 3000 rads of X-rays, compared with controls. In both irradiated groups, growth delay includes an initial phase of no volume change with time, followed by regrowth at similar reduced rates, up to 50 days posttransplant (37 days postirradiation).

The percentage of labeled mitoses experiments were done on control tumors at 14 days posttransplant and on regrowing irradiated tumors at 14 days after treatment (27 days posttransplant); control and X-irradiated tumors were about the same average size at the time these experiments were done; neutron-irradiated tumors were slightly more than twice as large (Table 1). However, cell kinetic differences are probably not due to these size discrepancies. The X-irradiated tumors grew half as fast as control tumors of essentially identical size. The larger neutron-irradiated tumors actually grew slightly faster than smaller recurrent X-irradiated tumors. Thus cell kinetic differences are not consistent with size discrepancies, as would be expected if reduced growth rate were due to increased tumor volume. Furthermore, the doubling time of control C3HBA tumors does not change detectably over the size range 10 to 150 cu mm.

The 14-day point after irradiation was chosen for other reasons also. At this time, tumors were regrowing steadily, and presumably reproductively doomed cells no longer represented a significant proportion of the cycling cells.

The percentage of labeled mitoses curves show that control and X-irradiated tumors have very similar cell cycles, while neutron-irradiated tumors may have a longer $T_c$ (Charts 4 and 5). The cell cycle distribution times for the 3

---

Chart 1. C3HBA tumor volume versus days postirradiation for graded doses. Each point is the mean value for 6 to 8 mice. The vertical bars are standard error of the mean and were calculated for all mean values but were drawn on only every 3rd point for clarity. Tumor volumes were normalized to 1.0 on the day they were irradiated.

---

J. S. R. Nelson et al.
groups of tumors are shown in Chart 6. Table 1 summarizes the duration of cell cycle compartments and kinetic values calculated from them, using median \( T_g \) and \( T_c \) values. While neutron- and X-irradiated tumors are regrowing at nearly the same rate, the underlying mechanisms may not be the same. Fast-growing controls have a higher growth fraction and a smaller cell loss factor than either of the irradiated groups.

In an attempt to elucidate other factors determining tumor growth rate, the average distance between tumor parenchymal cells and blood vessels was determined for the 3 groups of tumors. Within each group, mitotic cells are significantly closer to blood vessels than are interphase cells (Table 2; paired t test; \( p < 0.01 \)). However, mitoses in rapidly growing control tumors are not significantly closer to blood vessels than are mitoses in the more slowly growing irradiated tumors. However, control tumor interphase cells are significantly closer to blood vessels (average distance, 60.1 ± 2.7 \( \mu \text{m} \)) than are parenchymal interphase cells in X-irradiated tumors (average distance, 75.0 ± 2.5 \( \mu \text{m} \); \( p < 0.01 \)) or neutron-irradiated tumors (average distance, 79.6 ± 2.3 \( \mu \text{m} \); \( p < 0.01 \); Table 2). Because these distances were determined in 2 rather than 3 dimensions, they are best considered as measurements of relative differences within these 3 groups, not as absolute measurements of cell-to-blood-vessel spacing. While the median duration of the cell cycle is longer and there is a wider spread of values of \( T_c \) in

---

**Slow Tumor Regrowth Following X-rays or Neutrons**

---

**Table 1**

<table>
<thead>
<tr>
<th>Cell kinetic parameters for control and irradiated C3HBA tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control C3HBA tumors</strong></td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>( T_{cm} ) (hr)</td>
</tr>
<tr>
<td>( T_s ) (hr)</td>
</tr>
<tr>
<td>( T_{cm} ) (hr)</td>
</tr>
<tr>
<td>( T_c ) (hr)</td>
</tr>
<tr>
<td>Tumor size (mg)</td>
</tr>
<tr>
<td>( T_d ) (hr)</td>
</tr>
<tr>
<td>Labeling index</td>
</tr>
<tr>
<td>Mitotic index</td>
</tr>
<tr>
<td>( T^* ) (hr)</td>
</tr>
<tr>
<td>Growth fraction</td>
</tr>
<tr>
<td>( \phi )</td>
</tr>
<tr>
<td>( K_a )</td>
</tr>
<tr>
<td>( K_c )</td>
</tr>
</tbody>
</table>

---

* Mitosis is equally divided between G1 and G2 in this computer analysis performed by Dr. G. G. Steel, British Institute for Cancer Research.

* \( T_d \) was calculated as \( \ln 2/b \), where \( b \) is the slope of the growth curve as determined by a computerized best fit according to the equation \( y = ae^{br} \). T, growth fraction, \( \phi \), \( K_a \), \( K_c \), were calculated by the method of Steel (1968).
Distribution of cell cycle times for control, X-irradiated, and neutron-irradiated C3HBA tumors. These distribution times were determined from a computer analysis performed by G. G. Steel.

The microarchitecture of the regrowing irradiated tumors is different from that of similar-sized control tumors. Unirradiated controls of less than 100 cu mm (Fig. 1) have an anaplastic architecture with a large proportion of well-vascularized parenchyma, very little necrosis, and supportive stroma and large blood vessels located mostly around the tumor's periphery. Occasionally, tumor cells are seen arranged in cords surrounding a central blood vessel; this corded structure is seen much more frequently in larger unirradiated tumors (volume > 400 cu mm) and has been described in other experimental neoplasms (23–25). In regrowing neutron- and X-irradiated tumors of less than 100 cu mm, this arrangement is common. Viable-appearing tumor cells are frequently limited to a cord around a central blood vessel, the cord itself being surrounded by necrosis (Figs. 2 and 3).

DISCUSSION

There is conflicting evidence on differential repopulation rates after high- and low-linear-energy transfer radiation doses matched for equal cell killing or other equal acute effects presumably based on radiation-induced cell death. The relevant studies concern clone size analysis in vitro (18, 31), rate of healing of skin reactions (9), solid tumor-growth rate (11), survival time of mice containing equal numbers of surviving P388 ascitic leukemia cells irradiated with X-rays or neutrons (2), repopulation rates of CFU’s in irradiated mice (13), and initiation of regrowth of rat rhabdomyosarcomas after X-rays or 15 MeV neutrons (7). The latter 3 situations conflict with the preceding in that they indicate a reduced repopulation rate (2, 13) or delay in initiation of regrowth (7) after fast neutrons, while the other studies indicate equality of rates. While all these studies have yielded useful information on the question of repopulation,
none has thoroughly investigated the mechanisms underlying the observed posttreatment repopulation rate.

In ascites tumors, growth rate decreases with increasing tumor age, and in several systems (L1210, Ehrlich, Sarcoma 180, and NCTC), this is accompanied by an increasing $T_d$, while the growth fraction remains high and cell loss is often minimal, at least during early growth (15, 19). Thus it is possible that, in an ascites tumor regrowing from irradiated cells, a lengthened cell cycle following neutron irradiation could cause an observable reduction in growth rate, because lengthening $T_d$ appears to be a major factor in slowed growth. The slowly changing growth fraction and cell loss factor allow changes in $T_d$ to be expressed.

In the case of CFU’s assayed by the endo- and exocolony techniques after neutron- and X-irradiation (13), it is possible that slower growing neutron-irradiated survivors could be selected against when transplanted into heavily irradiated animals (exocolony technique), but not in the endocolony assay. In the heavily irradiated transplanted animal, competition between differentiation and reproduction of CFU’s occurs; slower growing CFU’s represent a larger “window” for the differentiation stimulus and hence are culled out of the colony-forming line. This could be reflected in reduced plating efficiency of slowly growing survivors, as these cells would not form large spleen colonies to be counted 8 to 10 days later. At the same time, the differentiation stimulus would be much less in the endocolony assay in which animals are sublethally irradiated with 450 rads of X-rays or 350 rads of neutrons. Thus many CFU’s which might be selected against in an exocolony assay will survive to form colonies, even if they initially have slow cell cycles (J. P. Okunewick, personal communication).

In many solid tumors, growth rate is not well correlated with either $T_d$ or growth fraction but is closely related to cell loss (8). Thus neutron-irradiated solid tumors with a lengthened $T_d$ could grow at a mate very similar to that in X-irradiated tumors, which themselves have a $T_d$ very similar to that in controls. The similar $T_d$ seen here in control and X-irradiated C3HBA tumors agrees very well with the report of Denekamp and Thomlinson (10). These authors showed that, in 4 rodent tumors irradiated with X-rays, the cell cycle duration measured at 7 to 28 days after irradiation was in all cases virtually identical to control values, although all of the irradiated tumors were growing more slowly than their unirradiated counterparts. These authors attributed the slower growth principally to greater cell loss in the irradiated tumors. In that study and in the experiments discussed here, the concept of reduced growth rate being due to increased cell loss is a very useful one. However, the calculation of the cell loss factor, as originally described by Steel (21), requires certain assumptions. One of these is that, in calculating cell loss by comparing actual tumor volume-doubling time to the potential parenchymal cell population-doubling time, the proportion of the tumor occupied by necrosis must not change during the time period over which the $T_d$ is measured. This has not been determined for regrowing
irradiated tumors in these experiments and so the estimates of cell loss factor must be regarded as approximations. However, the irradiated tumors do contain significantly more necrosis than the controls, where the amount of necrotic tissue is minimal. If the proportion of necrotic tissue in irradiated tumors increased during the course of measurements to determine volume-doubling time, growth would appear more rapid than it actually is and cell loss would be underestimated.

While the median \( T_e \) is longer in recurrent neutron-irradiated tumors than in control or X-irradiated tumors, it is difficult to assess whether this discrepancy is significant. The method used for analyzing the data gives no information on the statistical significance of the differences between 2 curves, and the scatter in the data limits the precision of the results. Thus, the longer \( T_e \) in neutron-treated tumors may be more apparent than real. While a longer \( T_e \) would be consistent with some reports (2, 3, 13), it may be pointed out that, for all 3 groups of tumors, the actual volume-doubling time is longer than the potential parenchymal cell population-doubling time calculated from cell cycle and pulse-labeling index data. This indicates that, in both control and treated tumors, factors modifying cell loss are of major importance in determining growth rate.

Tannock (25) and Tannock and Hayashi (27) argue that the limiting factor in tumor growth might be the inability of the vascular system to grow as fast as the parenchymal cells could multiply. Tannock’s estimate (24, 25) of a turnover time of 50 to 60 hr for endothelial cells in capillaries of a C3H mouse mammary tumor agrees well with the observed \( T_e \) of 61.4 hr for control C3HBA tumors in these experiments. The greater cell-to-blood-vessel distance in irradiated tumors suggests that radiation damage to endothelial cells may have decreased the available endothelial cell population so that the circulatory system is even less adequate than in controls. The average interphase cell-to-blood-vessel distance determined in 2-dimensional space is 75 to 79 \( \mu \)m in regrowing irradiated tumors and the average cord radius is 65.9 ± 3.7 \( \mu \)m for X-irradiated tumors and 64.5 ± 3.7 \( \mu \)m for neutron-irradiated tumors. While cell-to-blood-vessel distances cannot be completely assessed from measurements in only 2 dimensions, this distance, as well as the average cord radius, approaches the maximal \( O_2 \) diffusion distance of 70 to 80 \( \mu \)m for mouse adenocarcinomas of a corded structure surrounding a central blood vessel (26). The much larger percentage of necrosis in irradiated tumors (Table 3) also argues for circulatory system inadequacies in clearing cellular debris and is consistent with the observations of Hilmas and Gillette (14) on necrosis and circulatory system parameters in regrowing irradiated mouse mammary tumors. The observed increase in necrosis in the irradiated C3HBA tumors also correlates with the increased cell loss factor, as cell death leading to necrosis is a major form of cell loss in tumors (6, 21, 23). Thus the growth capabilities of the circulatory system may limit tumor growth, and structural and/or functional limits may cause less effective removal of necrotic debris (14). The change from a relatively disorganized histological structure in controls to a corded structure in regrowing irradiated tumors of similar size is also consistent with the altered circulatory system parameters. Tannock (24) argues that tumor cords are the result of large spacing between blood vessels. Since the neutron- and X-irradiated tumors in these experiments undergo identical growth delay, have similar growth rates at 14 days posttreatment, and have virtually identical blood vessel spacing and similar percentage of necrotic volume, the theory implicating inadequacies of the circulatory system as a factor limiting tumor growth has support.

The total-body dose of approximately 50 to 70 rads of neutrons which tumor-bearing mice received may also have had a systemic effect that could, in turn, have affected tumor growth. While this cannot be ruled out, the similar structure of the tumor circulatory systems in neutron- and X-irradiated animals, coupled with information from other studies of tumor microvasculature (14, 23, 24), indicate that these changes adequately explain the reduced growth rate. Furthermore, whole-body irradiation can suppress the host’s immune system, which would tend to increase, not decrease, the tumor growth rate.

Broeze et al. (4) reported an RBE for endothelial cell survival of 1.8 for 15-MeV neutrons, a value less than the RBE of 2.0 to 3.8 determined for growth delay in the C3HBA tumor following single doses of 8-MeV cyclotron neutrons (17). While this may argue against the importance of radiation effects on the capillary endothelium in limiting tumor growth and causing reduced growth rate, 2 factors should be pointed out. The RBE of 1.8 for endothelial cell survival was obtained in a model system where these cells were apparently well oxygenated. Blood stasis occurring in tumor capillaries (28) could mean that tumor capillary endothelium is not as well oxygenated as its structurally privileged location would indicate. A partial hypoxic condition in endothelium would tend to raise the neutron RBE. The disagreement between growth delay and capillary endothelial cell killing RBE is certainly also partly due to the fact that radiation-induced tumor-growth delay depends on cell killing as well as on reduced posttreatment growth rates. The disagreement between these 2 RBE rates may also serve as an indictment of tumor volume measurements as an end point of radiation response. Limitations of these measurements have been discussed by others (1).

In vivo small colony formation has been invoked to explain reduced tumor growth rates following irradiation (22, 30). The characteristics of in vitro small colony formation are increased generation time, reduced plating efficiency of the progeny of small colonies, inheritability of the tendency to form small colonies, and increased radiosensitivity (20). May these factors be invoked in the reduced growth rate seen after irradiation? There may be a longer median \( T_e \) in regrowing neutron-irradiated tumors, but not in the X-irradiated ones. The reduced growth rate has been shown not to be inheritable (16), and radiosensitivity of tumors transplanted from irradiated donors is under investigation. The large increase in necrosis in small regrowing tumors may argue for a reduced in situ plating efficiency of surviving tumor cells. This question remains to be studied further. The present evidence argues for an important and even limiting role of the tumor circulatory system in determining tumor growth rate after irradiation.
Slow Tumor Regrowth Following X-rays or Neutrons


Acknowledgments

We wish to thank Dr. Juri Eenmaa and Dr. Keith Weaver of the Division of Medical Radiation Physics for performing the neutron dosimetry and doing part of the neutron irradiations. We also thank Dr. G. Gordon Steel of the Department of Biophysics, British Institute of Cancer Research, for providing the computer analysis of the percentage of labeled mitoses curves.

References


Fig. 1. Control C3HBA mammary adenocarcinoma fixed at 14 days posttransplant. Luxol fast blue-PAS-hematoxylin stain, x 265.

Fig. 2. Regrowing C3HBA tumor fixed 14 days after receiving 3000 rads of 250 kVp X-rays. The tumor microarchitecture has changed from an anaplastic, disorganized structure to a corded structure with tumor parenchyma in many cases limited to a cylinder surrounding a central blood vessel. TC, tumor cord; BV, blood vessel; N, necrosis. Luxol fast blue-PAS-hematoxylin stain, x 265.

Fig. 3. Regrowing C3HBA tumor fixed 14 days after receiving 1000 rads of 8 MeV (mean energy) neutrons. Luxol fast blue-PAS-hematoxylin stain, x 265.
Mechanisms Underlying Reduced Growth Rate in C3HBA Mammary Adenocarcinomas Recurring after Single Doses of X-rays or Fast Neutrons

Janet S. R. Nelson, Rita E. Carpenter and Dianna Durboraw


Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/36/2_Part_1/524

Sign up to receive free email-alerts related to this article or journal.

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/36/2_Part_1/524. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.