ABSTRACT

Hypoxia is considered to be a major cause of tumor radioresistance. Reoxygenation of previously hypoxic areas after a priming dose of radiation is associated with an increase in tumor radiosensitivity. In a study of a hypoxic mammary carcinoma, $^3$P nuclear magnetic resonance spectra showed statistically significant increases in metabolite ratios (phosphoethanolamine/Pi and nucleotide triphosphate/Pi) after 65 and 32 Gy. The maximum changes in metabolite ratios after 32 Gy occurred at 48 h, although significant changes were detected at 24 h. A corresponding increase in the mean tumor pO$_2$ (polarographic microelectrode measurements) and a decrease in hypoxic cell fraction (changes in paired versus unclamped tumor control dose for 50% of tumors) were also shown to occur 48 h after a priming dose of 32 Gy. A significant increase in the mean tumor pO$_2$, phosphoethanolamine/Pi, and nucleotide triphosphate/Pi, compared to initial values, was noted at 24, 48, and 96 h post 65-Gy radiation. An increase in the downfield component of the phosphomonoester peak relative to the upfield component (phosphoethanolamine), is also noted after doses of 65 and 32 Gy. These are likely to be due to cell kill and/or decreased cell proliferation. In this tumor model, $^3$P nuclear magnetic resonance spectroscopic changes postradiation are temporally coincident with and may be indicative of tumor reoxygennation as measured by the tumor control dose for 50% of tumors and oxygen-sensitive microelectrodes.

INTRODUCTION

The potential importance of tumor hypoxia as a cause of resistance to radiotherapy and chemotherapy has long been recognized (1–12), and there is clinical evidence suggesting that tumor hypoxia is a cause of radioresistance in patients. Experimental evidence supporting the concept of radiobiological hypoxia (pO$_2$ < 2.5 mm of Hg) in murine and human (4, 6) tumor xenografts has further implicated hypoxic clonogenic cells as a major influence in the overall response rates to radiation and possibly chemotherapy (8).

Numerous physical and chemical attempts have been used to overcome tumor hypoxia for improving the therapeutic ratio of fractionated external-beam radiation therapy. Initially after a dose of radiation, oxygenated cells die, and the more radioresistant hypoxic cells survive, causing an increase in tumor hypoxia (3, 5, 7, 12). Subsequently, these remaining tumor cells undergo “reoxygenation” (9), which has been defined as the “reacquisition of radiosensitivity by those clonogenic cells . . . that were hypoxic at the time of exposure” (9). The rate and extent of radiobiological reoxygenation have been shown to be very variable in numerous experimental investigations of this phenomenon (1, 3, 5, 11, 12) but are recognized to be important, because murine tumors are more radiosensitive after reoxygenation.

Several methods have been used to measure and quantitate the partial pressure of oxygen in tumors including oxygen electrodes (13), fluorescent antibodies to hypoxic sensitizers (14), $^3$H- and $^{14}$C-labeled hypoxic cell sensitizers (15, 16), and cryospectrophotometry (17). Generally, these procedures are invasive and do not allow repetitive measurements without compromising the tissues under investigation. A noninvasive method of detecting and monitoring tumor reoxygenation could theoretically improve the efficacy of radiation treatment by optimizing the interval between radiation fractions and or chemotherapy.

Previous studies have demonstrated that NMR$^3$ spectral changes are related to tumor hypoxia. Investigations of metabolic effects induced by radiation have shown a range of spectral changes. We (18) and other investigators (19, 20) have demonstrated an increase in PCr/Pi and NTP/Pi, at 24 to 72 h postradiation. Irradiation of a hypoxic tumor (FSall) caused changes in tumor metabolism, as evidenced by an increase in PCr/Pi. (18). Similar effects have been noted by Rajan et al. (19) and Tozer et al. (20) studying the RIF-1 tumor model. Tozer et al. (20) suggested that the spectral changes could be ascribed to changes in tumor blood flow. Majors et al. (21) noted a decrease in P/β-ATP. These studies did not relate the observed metabolic changes postradiation to alterations in radiosensitivity or pO$_2$. This study was undertaken to determine if radiobiological reoxygenation and changes in pO$_2$ are temporally coincident with and related to noninvasively detected NMR spectral changes. Tumor pO$_2$ values and changes in radiobiological hypoxia were measured at the time point of maximum changes in bioenergetics as detected by $^3$P NMR spectroscopy. Radiation doses were selected to study the metabolic and radiobiological effects of curative and subcurative doses of radiation which induce substantial cell kill.

MATERIALS AND METHODS

NMR. $^3$P NMR spectra were obtained using previously described techniques (22–24) on an NT-300 wide-bore magnet (General Electric NMR, Fremont, CA) operating at 121.5 MHz for $^3$P. Experimental parameters included a spectral width of ±12,000 Hz, recycle delay time of 2 s, 60-degree spin flip angle, 1024 data points, and 256 to 728 signal-averaged FIDs. Using these acquisition parameters, the spectra are partially saturated. Four-turn solenoid coils were used for these studies; the size of the coil was chosen to fit closely around the tumor.
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The field was shimmed prior to spectral acquisition using the 1H water signal from the tumor. The typical width of the water resonance ranged from 0.2 to 0.3 ppm. Signal-averaged FIDs were zero filled to 4096 data points and multiplied by 15-Hz line-broadening filter, prior to Fourier transformation. Spectral peak areas were estimated by fitting the spectra to a series of Lorentzian peaks, using a program (GEMCAP) available on the spectrometer, after fitting the baseline to a third-order polynomial (using standard General Electric software). The 1H peak was used to estimate the NTP/Pi ratio, since the a and g peaks overlap other resonances. Tumor pH was estimated from the chemical shift of Pi, relative to that of PCr (26). Because the exact intracellular concentrations of substances which could affect the PCr-Pi chemical shift are not known, the derived pH-chemical shift curve has an uncertainty of ±0.1 pH units (27). Each of the parameters derived from the NMR data was statistically analyzed using a two-way analysis of variance with repeated measures (28), with radiation dose and time as the two independent variables. Scheffe tests were used for multiple comparison testing to identify statistical significance. No anesthesia was used during NMR experiments.

Tumor Transplantation and Irradiation. The MCa tumors were removed aseptically from tumor-bearing animals by previously described techniques (29, 30). Briefly, a single-cell suspension was prepared from a solid tumor by teasing and abrasion against stainless steel mesh immersed in iced minimal essential medium (Earle's balanced salt solution) containing 2% heparin. Cell suspensions were additionally disrupted by aspiration through an 18 gauge needle, and the final suspension was agitated constantly by a magnetic spin bar. A tumor inoculum of 0.025 to 0.04 ml (approximately 10^6 cells) was injected s.c. into the dorsum of the foot of male C3H/He mice (Jackson Laboratory, Bar Harbor, ME), with a 26-gauge needle. Greater than 75% of the MCa tumor cells were viable based on trypan blue exclusion. Tumor volume determinations were performed using the formula V = a x b x c, where a, b, and c are three orthogonal diameters. Separate cohorts were used for NMR, radiobiological experiments, and pO2 measurements. Parallel cohorts of tumor-bearing mice were used for pO2 measurements on different days postradiation to eliminate the possibility of artifacts induced by previous microelectrode insertion. Tumor growth was quantified using the tumor-bearing mice studied by NMR spectroscopy.

Irradiation studies were performed using previously described techniques (24). Animals were anesthetized with 35 mg/kg of pentobarbital and confined to a circular plastic jig radially divided into 10 compartments, with a removable plastic top and 0.5-cm lead cover shield. The top center of this Lucite jig was open to allow the tumor-bearing feet to project through and measured 7.5 cm in diameter, the port size for irradiation. The legs were additionally immobilized by a string tie to the toe and taping at the toe to the plastic jig base which had a double-adhesive tape surface. This increased the anchoring of the posterior footpad and averted physical compression of the tumor mass.

Irradiation was performed using a Philips MG 324 irradiation unit (Mahwah, NJ) operating at 320 kVp and 10 mA with 0.5-mm copper filtration. The field size was 10 x 10 cm at a distance of 35-cm source-to-surface distance for a dose rate of 304 cGy/min. Calibration of the field was performed using a Victoreen ionizing chamber at 50-cm source-to-surface distance, and the exposure rate at the above setting was 0.0405 C/kg/min (157 R/min). Radiation treatments were given when tumors reached a volume of 150 to 325 mm^3 on parallel cohorts of mice. All volumes were determined by caliper measurements of orthogonal diameters.

Animal tumors irradiated under hypoxic conditions had the tumor-bearing leg clamped 10 min before and during the time period of irradiation. Clamping was achieved by a waxed-string tourniquet (Johnson and Johnson, Kalamazoo, MI) secured and tightened proximal to the knee joint. Animals in different groups were palpated or visually inspected for possible tumor recurrence at least 2 times/wk for the first 6 wk and once a week postirradiation for 45 to 90 days (for the recurrence tumor distribution). The data were calculated by logit analysis and are represented as TCD50 values. The program version written by M. Stracher for the MacIntosh P.C. (7/88) is the most recent update of the original contribution by H. Haber, S. Urano, and M. Stracher. Single radiation dose-response curves were based on no less than six animals/radiation dose, and a minimum of six doses were studied per TCD50 measurement. Cell HFs were calculated from the equation (31)

\[ HF = \exp\left(\frac{TCD_{50\,\text{assay}} - TCD_{50\,\text{norm}}}{D_{\text{norm}}}\right) \]

using a D_{\text{norm}} value of 320 rads (3). Selection of a different D_{0} value would alter the absolute estimate of the hypoxic fraction, but would not significantly alter the interpretation of the data.

To determine the magnitude of reoxygenation after a priming dose of radiation, TCD50 assays were done. Two cohorts of mice received a priming dose (32 Gy) under aerobic conditions. This was followed by a second dose (the graded dose for determination of the TCD50) at 48 h. This interval coincided with peak changes of NMR and microelectrode measurements. One cohort received the second dose under anoxic conditions, and the other cohort was irradiated under oxygenated conditions (31). The difference between the hypoxic fractions obtained with and without a priming dose of radiation was calculated to estimate the magnitude of reoxygenation. Statistical significance was determined by using Student's t test for comparison of the difference between means.

pO2 Measurements. Mice were anesthetized with 35 to 50 mg/kg of pentobarbital (i.p.). Tumor pO2 values were measured polarographically (32). Insulating glass tubing was pulled to the 50- to 80-µm tip, and gold wire (20-µm in diameter) was inserted into the glass tube leaving a recess. The tip was partially melted to give a good contact between glass and gold, and the surface of the gold wire facing the tip of the microelectrode was plated with gold to ensure proper conduction. The best operating voltage for oxygen current was 0.75 negative V. Each pO2-sensing electrode was calibrated against a reference electrode by immersing it in saline solutions saturated with N2 gas or 5 to 95% oxygen. The electric current for anoxia (0% pO2) was confirmed by depleting oxygen in the saline solution with glucose oxidase and a sufficient amount of glucose. The performance of electrodes was regularly checked before and after measurements of tumor pO2. Oxygen current was amplified with a Chemical Microsensor (Diamond-General Development Corp., Ann Arbor, MI), and the values were stored on a chart recorder or digitized.

Verification of Phosphate Metabolites. The peak assignments for the prominent resonance bands in the 31P NMR spectral profiles of this study are based upon the measurements of chemical shifts. Specific phosphate metabolites contributing to these resonance bands were identified through the perchloric acid extraction of the phosphate metabolites in harvested tumors and high resolution 31P NMR spectroscopic analysis (33). MCa tumors were harvested for extraction by incising, at right angles, the dorsal surface of the skin surface adjacent to the tumor. The tumors were then either freeze-clamped between metal tongs prechilled to liquid nitrogen temperature, or immediately scooped into liquid nitrogen after tumor excision to prevent hydrolysis of high-energy phosphates (34). The tissue was extracted and analyzed according to methods previously described (33).

RESULTS

Fig. 1 summarizes the effect of a single dose of radiation on tumor growth. Unirradiated tumors (n = 12) continue to grow from a mean volume of 215 ± 18 mm^3 (mean ± SEM) at the time of initial study to a volume of 1018 ± 87 mm^3 at Day 9. In contrast, animals treated with 65 Gy of radiation (n = 13) demonstrated a small decrease in tumor volume from their initial value over the course of 9 days (191 ± 14 mm^3 to 177 ± 22 mm^3). Tumors irradiated with 32 Gy (n = 14) continued to grow significantly (32).
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Fig. 1. Mean tumor volume as a function of time after 0, 32, and 65 Gy. As expected, there is a significant delay in tumor growth after irradiation ($P < 0.001$) as evidenced by differences in tumor volumes on Day 9. Points, mean; bars, SEM.

Grow, albeit more slowly [227 ± 16 mm$^3$ (Day 0) — 455 ± 27 mm$^3$ (Day 9)], than unirradiated tumors. At Day 9, the differences in tumor volume between animals receiving 0 and 32 Gy were significant ($P < 0.001$) as were the differences in tumor volumes of animals receiving 32 or 65 Gy ($P < 0.001$).

Fig. 2A demonstrates changes in tumor metabolism after 65 Gy as detected noninvasively by $^{31}$P NMR spectroscopy. The individual peaks are identified in the legend of Fig. 2A. Fig. 2B shows successive spectra taken from an untreated MCa tumor. A marked decrease in the relative intensity of the P$^i$ resonance is noted after 65 Gy. In contrast, the P$^i$ peak of untreated tumors is initially unchanged and subsequently increases.

Fig. 3A graphically summarizes the changes noted in the PCr/P$^i$ ratio after 0, 32, and 65 Gy of radiation. As expected, there were no significant differences in the value of PCr/P$^i$ among the three cohorts prior to treatment. An increase in PCr/P$^i$, at 24 h postirradiation is noted which is statistically significant for both the 65-Gy group ($P < 0.01$) and the 32-Gy group ($P < 0.05$). The PCr/P$^i$ value continued to increase in both irradiated groups, although further increases were not significantly different from the value measured at 24 h postirradiation. The spectra obtained from tumors treated with 32 Gy showed an increase in PCr/P$^i$ at 24 h with a subsequent increase (albeit not significant compared with the value 24 h postirradiation) to a maximum value at 48 h. This was followed by a decrease in PCr/P$^i$ over the next 10 days which was qualitatively similar to the effect noted in control animals which showed a significant decrease in PCr/P$^i$ over the course of the study ($P < 0.05$).

Fig. 3B shows a similar graph of the effects of radiation on tumor NTP/P$^i$ values. The initial values of NTP/P$^i$ for untreated tumors are noted to show no differences among the different groups. The changes in NTP/P$^i$, after irradiation parallel the PCr/P$^i$ data (Fig. 3A). Irradiated tumors (65 and 32 Gy) showed an increase in NTP/P$^i$ at 24 h ($P < 0.05$; $P < 0.05$). The tumors treated with 32 Gy showed a further increase at 48 h (albeit not significant), and a subsequent decrease occurred over the next 10 days. By Day 12 the value of NTP/P$^i$ for the group treated with 32 Gy is identical to the untreated group. Changes in PCr/P$^i$ and NTP/P$^i$ did not show a dose-related effect.

There were no significant differences observed in pH values between the 65-Gy group and control animals (data not shown).

A significant increase in pH was noted 48 h postradiation after 32 Gy ($P < 0.01$).

The PME resonance band is resolved into two principal components in the spectral profiles of this study (Fig. 2). Prior to irradiation, the PME component, identified in tissue extracts...
as consisting of phosphoethanolamine, inosine, and adenosine phosphate, the 2' phosphate of NADP and other minor phosphomonoesters, appeared with lower signal intensity than the upfield component of the resonance band. The upfield component of the PME resonance was determined to consist primarily of phosphocholine. The relative differences in peak heights of the two components were found to reverse following irradiation. The height of the peak corresponding to the resonance of phosphomonoesters, appeared with lower signal intensity than the upfield component of the resonance band. The upfield component of the resonance band. The upfield component of the resonance band.

Fig. 4 summarizes the changes induced by tumor irradiation on the phosphomonoester region (based upon relative peak height measurements) as reflected by the increase in the PME/PC ratio. A dose-response relationship is observed with the 65-Gy group having a significantly higher value than the 32-Gy group (P < 0.01) and the 32-Gy group having a significant increase compared with the control group (P < 0.01). After 65 Gy, a significant increase in PME/PC occurred from baseline to 24 h and again between 24 and 96 h (P < 0.01). For 32 Gy, the significant increase in PME/PC occurred from baseline to 48 h (P < 0.05).

Fig. 5 represents the distribution of pO2 values pre- and post-32 and 65 Gy of radiation. Prior to treatment, it was noted that 55.7% of the measurements demonstrated a pO2 < 2.5 mm of Hg. After 65 Gy, the distribution is shifted to the right and demonstrates the increase in tumor oxygenation. Table 1 summarizes the pO2 data. After 65 Gy, a significant increase in the mean pO2 is noted at 1, 2, and 4 days postirradiation. For animals treated with 32 Gy, there is a decrease in the percentage of measurements with a pO2 < 2.5 mm of Hg 24 h after irradiation. An increase in the mean pO2 is noted at 24 h although statistical significance is only attained at 48 h postirradiation. By 96 h post-32 Gy, the mean pO2 had declined to less than its initial value (Table 1), although the fraction of measurements with a pO2 < 2.5 mm of Hg remained lower than that of control tumors.

Table 2 and Fig. 6 summarize the TCD50 and hypoxic fraction measurements for the MCa tumor (mean volume ~250 mm3). The TCD50 value for this tumor model under normoxic conditions is 63.6 Gy. If the tumor-bearing leg is clamped prior to radiation, an increase in the TCD50 to 67.4 Gy is noted (Fig. 6), corresponding to a hypoxic fraction of 30.2%. Tumors treated with a priming dose of 32-Gy radiation and then retreated with graded doses of radiation for TCD50 measurements were found to have a TCD50 value (excluding the 32-Gy priming dose) of 25.7 Gy. Under clamped (hypoxic) conditions, tumors pretreated with a priming dose of 32 Gy of radiation had a TCD50 value (excluding the 32-Gy priming dose) of 38.4 Gy. The hypoxic fraction calculated from the differences between normoxic and clamped TCD50 values decreased to 1.9%. The standard error of the hypoxic fraction was estimated by the method of Grau et al. (35), and the change at 48 h is significant (P < 0.01). This decrease in the hypoxic fraction of normoxic tumors (48 h after a priming dose of radiation) is attributed to a significant increase in the oxygenation status of MCa tumors treated with 32 Gy.
DISCUSSION

$^{31}$P NMR spectroscopy has been used to monitor changes in tumor metabolism with growth, chemotherapy, radiation, hyperthermia, photodynamic therapy, etc. Studies have shown that tumor metabolism is sensitive to drugs which are under investigation as potential agents to enhance sensitivity to radiation (24, 36–41). Numerous investigators have demonstrated spectroscopic changes in response to changes in tumor oxygen status. We have previously shown that it is feasible to detect changes in tumor radiosensitivity induced by radiosensitizing agents (24). Specifically, a linear relationship between change in TCD$_{50}$ and change in PCr/P$_i$ was noted in MCA tumor-bearing mice receiving Fluosol-DA (24). Sostman et al. (36) studied a rhabdomyosarcoma and also noted a change in NMR spectra after treatment with oxygen and perfluorocytromid in conjunction with pO$_2$ and radiosensitivity measurements. They concluded that $^{31}$P NMR could be used to monitor oxygenation in that tumor model. More recently, Wood et al. (37) have also found that the time course of the changes in $^{31}$P NMR spectra after flunarazine and nicotinamide coincide with radiosensitivity. These data suggest that changes in radiosensitivity induced by certain agents may be detected noninvasively by $^{31}$P NMR.

Previous studies have indicated a relationship between tumor oxygenation and metabolic status. Okunieff et al. (25) noted that an increase in the hypoxic fraction occurs in parallel to a decrease in high-energy phosphates. This has been corroborated by other investigators subsequently. Rofstad et al. (42) noted a linear correlation between bioenergetic status and hemoglobin oxygen saturation in three tumor models, indicating a relationship between high-energy phosphates and tumor oxygen supply. This relationship was not identical for the different tumor models, indicating that intrinsic cellular properties of different tumor models influenced tumor hypoxia and energetic status. Mueller-Kleiser et al. (43) noted in biopsy samples a positive correlation between the ratio of NTP to the total phosphate measured by NMR spectroscopy with the modal ATP concentrations measured by bioluminescence. Vaupel et al. (44) noted a significant correlation between the partial pressure of oxygen as measured by oxygen electrodes and NMR-measured metabolite ratios and concluded that NMR could be used to detect changes in tumor energetics induced by changes in tumor oxygenation. Steen et al. (45) have noted that $^{31}$P NMR can detect metabolic changes associated with an increase in tumor radioreistance. Significant correlations between hypoxic fraction and metabolite ratios measured by $^{31}$P NMR spectroscopy were noted by Fu et al. (46) studying the EMT-6 murine tumor at different volumes. These investigators noted that the data showed considerable heterogeneity which precluded accurate prediction of hypoxic fractions from the NMR data. The heterogeneity may be inherent to the technique of paired cell survival comparisons and clonogenic hypoxic cells, where response is dictated by a small cellular component, or true biological diversity that is both tumor and volume dependent.

Several previous studies have noted an increase in high-energy phosphates after radiation (18–21). This investigation differs from previous NMR investigations of the effect of radiation on tumor metabolism by demonstrating that the NMR changes are temporally coincident with changes in radiobiological and molecular hypoxia. The increase in PCr/P$_i$ at 24 to 48 h after radiation is similar to our previous findings in which large hypoxic FSA11 tumors had an increase in PCr/P$_i$ at 48 h postradiation. Tozer et al. (20) also noted an increase in high-energy phosphates in the RIF-1 tumor which they noted to have a hypoxic fraction of 11% (tumor volume= 800 mm$^3$). This increase occurred at 24 h post-10 Gy and 48 h post-20 Gy. Similarly, Rajan et al. (19) also noted an increase in high-energy phosphates during the 24- to 72-hour interval postradiation. Sijens et al. (47) studied the NU-82 murine tumor and did not detect specific metabolic changes associated with an increase in tumor radiosensitivity.

In contrast, other studies have not noted a relative increase in high-energy phosphates during the 24- to 72-hour interval postradiation. Sijens et al. (47) studied the NU-82 murine tumor but noted that, after 10 Gy, ATP/P$_i$ rose at 10 h and dropped at 47 h. Kritjansen et al. (48) studied lung cancer xenografts and noted that low doses of radiation induced an increase in ATP/P$_i$; while higher doses of radiation (20 Gy) induced a decrease in PCr/P$_i$. Allavena et al. (49) studied a dimethylbenz[a]anthracene-induced fibrosarcoma in rats and did not detect spectral changes after a total of 15 Gy administered in 3 fractions of 5 Gy. Since reoxygenation has been shown to depend on both the tumor model and the radiation dose, these varying results
may be due to the different responses expected from the study of diverse tumor models and priming doses of radiation.

Table 1 Changes in tumor pO2 after 65- and 32-Gy irradiation

| No. of | No. of | pO2 values (mm of Hg) | Significance | pO2, 0 to 2.5 mm
|---|---|---|---|---|
| of | of | | | of Hg (frequency, %)
| measurements | measurements | Median | Av. | SEM | |
| Control | 10 | 291 | 1.7 | 5.0 | 0.4 | 55.7 |
| 65 Gy | | | | | | |
| Day 1 | 10 | 277 | 2.9 | 6.5 | 0.5 | 0.01* | 45.8 |
| Day 2 | 9 | 259 | 3.6 | 6.2 | 0.4 | 0.02* | 35.9 |
| Day 4 | 9 | 267 | 4.6 | 6.1 | 0.4 | 0.03* | 25.1 |
| 32 Gy | | | | | | |
| Day 1 | 9 | 240 | 3.1 | 5.7 | 0.4 | 0.14 | 39.6 |
| Day 2 | 9 | 233 | 3.2 | 6.5 | 0.5 | 0.01* | 45.5 |
| Day 4 | 11 | 299 | 2.5 | 4.1 | 0.3 | 0.03 | 48.5 |

*Statistically significant increase in pO2 (P < 0.05). The median and average pO2 data are based on statistical analyses and do not reflect the inaccuracies in individual measurements which is greater than 0.1 mm of Hg.

Table 2 TCD50 and hypoxic cell fractions with and without 32-Gy priming

<table>
<thead>
<tr>
<th>TCD50</th>
<th>n</th>
<th>Hypoxic fraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxic</td>
<td>63.6 ± 1.4*</td>
<td>159</td>
</tr>
<tr>
<td>Hypoxic (clamped)</td>
<td>67.4 ± 2.3</td>
<td>67</td>
</tr>
<tr>
<td>Normoxic*</td>
<td>25.7 ± 1.8</td>
<td>112</td>
</tr>
<tr>
<td>Hypoxic* (clamped)</td>
<td>38.4 ± 2.4</td>
<td>88</td>
</tr>
</tbody>
</table>

*Mean ± SEM.

*Graded dose administered 48 h post 32-Gy priming dose. The 32-Gy priming dose is not included in the TCD50 value.

*After a priming dose of 32 Gy, the hypoxic cell fraction is significantly less than the pretreatment value (P < 0.01).

The changes in the PME and PDE regions noted in Figs. 2 and 4 which we noted in a preliminary report of this investigation (56) have not been reported previously in tumor metabolic studies. Changes in the PME and PDE resonance bands result from alterations in cellular energy metabolism and processes involving the precursors and products of membrane metabolism (57). This is supported by the dose-response effect noted in this study, as outlined by the measured differences in the spectral profiles of tissues irradiated with 32 Gy versus 65 Gy. Scherer et al. (58) have recently noted an inverse correlation between phosphoethanolamine levels and the rate of cell proliferation using four cell lines and have suggested that the PE resonance may yield information about the proliferative capacity of the tumor. The in vivo findings of an increase in the PME/PC ratio (where PME is composed primarily of PE) after radiation doses that induce cell kill of greater than 99.99% are compatible with this suggestion. These spectral changes could potentially be detected in clinical spectroscopic studies for monitoring tumor response and may be indicative of alterations in cell cycle...
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kinetics. The changes in the PME and PDE resonances are undergoing further spectroscopic investigations utilizing both perchloric acid and lipid extracts.

In the MCa tumor, metabolic alterations as reflected by changes in PCr/Pi and NTP/Pi measurements occur concurrently with changes in radiobiological reoxygenation and changes in pO2. This study suggests that the metabolic changes detected by 31P NMR spectroscopy might be due to alterations in metabolism induced by preferential changes in oxygen concentration to surviving cells. Changes in the PME resonances, specifically the ratio of PME/PCr, are noted after high doses of radiation which induce loss of cell proliferation. Further studies with the MCa model at clinically relevant radiation doses are necessary to determine if NMR spectroscopic changes related to tumor oxygenation and proliferative capacity can be detected.

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Quantitative Changes in Tumor Metabolism, Partial Pressure of Oxygen, and Radiobiological Oxygenation Status Postradiation

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