indicate that these defects might be associated with impaired expression of the tyrosine kinase activity associated with the receptor.

GEORGE GRUNBERGER
YEHIEL ZICK
PHILLIP GORDEN
Diabetes Branch, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205

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14. Using the erythrocyte insulin receptor, F. Grigorescu and C. R. Kahn [*Diabetes* **32** (Suppl. 1), 1A (1983)] showed that a patient with diminished [^{125}I]insulin binding has decreased receptor kinase activity. In addition, we have shown, in another patient with type A insulin resistance and very low [^{125}I]insulin binding, a marked decrease in insulin-stimulated phosphorylation of the β subunit.
15. It has been speculated that binding of insulin to the α subunit of its receptor leads to phosphorylation of the β subunit, which in turn initiates a cascade of events leading to the terminal action of the hormone [Y. Zick, R. W. Rees-Jones, J. Roth, in *Proceedings of the 11th Congress of the International Diabetes Federation* (Excerpta Medica, Amsterdam, 1982), p. 161; M. Kasuga, Y. Fujiti-Yamaguchi, D. L. Blithe, C. R. Kahn, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2137 (1983); O. M. Rosen, R. Herrera, Y. Olowe, L. M. Petruzzelli, M. H. Cobb, *ibid.*, p. 3237].
16. Preliminary studies with extract from Epstein-Barr virus (EBV)–transformed lymphocytes from the study patient do not show this severe defect in insulin receptor phosphorylation. We have, however, observed situations in which binding to freshly isolated monocytes, EBV-transformed lymphocytes, and cultured human fibroblasts is discordant (S. I. Taylor, J. Podskalny, G. Grunberger, P. Gorden, in preparation). The meaning of this discordance, either in binding or in phosphorylation, is not known.
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Perfluorochemical Emulsions Can Increase Tumor Radiosensitivity

Abstract. *An oxygen-carrying perfluorochemical emulsion enhanced the effectiveness of radiation therapy in two transplantable solid tumors in mice. The perfluorochemical emulsion had no effect on tumor growth after x-irradiation, but delayed tumor growth significantly when administered to oxygen-breathing mice before or during irradiation.*

The cytotoxicity of ionizing radiation is modified by the presence of molecular oxygen (*1*). Hyperbaric oxygen treatment augments the therapeutic effect of radiation in animals and humans (*2, 3*), but is difficult to implement clinically (*3*). Although several chemicals, including nitroimidazole derivatives (*4*) and platinum complexes (*5*), sensitize hypoxic cells to cytotoxic agents, none has proved to be clinically useful (*6*).

In both normal and tumor tissue, oxygen tension is dependent on delivery through the vascular system and on utilization by tissues (*7*). Tumors often have inadequate vasculature and areas of intermittent and irregular blood flow because blood vessels in tumors can constrict and collapse (*8*). This leads to zones of necrosis and areas of hypoxia.

Radiotherapeutic efficacy may be limited by the radioresistance of hypoxic cells (*1*). Three times more radiation is required to kill fully anoxic cells than oxygenated cells. Perfluorochemicals dissolve large amounts of oxygen (*9*), and perfluorochemical emulsions are being tested as oxygen transport agents for use during surgery after hemorrhage or to minimize ischemic damage after stroke or myocardial infarction (*10*). The volume of oxygen dissolved in perfluorochemical emulsions changes linearly with the partial pressure of oxygen. Therefore, to fully exploit the oxygen-carrying capacity of these materials in vivo, high partial pressures of oxygen are used. As with hemoglobin, the uptake and release of oxygen from perfluorochemical emulsions are completely reversible, and the rate is twice as fast as that of hemoglobin. Ninety percent or more of the emulsion particles in the preparation that we used are less than 0.2 μm in diameter—much smaller than red blood cells (average diameter, 5 to 10 μm). We reasoned that these small oxygen-carrying particles would be able to deliver sufficient oxygen to hypoxic regions of solid tumors to significantly increase the radiosensitivity of these tumor regions. we report that a perfluorochemical emulsion and a 95 percent oxygen atmosphere markedly delayed the growth of two solid tumor lines exposed to x-rays in vivo. Using a tumor excision assay, we were able to measure the enhancement of cell killing in one of the lines.

Lewis lung tumor (*11*) was carried in C57BL/6J male mice (Jackson Laboratory). FSa-II fibrosarcoma (*12*), which had been adapted for growth in culture (FSa-IIC), was carried in C3H/Be/FeJ male mice (Jackson Laboratory). For the experiments 2×10^6 tumor cells prepared from a brei of several stock tumors were implanted intramuscularly in the legs of mice. The Lewis lung tumor was grown in B6D2F1/J male mice (Jackson Laboratory) and the FSa-IIC fibrosarcoma was grown in C3H/Be/FeJ male mice 8 to 10 weeks of age. When the tumors were approximately 50 mm^3 in volume (about 1 week after tumor cell implantation), the perfluorochemical emulsion, Fluosol-DA (20 percent) (*13*), in volumes ranging from 0.2 to 0.5 ml, was injected into the tail vein. The animal was then allowed to breathe air or was placed in a circulating atmosphere of 95 percent oxygen and 5 percent carbon dioxide. One hour later the tumor-bearing limb was given a single dose of x-rays of 1000, 2000, or 3000 rads with a Gamma Cell 40 (Atomic Energy of Canada; dose rate, 88 rads per minute). The shielded portion of the animal received less than 2 percent of the delivered dose. Animals were anesthesized during the radiation treatment.

In experiments to determine the delay in tumor growth, the progress of each tumor was measured thrice weekly until it reached a volume of 500 mm^3. Untreated Lewis lung tumors reach 500 mm^3 in about 14 days and untreated FSa-IIC tumors reach 500 mm^3 in about 12 days. Statistical comparisons were made with the Dunn multiple comparisons test (*14*).

In experiments to determine the surviving fraction of FSa-IIC fibrosarcoma cells, tumors were excised 24 hours after x-ray treatment and single-cell suspen-

sions were prepared by treating the brei with trypsin and deoxyribonuclease for 10 minutes. The number of viable single tumor cells was determined by trypan blue exclusion (*15*). Survival of a known number of tumor cells was determined with a colony-forming assay. Cultures were incubated for 8 days in an atmosphere of 95 percent air and 5 percent carbon dioxide. Only colonies of > 50 cells were counted.

Fluosol-DA and the 95 percent oxygen atmosphere significantly affected the response of both tumors to x-irradiation. The perfluorochemical emulsion had no significant effect on the response of either tumor to x-irradiation in an air atmosphere. For clarity, results produced with the various doses of Fluosol-DA were pooled, since no significant dose-response effect was observed.

Table 1 shows the results obtained with the Lewis lung tumor. Oxygen alone had little influence on the radiosensitivity of this tumor. Tumor growth delay (the number of days beyond 14 days required for the tumor to achieve a volume of 500 mm^3) was greater in animals exposed to Fluosol-DA and 1 hour of oxygen before x-irradiation than in animals exposed only to oxygen and x-rays, but the difference was significant only at the 3000-rad dose. The delay in tumor growth was still greater when oxygen was breathed during irradiation. The greatest delay was observed when Fluosol-DA-treated animals were exposed to oxygen for 1 hour before and during the radiation treatment.

Of the mice given Fluosol-DA and oxygen before and during radiation at 3000 rads, 70 percent died on or about day 60 of metastatic disease, with no tumor palpable at the primary site. With 2000 rads and otherwise identical conditions, 46 percent died. Therefore, 60 days was used as a ceiling point for the growth delay that causes an artificial leveling of data obtained at 2000 and 3000 rads. In all other groups there were no apparent cures by 60 days. On the basis of data obtained in mice given Fluosol-DA and 1000 rads, the dose-modifying factor [DMF (*16*)] with oxygen before, during, and before and during irradiation was 1.75 ± 0.23, 2.58 ± 0.47, and 2.76 ± 0.56, respectively.

Similar delays were observed in the growth of the FSa-IIC fibrosarcoma. At 1000 rads the DMF produced by 0.5 ml of Fluosol-DA and oxygen exposure before and during irradiation was 1.83 ± 0.32. Since the FSa-IIC fibrosarcoma is adapted to culture, we were able to determine the fraction of tumor cells killed by using a colony-forming assay.

Table 1. Effect of the perfluorochemical emulsion and the 95 percent oxygen atmosphere on the growth of Lewis lung tumors treated with various doses of x-irradiation. Values (means ± standard errors; $N = 55$ for each group) are days beyond 14 days necessary for the tumors to reach 500 mm^3.

Fluosol-DA	Oxygen exposure	Delay in tumor growth (days)		
		1000 rads	2000 rads	3000 rads
No	Before and during irradiation	1.6 ± 0.5	6.2 ± 1.0	8.8 ± 1.0
Yes	Before irradiation	3.8 ± 0.5	10.0 ± 0.7	18.8 ± 1.5*
Yes	During irradiation	8.3 ± 1.2†	18.5 ± 1.5†	26.5 ± 2.0‡
Yes	Before and during irradiation	8.5 ± 1.5†	28.5 ± 2.5‡	36.3 ± 2.0‡

*Significantly different from result for corresponding control group ($P < 0.0005$, Dunn multiple comparisons test). †$P < 0.001$. ‡$P < 0.0001$.

The survival of tumor cells from animals inspiring oxygen for 1 hour before and during x-ray treatment is shown by the closed circles in Fig. 1. A larger fraction of tumor cells died when 0.5 ml of the perfluorochemical emulsion was administered and oxygen was given for 1 hour before and during x-ray treatment (open circles in Fig. 1). The ratio of the slopes of the two lines indicates a DMF of 1.41 due to the addition of Fluosol-DA.

These experiments demonstrate that an intravenously administered perfluorochemical emulsion enhances the effectiveness of radiation therapy when an oxygen-rich atmosphere is breathed before or during irradiation. From the data presented in Fig. 1, it appears that the most immediate effect of the oxygen-carrying perfluorochemical emulsion is to make a previously resistant subpopulation of tumor cells susceptible to the lethal effects of radiation. The discrepancy in the DMF's between the tumor growth delay assay and the survival assay indicates that some additional cytotoxic events, triggered by or involving the oxygen-carrying perfluorochemical emulsion, occur later than the first day after treatment, since the in vitro assay measures survival at that time. Alternatively, the perfluorochemical emulsion may alter the regrowth kinetics of surviving tumor cells by retarding cell proliferation, thus implying a greater cell kill than that measured by the tumor excision assay.

The small size of the emulsion particles may allow delivery of oxygen to sites that red blood cells cannot reach. Since the overall oxygen concentration of the body is changed very little by the perfluorochemical emulsion, x-ray toxicity to most normal tissues should not be enhanced (*17, 18*). These findings are relevant to cancer therapy because they suggest a nontoxic method of delivering molecular oxygen to tissues. Because the efficacy of several drugs as well as radiation is enhanced by oxygen and oxygen-mimicking substances (*19*), oxygen-carrying perfluorochemical emulsions may provide a means of increasing the effectiveness of radiation therapy and of certain chemotherapeutic agents.

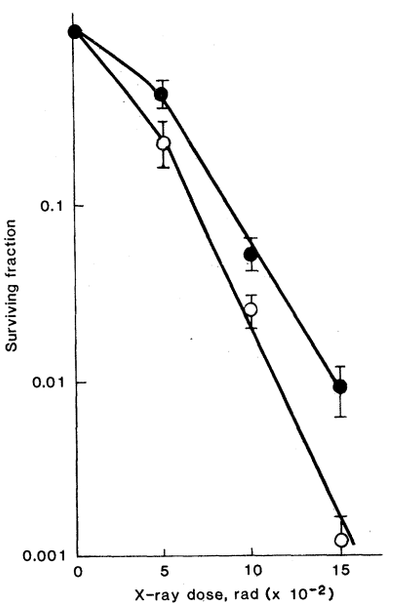

Fig. 1. Mean surviving fraction of FSa-IIC fibrosarcoma tumor cells at each dose of x-rays. Mice were given 95 percent oxygen for 1 hour before and during irradiation (●) or intravenous Fluosol-DA followed by oxygen exposure for 1 hour before and during irradiation (○). Each point represents the mean ± standard error for five experiments.

BEVERLY A. TEICHER
CHRISTOPHER M. ROSE
Dana-Farber Cancer Institute, Boston, Massachusetts 02115, and *Department of Radiation Therapy, St. Joseph Medical Center, Burbank, California 91505*

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9. Perfluorochemicals are compounds in which all of the hydrogens have been replaced by fluorine atoms. While water or blood plasma dissolve only about 2.0 percent oxygen by volume and whole blood about 20 percent, perfluorochemical liquids dissolve 40 percent or more. However, perfluorochemicals have limited oxygen-transport capability at ambient oxygen pressures. Blood delivers approximately 6 percent oxygen by volume to tissues at ambient pressure, whereas Fluosol-DA (20 percent) can deliver only about 2 percent. If the oxygen pressure is increased to one atmosphere of inspired gas (F_IO_2-1.0), then the amount of oxygen that can be delivered by Fluosol-DA (20 percent) is about 6.5 percent by volume [R. P. Geyer, in *Blood Substitutes and Plasma Expanders*, G. A. Jamieson and T. J. Greenwalt, Eds. (Liss, New York, 1978), vol. 19, p. 1; *New Engl. J. Med.* **307**, 304 (1982)].
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13. Fluosol-DA 20 percent (Green Cross Corporation) was obtained from Alpha Therapeutics Corporation. The emulsion consists of 20 percent (weight to volume) perfluorochemicals—that is, seven parts of perfluoro-Decalin and three parts of perfluorotripropylamine, Pluronic F-68 (2.7 percent, weight to volume), and yolk phospholipids (0.4 percent) as the emulsifier and glycerol (0.8 percent) as a cryoprotecting agent. Krebs-Ringer bicarbonate solution and hydroxyethyl starch (3.0 percent, weight to volume) were added to give the preparation physiological osmolarity and oncotic pressure. The combined surface area of the emulsion particles available for oxygen diffusion is 1.82×10^8 cm^2/liter (about 100 times the surface area of whole blood). The half-life of Fluosol-DA in vivo is about 12 hours.
14. Data on the delay of tumor growth were analyzed with a Basic program for the Apple II+ microcomputer. The program derives the best fit curve for each set of data, then calculates the median, mean, and standard error of the mean for individual tumor volumes and the day on which each tumor reached 500 mm^3. Statistical comparisons were carried out with the Dunn multiple comparisons test after a very significant effect was found by analysis of variance.
15. For the tumor excision assay, all reagents were sterilized with 0.22-μm Millipore membranes and were added aseptically to the tumors. Mice were killed and soaked in 70 percent ethanol. Their leg tumors were excised under sterile conditions in a laminar flow hood and were minced to a fine brei with small curved scissors. Four tumors were pooled to make each treatment group. Approximately 1 g of tumor brei was used to make each single-cell suspension. Each sample was suspended in 20 ml of Dulbecco's phosphate-buffered saline (Gibco) containing deoxyribonuclease (93 μg/ml; Sigma) and trypsin (1.85 mg/ml; Gibco) in a 50-ml plastic centrifuge tube. The samples were incubated for 10 minutes at 37°C, after which the liquid was gently decanted and discarded. Tumor homogenates were resuspended in the enzyme-containing phosphate-buffered saline, mixed (Vortex mixer), incubated and rocked for 10 minutes, and mixed again. The deoxyribonuclease concentration was then increased to 2.5 mg/ml in each tube. After being thoroughly mixed, each sample was filtered through a 135-μm stainless steel mesh in a Nuclepore Swin-Lok holder into a 50-ml plastic centrifuge tube. The samples were centrifuged (E-C model PR-5) at 1000 rev/min and 4°C for 10 minutes, at which time the supernatant was decanted and the pellet resuspended in Eagle's MEM containing 10 percent fetal bovine serum and antibiotics (Gibco). The samples were centrifuged again, the supernatants decanted, and the pellets resuspended in Eagle's MEM containing 10 percent fetal bovine serum and antibiotics. These single-cell suspensions were counted and plated for the colony-forming assay.
16. The influence of oxygen on radiosensitivity appears to be largely quantitative rather than qualitative, since it affects mainly the dose required to produce a given end point. When the effect of oxygen is strictly quantitative, the degree of its influence can be specified by a constant DMF. Thus, dose-effect curves at different oxygen concentrations are related by a constant multiplier, and their shape is independent of local oxygen concentration. We assumed zero growth delay with 0 rad and calculated the factor that related the control dose to the same growth delay achieved with 1000 rads, Fluosol-DA, and 95 percent oxygen.
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Partial Characterization of 21.5K Myelin Basic Protein from Sheep Brain

Abstract. *The 21,500 molecular weight (21.5K) variant of myelin basic protein (MBP) was isolated from sheep brain and partially characterized. Digestion with cyanogen bromide and trypsin yielded peptides which showed that approximately 30 additional amino acids were inserted at the equivalent of the amino acid at position 57 in the bovine 18.5K MBP sequence. An unusually hydrophobic peptide Pro, Val, Leu, Trp, Lys was present in this region. Ornithine was present in hydrolyzates of 21.5K MBP, but it was not detected in any of the peptides.*

In 1977, Barbarese *et al.* (*1*) showed that mouse brain contained four myelin basic proteins (MBP) with apparent molecular weights of 21.5K (21,500), 18.5K, 17K, and 14K. Subsequently, these variants were found in myelin from other species (*2*). Originally it was thought that 21.5K and 17K MBP's were precursors of 18.5K and 14K MBP's, respectively (*1*). However, studies on the synthesis of the MBP's indicated that they were more likely to be the products of separate messenger RNA's (*3, 4*).

Sheep brain was chosen for the isolation of 21.5K MBP for our study. An established method (*5*) for the preparation of MBP was followed, except that Sephadex G-75 (superfine) with 0.2*M* KCl-HCl buffer, *p*H 2.0, was used. Separation was monitored by gel electrophoresis and by an immunoblotting technique (*6*). A monoclonal antibody (*6, 7*) that reacts with the amino acid sequence Ala-Ser-Asp-Tyr-Lys-Ser in MBP was used to locate 18.5K and 21.5K sheep MBP in the fractions. From repeated chromatography on Sephadex G-75 (superfine), approximately 18 mg of 21.5K MBP was isolated from 200 g of brain.

Duplicate samples of 21.5K MBP were hydrolyzed and analyzed (Table 1). Surprisingly, ornithine was found in all hydrolyzates of 21.5K MBP but not in any of the peptides (*8*) nor in hydrolyzates of sheep 18.5K MBP. The association of ornithine with 21.5K MBP requires further study.

As 21.5K MBP contained three methionine residues, digestion with cyanogen bromide (CNBr) was used to ascertain their positions. The digest was fractionated by gel filtration (*9*). Electrophoresis of the fractions and transfer of the peptides to nitrocellulose revealed three peptides (as seen by staining with amido black) with apparent molecular weights of approximately 14K, 6K, and 2K; these values were determined from a comparison with a CNBr digest of myoglobin. Only the 14K peptide reacted