Effect of Pre-irradiation on the Ratio on Oxygenated and Anoxic Cells in a Transplanted Mouse Tumor

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It is attractive to propose that the increased curative efficiency of radiation delivered by a fractionated scheme is greater than by single acute doses because of radiation-induced changes in the proportion or extent of anoxic regions within a tumor. Thus, early irradiation should tend preferentially to destroy those tumor cells which were located within a critical radius from capillaries and this would lead to a redistribution of oxygenated sites owing perhaps to progressive passive migration of formerly anoxic foci to the radioresistant capillaries. Attempts to test this hypothesis directly by the implantation of oxygen-sensitive electrodes within tumors have been incapable of yielding reliable or unambiguous results. The present study was undertaken as an indirect approach to this question.

Mice of the strain C3H/Km were inoculated subcutaneously with an isogenic transplanted sarcoma of spontaneous origin, and the tumors were allowed to grow for 10–14 days. Tumors were then irradiated in situ with 250 kv X-rays in either of two conditions: (a) in mice breathing air during irradiation, or (b) in mice which had been killed by nitrogen asphyxiation 5 minutes prior to the start of irradiation. Tumor cell surviving fractions were then obtained by endpoint dilution assay: the tumors were excised from their hosts, minced in Hanks' solution, and made into a single cell suspension with the aid of mild trypsinization. The counted suspension was serially diluted in 5-fold steps and appropriate dilutions were inoculated into four separate subcutaneous sites in isogenic normal recipient mice (16 inocula/cell dilution). After scoring tumor takes in all sites for at least 100 days after in-

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Sponsored by the Zellerbach Family Fund

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oculation, estimates were made of the TD₅₀, the number of cells necessary to produce a take (growth of a solid tumor at the site of inoculation) in 50 % of the cases in a given series of cell dilutions. By computing TD₅₀ unirradiated/ TD₅₀ irradiated, survival curves were constructed. These survival curves were of the classic sigmoid shape and exhibited a D₀ of 465 R (D₁₀ = 1070 R). The survival curves of tumors irradiated in air-breathing mice and in dead mice had essentially the same terminal slopes, but significantly different O-dose extrapolates. If it is assumed that two extremes of radiosensitivity are exhibited by oxygenated vs. anoxic cells and that this sensitivity difference is the only one which is relevant to the present study, it may be deduced that approximately 15% of the cells of this tumor are anoxic in the air-breathing animal.

In a second group of experiments, tumors were subjected to a series of daily localized irradiations of 200 R for 5 days, a treatment which could be expected to eliminate preferentially the more radiosensitive well-oxygenated cells thereby reducing the number of these cells to less than 5 % of their original number. On the third day after exposure to the fifth 200 R fraction, test doses of 0–2300 R were administered to the pre-irradiated tumors in situ in air-breathing and nitrogen-killed mice. Survival curves were subsequently constructed on the basis of TD50's as described above. The terminal slopes of these survival curves were the same as those of the tumors which were not previously irradiated; and analysis of these curves indicated that after this course of prior irradiation tumors in living mice contained, on the average, 19% (range, 6–65%) anoxic cells. An additional set of tumors which were pre-irradiated with whole body doses (4 X 200 R/day) gave similar results: average of 24% anoxic cells.

Although the experimental results are not yet complete, it is evident that in the course of fractionated radiotherapy new cells must have entered the well-oxygenated compartments, thus tending to maintain the same radiosensitivity status of the tumor with respect to the oxygen effect. These observations are limited to a single, fast growing transplantable tumor, and confirmation in other systems is desirable. But the observed phenomenon may well be of a general nature and could contribute materially to a valid explanation of some advantages of fractionated radiotherapy.

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The Effect of Anoxia on Radiation Injury and Recovery

By T. L. Phillips and G. E. Hanks

The absence of oxygen enhancement and the absence of recovery between exposures associated with high LET radiations suggest that low LET radiations associated with hypoxic protection could cause damage having a similar absence of recovery. LAF^o Mice were irradiated with a 250 kvp. X-ray machine in an atmosphere of either 4.2% oxygen or in air. The LD 50/30 dose under hypoxic