ered when interpreting the ratios of various rock types of any given age. In other words, the total mass of sediments of all ages existing at any given time in the geologic past may have had about the same ratios of rock types that we observe today. Differences in the ratios of rock types as a function of age in the rock mass existing today may depend on differential cycling rates of the components. We do not contend that all age differences in ratios of rock types existing in sedimentary rocks are caused by differential removal and deposition of various components of the sedimentary mass, but that geological conclusions based on use of today's ratios as indications of the ratios at the time of sedimentation should be tempered by consideration of the effect of differential cycling.

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Xenon: Effect on Radiation Sensitivity of HeLa Cells

Abstract. *HeLa cells, plated onto plastic petri dishes, were exposed to various atmospheres composed of air and carbon dioxide; helium, oxygen, and carbon dioxide; and xenon, oxygen, and carbon dioxide in a pressure vessel. Survival curves with x-rays, 280 kilovolts (peak), show that air and helium have the same effect, but that xenon potentiates x-irradiation to the extent that the dose to produce a given level of survival with xenon is 0.58 of the dose required with air.*

Ebert and Howard (*1*), reporting on the growth rate of *Vicia faba* irradiated under atmospheres of xenon and air, showed that the presence of xenon produced a hypoxic type of protection, as measured by growth rate. Later experiments by Evans, Roberts, and Orkin (*2*) on the 30-day survival of mice irradiated under Xe-O_2 atmospheres produced similar results. In conjunction with a study of the distribution of xenon gas within mammalian cells exposed to Xe-O_2-CO_2 atmospheres at various pressures and periods of exposure, we investigated the influence of xenon and helium gases on the radiation sensitivity of the HeLa cell.

HeLa S-3 cells (*3*) were maintained in sterile, glass prescription bottles in Eagle's minimum essential medium supplemented with 10 percent fetal bovine serum, after the methods of Puck and Marcus (*4*). Cells were treated with a mixture of trypsin and EDTA to remove them from the glass surface and were plated onto Falcon plastic petri dishes (tissue culture grade). The number of cells plated was varied so that the fraction of cells expected to survive irradiation would produce between 100 and 200 clones. Control plating efficiencies were between 45 and 75 percent. Plates were incubated at 37°C under a charcoal-filtered, water-saturated atmosphere of 95 percent air and 5 percent CO_2 for 4 to 24 hours prior to experimentation. Initially all irradiations were performed 4 hours after plating, but, based on the findings of Barendsen and Walter (5), we shifted to 24 hours after plating to allow cells to recover from the traumatic effects of trypsinization. In terms of clone formation, cells irradiated at 24 hours will appear less sensitive than those irradiated at 4 hours, because of cell multiplicity. Methods for correcting for cell multiplicity have been described by Sinclair and Morton (*6*); however, these corrections affect mainly the shoulder of the survival curve without changing the value of D_0, the dose that, on the straight line portion of the survival curve, will reduce survival to 37 percent.

Cells were irradiated in a pressure vessel that holds four 60-mm petri dishes. A gassing system was designed that allows for precise delivery of each gas to the pressure vessel. Control plates were gassed with premixed air (95 percent) and CO_2 (5 percent). Plates treated with experimental gases were first flushed with a mixture of O_2 (80 percent) and CO_2 (20 percent) at a reduced pressure of 0.25 atm. The vessel was then sealed at this reduced pressure and helium or xenon (*7*) admitted to produce total pressures from 1 to 7.75 atm. The pressure vessel was held in a reproducible position 47 cm from the target of an x-ray machine, 280 kv (peak), by a support jig that was locked to the body of the machine. The radiation field was made uniform to ±1.5 percent at the level of the petri dishes. The quality of the x-ray beam inside the pressure vessel was 1.1 mm Cu half-value layer and the exposure rate was approximately 190 r/min at the level of the cells, as determined by LiF thermoluminescent dosimeters held in the pressure vessel.

Following irradiation, the four plates used for each experimental point were removed from the pressure vessel and returned to the incubator. Survival was measured by clone formation 12 to 14 days after irradiation. Colonies were stained with methylene blue and scored according to criteria for distinguishing viable from abortive clones (*8*).

Initially, the HeLa cells were irradiated approximately 4 minutes after pressurization with the gases, pressures of the gas mixtures ranging from 1.0 to 7.75 atm. The survival of cells irradiated under a He-O_2-CO_2 mixture at the above pressures was the same as for cells irradiated in air at a pressure of 1 atm. Pressure, per se, does not

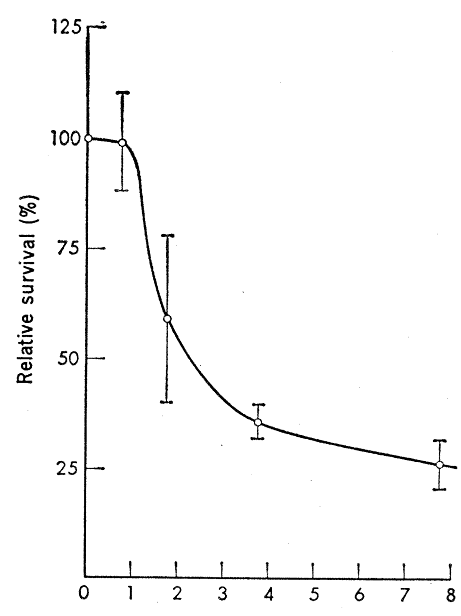

Fig. 1. Survival of HeLa cells in xenon, relative to cells in air, versus the partial pressure of xenon. The x-ray exposure was 430 r. Partial pressures of O_2 and CO_2 were maintained at 0.20 and 0.05 atm, and irradiation was 4 hours after plating. Vertical lines about each mean value represent 95 percent confidence intervals.

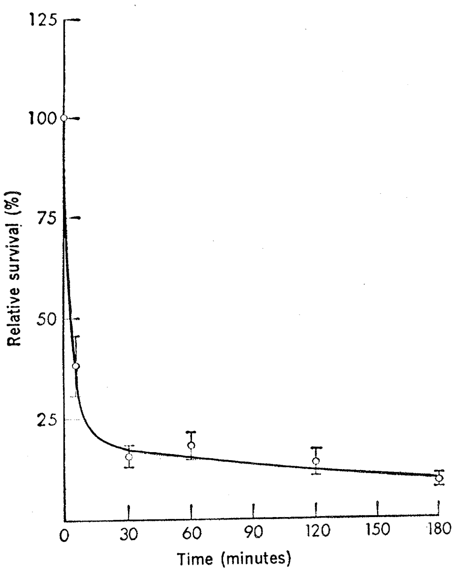

Fig. 2. Survival, relative to air, of HeLa cells exposed to 430 r versus the time of exposure to an atmosphere composed of Xe (3.50), O_2 (0.20), and CO_2 (0.05); numbers in parentheses are partial pressures. Irradiation was 24 hours after plating.

appear to affect survival after irradiation. However, cells irradiated under Xe-O_2-CO_2 atmospheres showed an increased sensitivity to radiation that was a direct function of the partial pressure of xenon. The survival of cells exposed to 430 r under various pressures of xenon is related to their survival under air (see Fig. 1). Irradiation of these cells took place 4 hours after plating.

The experimental results depicted in Fig. 1 are the opposite of what we had anticipated from the reports of Ebert

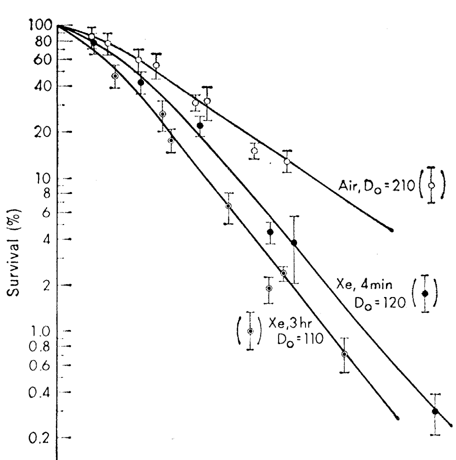

Fig. 3. Survival curves for HeLa cells irradiated under atmospheres of air and xenon. The xenon curves are for 4-minute and 3-hour periods of exposure to the gas prior to irradiation. Irradiation was 24 hours after plating; D_0, mean lethal dose.

and Howard (*1*) and Evans *et al.* (*2*) and prompted further investigation of this phenomenon. Since radiation sensitivity increased with the partial pressure of xenon, it appeared to be proportional to the amount of xenon in or surrounding the cells at the time of irradiation. Estimates of the rate of diffusion of xenon through the 2 mm of growth medium that overlies the HeLa cells attached to the bottom of the petri dishes indicated that approximately 2 hours are required for 95 percent saturation. Therefore, we tested next the radiation sensitivity of HeLa cells exposed for varying periods of time to an atmosphere composed of Xe (3.50), O_2 (0.20), and CO_2 (0.05), the numbers in parentheses being the partial pressures of the gases. In this experiment the cells were exposed to 430 r 24 hours after plating (Fig. 2). The increase of sensitivity with increased exposure time is clearly demonstrated.

Because of the relatively high atomic number of xenon, the absorbed dose in growth medium overlain with an atmosphere of xenon will be greater than that in the same medium overlain with air. By use of the solubility coefficient of xenon in plasma determined by Ladefoged and Anderson (*9*), it was calculated that the absorbed dose in medium overlain by air, 0.97 rad/r, could, under conditions of equilibrium, increase to 1.23 rad/r when the medium was overlain by xenon at a partial pressure of 3.5 atm. To test these results, a fine grade of LiF thermoluminescent powder (average particle size, 21 μm) was irradiated under conditions identical to those used for the cells. The dose at the level of the cells was measured as a function of time of exposure to the xenon atmosphere. As the time of exposure to xenon prior to irradiation was increased, the absorbed dose in LiF increased, reaching a maximum value of 1.11 rad/r in approximately ½ hour. The curve of the increased dose of LiF with increased time of exposure to xenon showed almost an exact mirror image of the survival curve in Fig. 2. This remained unchanged for exposures up to 3 hours, the longest time studied. Discrepancies between calculated and measured doses may be due to such factors as the wrong x-ray spectrum or absorption coefficients, the solubility coefficient of xenon in growth medium, nondiffusibility of xenon in LiF, and others. Because we place greater reliance on our experimental dosimetry, the absorbed dose scale in Fig. 3 is based on the LiF measurements.

Survival curves for HeLa cells irradiated in air and xenon at a partial pressure of 3.5 atm, 24 hours after plating, are shown in Fig. 3. The curves for 4-minute and 3-hour periods of exposure to xenon prior to irradiation are qualitatively the same as the "air" curve but with the mean lethal doses (D_0) reduced from 210 rads to 120 and 110 rads, respectively. The effect of exposure to xenon gas for periods as short as 4 minutes is to potentiate the effects of x-rays on the HeLa cells by a factor of 1.75, and this is obtained after correcting the absorbed dose for the increased absorption due to xenon. Administration of xenon after irradiation has not produced any alteration in survival.

The explanation of this effect may be that the concentration of xenon in the HeLa cell is much greater than in the surrounding growth medium. Except for its high lipid solubility, we cannot suggest any mechanism that would explain high intracellular concentrations of a so-called inert gas. Exclusion of a purely dosimetric effect requires that we postulate either a metabolic or a chemical action for xenon, and these would have to be synergetic with radiation since exposure to xenon alone, for the short periods utilized here, has shown no effects on cell survival.

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