IRRADIATION OF CELLS IN TISSUE CULTURE
VI. THE INFLUENCE OF OXYGEN ON INJURY TO CELLS BY GAMMA
IRRADIATION FROM A COBALT** SOURCE*

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With 13 Figures in the Text
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Introduction
Radiosensitivity, in relation to increase in oxygen availability, has been
explored since the publication of the classic paper by HÖLTHUSEN (1921). Recent
findings reviewed by GRAY (1957) indicate that a variety of biological systems
show this oxygen effect as measured in terms of lethality, mitotic inhibition or
chromosomal alterations. While there is general agreement that cells become
more vulnerable to irradiation with increases in the level of oxygen in their
immediate environment, there is considerable speculation regarding its mechanism
of action (PATT 1953 and LASER 1956).

Giant cell formation resulting from X-irradiation of cell strains has been
reported by PATerson (1942) and by PUCK and MARCUS (1956). This biological
effect was subsequently shown by POMERAT, KENT and LOGIE (1957) to be
typical for established cell strains, but not for cells in primary outgrowth. It
is believed that this phenomenon may be correlated with increased numbers of
chromosomes in cell strains, although PUCK et al. (1957) reported giant cell
formation in a freshly isolated line of fibroblast-like cells. In addition, giant
cells have been observed in irradiated human uterine carcinomata (ATKIN and
RICHARDS 1958). Preliminary studies in this laboratory have indicated that
giant cell formation also demonstrates a sensitivity to irradiation which is depend-
ent upon the oxygen concentration.

The purpose of the present report was to establish the population curves of
standard cultures which had been subjected to varying irradiation dosages in
terms of normal and giant cell survival. In addition, the experiments were
designed to estimate the effect of varying oxygen tensions in relation to lethality
and to giant cell production.

Materials and Methods
All experiments were performed in Rose chambers (ROSE 1954) with a standard number
of 10,000 amnion cells per chamber. The strain of human amnion developed by FERNANDES

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(1958) was cultured in Eagle's medium plus 10% horse serum for two days prior to irradiation. These resemble epithelial elements with approximately 0.25% showing giant cell form. The chromosome count has a modal value of 75 according to Nakanishi et al. (1959). The Cobalt60 facility employed was described in the first paper in this series (Pomerat, Kent and Logie 1957).

The setup for replacing the fluid with gas is shown in Figure 1. A short plastic tube and a sterile needle (no.1) were flushed with the gas mixture being used. The pressure was regulated at two pounds per square inch and the gas was permitted to flow continuously. The gas flowed through a Millipore (HA) filter held in a Swinney adapter to prevent introduction of microorganisms into the cultures. Prior to flushing the chamber, a second sterile outlet needle (no. 2) was inserted and the chamber rotated so that needle no. 2 was down. With the gas flowing, needle no. 1 was inserted and the gas was permitted to displace the fluid from the chamber, leaving only a film of culture medium over the cells. Needle no. 2 and then needle no. 1 were quickly removed. The chambers were then ready for irradiation. Immediately after irradiation, they were refilled with Eagle's medium.

The test gases consisted of commercial supplies of 100% oxygen, air and nitrogen. The nitrogen was passed through a pyrogallol solution in a gas washing bottle (see Fig. 1) in an attempt to remove the residual traces of oxygen. Four chambers each were subjected to the following gamma radiation dosages: 0, 100, 300, 500, 600, 800, 1000, 2000, 4000, 6000, 8000, 10,000 and 25,000 r. Unirradiated control chambers were filled with the test gas for a period which was equivalent to the maximum irradiation time. Cultures were incubated for 5 days post irradiation, fixed with absolute methyl alcohol and stained with the Jacobson's method (May Grinwald-Giemsa).

For quantitative studies of these stained preparations, total population counts (consisting of both non-giant and giant cells) were determined in terms of the number of cells in a standard area. This area, which was defined by a reticle, consisted of a lined strip 0.5 mm wide across the diameter of the chamber, or a total area of 14 mm² when used with a 10× ocular and a 10× objective. Due to the fact that the cells were not equally distributed throughout the chamber, it was necessary to determine the average of counts across four different axes of each chamber to insure an accurate sampling. These average values were used to calculate the number of surviving cells in relation to a standard of 1000 cells in the unirradiated control cultures.

Giant cells were arbitrarily defined as being elements which had at least twice the area of the average non-giant cell. The percentage of cells which were considered giants was determined as a result of counting a minimum of 2000 cells for each experimental condition. It was then possible to estimate the average number of surviving giant and non-giant cells as compared to the standard number (1000) of cells in control cultures.

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